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Use of β-glucuronidase reporter gene for gene expression analysis in turfgrasses

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Abstract

The β-glucuronidase (GUS) gene has been successfully used as a reporter gene in innumerable number of plant species. The functional GUS gene produces blue coloration in plants upon integration into the plant genome. Because of the ease it provides to analyze the gene expression (as no expensive equipment is needed), GUS gene is surely plant biotechnologist's first choice as a reporter gene. The turfgrass family contains the world's most economically important horticultural crops. There is a world-wide drive for genetic modification of grasses due to its huge economic importance. GUS gene can be transiently or stably expressed in grasses for the purpose of promoter analysis and to study tissue-specific and developmental gene expression. This paper summarizes the use of GUS gene for transient and stable expression studies in various turfgrass species.
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The choice of a good reporter gene is an integral component for the success of plant transformation studies. Scientists have studied chimeric GUS reporter gene fusion systems for various purposes including: (1) to understand developmental patterns of gene expression; (2) to optimize the particle bombardment parameters; (3) to compare different transformation methods; (4) for promoter analysis (to identify the ideal promoter useful for a specific plant species transformation); and (5) to compare the best tissue culture media for selection and regeneration. A wealth of literature is already available on in-depth analysis of GUS gene expression studies in many different types of plant species. The purpose of this review is to summarize GUS gene expression studies done in turfgrass species only.

Genetic improvement of turfgrass

Turfgrasses are monocotyledonous plants. They belong to the family Poaceae (Graminae). All cereal crops fall into this 'grass' category. There are many types of turfgrasses, including ryegrass, orchardgrass, tall fescue, timothy, Kentucky bluegrass, quackgrass, smooth bromegrass, and creeping bentgrass, etc.

Turfgrass management and production is one of the fastest growing areas of agriculture. The lawn care industry serves 35.5 million single family detached households (SFDH) in the USA [1]. There has been an exponential growth in the lawn care industry since 1960 [1]. The USA turfgrass seed industry has $580 million in annual seed sales [2,3]. Given the magnitude of the nation's turf enterprise, it is not surprising that the impact of turf on the environment and the resources committed to its maintenance should receive critical attention. This attention challenges turfgrass scientists to develop a low input, sustainable turf ecosystem.

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Biotechnological approaches for turfgrass improvement

Previously, turfgrass improvement was confined to the use of conventional breeding techniques which are laborious, time consuming, and solely dependent upon the production of new germplasm through sexual reproduction [4]. Now, with the advent of biotechnology approaches, new and more efficient molecular tools for genetic manipulation are available to the plant breeder [5]. The importance of biotechnology in turfgrass improvement was highlighted in the 6th International Turfgrass Conference in Tokyo, Japan, in 1989 [6]. Subsequently, Zhong et al. [7] reported one of the first successful stable transgenic turfgrass plants generated using microprojectile bombardment [8]. Progress has been rapid, and Chai and Sticklen [4] pointed out four major breakthroughs in techniques utilized in the application of biotechnology for turfgrass improvement including molecular markers, in vitro tissue culture, direct gene transfer, and use of fungal endophytes.

Use of GUS as a reporter gene in turfgrass improvement programs

The *Escherichia coli uidA* gene encoding β-glucuronidase (GUS) [9] is widely used as a reporter gene in plant transformation studies [10] because the gene expression patterns can be quantified by fluorometric and spectrophotometric analysis. Additional advantages of the GUS assay are that it is very straightforward and requires no expensive equipment. The major disadvantage of the GUS assay is that the chemicals necessary for this assay are expensive. Also the GUS assay is lethal for the plant tissues. With these limitations, however, the GUS assay is still one of the most effective reporter gene systems used by the scientists in plant gene expression studies.

Plant scientists have bombarded both embryogenic and non-embryogenic tissue-cultured cells and used chimeric GUS reporter gene fusions for transient and stable expression studies. The following section outlines some examples of use of GUS as reporter gene in various turfgrass species.

Tall fescue (Festuca arundinacea)

Ha et al. [11] electroporated tall fescue protoplasts with a plasmid (pZO1052) carrying a hygromycin-resistance gene and a GUS reporter gene (both driven by the CaMV 35S promoter) and observed GUS activity in most of the transformed cells. Penmetsa and Ha [12] also worked on factors and parameters of electroporation that influence transient gene (GUS) expression in tall fescue protoplasts and observed highest GUS activity 24 h after electroporation. Kuai and Morris [13] performed polyethylene glycol mediated transformation of tall fescue suspension cell cultures with the plasmid pAct1-D. They noticed a gradual decline in both transient and stable GUS (driven by the rice actin 1 promoter) expression in suspension cell cultures when the cells were cultured for long periods of time [13]. Kuai and Morris [14] even observed different types of GUS expression (light blue and dark blue) from two types of callus clones of tall fescue when they were screening for stable transformants (after polyethylene glycol mediated transformation). Bettany et al. [15] observed GUS expression (driven by the rice actin 1 promoter) at different vegetative developmental stages of the fescue plant (after they stably transformed the protoplasts using polyethylene glycol mediated transformation) and found unstable GUS expression during early stages of tillering. When Kuai et al. [16] regenerated fescue plants from tissue culture of callus bombarded with constructs including the *bar* gene and a GUS gene under the control of rice actin 1 promoter, surprisingly they were not able to detect GUS activity although they detected the activity of the *bar* gene. Cho et al. [17] produced transgenic fescue plants by microprojectile bombardment with the *uidA*, a GUS reporter gene along with the *bar* gene (driven by the rice actin 1 promoter).

