Transient gene expression in protoplasts from switchgrass (*Panicum virgatum* L.): A potential bioenergy crop.

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Abstract:

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass that is a major component of the prairies of North America. It is grown in monoculture for hay, grazing, erosion, and is a potential source for biofuel production as a feedstock. Genetic manipulation of the growth and development of switchgrass is needed for better cellulosic ethanol production, especially to improve cellulose-to-lignin ratios. Establishment of protoplasts techniques for switchgrass is of crucial importance to bioenergy biotechnology. Several genes can make significant improvements in agronomic and feedstock traits of switchgrass, such as, altered cellulose levels, dwarfism, drought resistance and pollen alterations that can be introduced via transgenesis. Although there is no substitute for stable transformation, current procedures are time-consuming, laborious, inefficient, and not suited for high-throughput assays. Conversely, the use of transient gene expression assays offers an opportunity to study large numbers of genes quickly, which would be advantageous for evaluating the transcriptional activity of different promoters, and might be especially useful for assaying cell biology and cell wall traits.

Here we report the first protocol for protoplasts isolation and transgene expression based on leaf- or root-derived protoplasts of two switchgrass genotypes. We demonstrated transient expression of polyethylene glycol (PEG)-mediated DNA uptake in the isolated protoplasts by measuring the activity of b-glucuronidase (GUS) reporter gene driven by either the Cauliflower mosaic virus (CaMV) 35S promoter or the maize ubiquitin-