Orchardgrass (Dactylis glomerata), timothy (Phleum pratense), and zoysiagrass (Zoysia japonica Steud.)

Horikawa et al. [18] delivered foreign genes to orchardgrass by biolistics (using the pBI221 plasmid from Clontech, Palo Alto, CA, USA) and assayed the callus for GUS expression. Denchev et al. [19] produced transgenic orchardgrass by microprojectile bombardment and observed transient GUS expression (driven by the maize ubiquitin promoter ubi-1) in the leaf tissues and in the somatic embryos. Toyama et al. [20] produced herbicide tolerant zoysiagrass by *Agrobacterium*-mediated transformation. They reported that removal of calcium from the tissue culture media during co-cultivation with *Agrobacterium tumefaciens* enhanced GUS expression.

Ryegrass (Lolium perenne, Lolium multiflorum)

Hensgens et al. [21] studied the transient and stable expression of GUS (under the control of the CaMV 35S promoter) using particle bombardment in rice, barley, and perennial ryegrass. They studied the activity of a GUS fusion with the rice gene *GOS2* (involved in translation initiation) and another rice gene *GOS5* (a light inducible gene) [21]. They observed that insertion of the described transcriptional, translational, intron, and exon sequences in a plasmid construct (PORCH-EHyg) resulted in higher GUS activity [21]. Van Der Mass et al. [22] stably transformed perennial ryegrass by

microprojectile bombardment and observed stable integration of the *uidA* gene (GUS) (regulated by a constitutive promoter of the rice gene *GOS2*) in the plant genome. Using transient GUS gene expression profiling in suspension culture cells, Spangenberg et al. [23] optimized the bombardment parameters for the purpose of delivery of foreign genes to ryegrass. Wang et al. [24] transformed ryegrass protoplasts and showed that the GUS gene was integrated into the genome and the gene was transferred into the progeny. Dalton et al. [25] stably transformed ryegrass with a GUS reporter gene (driven by the CaMV 35S promoter) using biolistics and regenerated the plants from the suspension cultures.

Bentgrass (Agrostis palustris)

Zhong et al. [7] analyzed transgenic creeping bentgrass for GUS expression after microprojectile bombardment under the control of rice actin promoter. They observed differential GUS activities in different tissue types [7]. They observed highest GUS activity in the stem node and lowest GUS activity in the root hair zone [7]. Lin et al. [26] electroporated bentgrass protoplasts with pZO1052 (a plasmid containing the CaMV 35S promoter, maize *Adh1* intron 6, and *uidA* as a reporter gene). They optimized the electroporation conditions for GUS expression in bentgrass protoplasts [26]. GUS expression reached its highest peak at a KCl concentration of 120 mM in the electroporation medium and then the GUS expression decreased sharply with more KCl in the medium [26]. Basu et al. [27] bombarded GUS gene driven by four different promoter constructs (ubiquitin rice, ubiquitin corn, ubiquitin-3-potato, and CaMV 35S) onto turfgrass leaves, roots, and callus tissues. From the transient gene expression profiling at various turfgrass tissues they concluded that ubiquitin rice expressed highest number of GUS irrespective of the tissue bombarded [27]. Basu et al. [27] also showed that GUS gene expression varies between genotypes and the transient GUS expressing protein degrades over time in creeping bentgrass callus tissues. It was also shown by Basu et al. [28] that turf tissues had enough nuclease activities to degrade foreign DNA containing GUS gene. But when they treated the turf tissues with nuclease inhibitors there was more than 1000-fold higher GUS expression compared to the control tissue [28]. Luo et al. [29] reported the production of phosphinothricin resistant creeping bentgrass via *Agrobacterium*-mediated transformation. They used transient GUS expression patterns to monitor efficiency of transformation and observed 60% of embryogenic callus of creeping bentgrass expressed GUS following *Agrobacterium* inoculation and co-cultivation with the bacteria [29].

It is interesting to note that when Dalton et al. [30] transformed annual ryegrass, tall fescue, and redtop (*Agrostis stolonifera*) by silicon carbide fiber-mediated transformation with a GUS reporter gene, one of the *A. stolonifera* and three annual ryegrass transgenic plants did not show any GUS activity.

Conclusion

There is no doubt that GUS expression patterns are complex in plant cells. GUS gene expression is affected by various biochemical, molecular, and biological factors. GUS gene can be effectively used as a reporter gene in grasses. From the transient gene expression studies, it is possible to evaluate a set of novel promoters so a choice can be made for the purpose of precisely controlling expression patterns of genes that can be used to confer trait enhancement (e.g., herbicide tolerance, pathogen resistance, etc.) in turfgrass species. Our previous experiments were designed to analyze two basic components of plant gene transfer methods: (1) what promoter most effectively regulates high expression levels of foreign genes in turfgrass, and (2) how chosen promoters regulate gene expression in different tissues and at different developmental stages (Basu et al. [27]). We have also reported use of GUS gene to analyze in vivo nuclease activities in turfgrass (Basu et al. [28]). The use of GUS genes in grasses will open new research avenues which will lead to further work on germplasm enhancement and functional genomics of grass species.

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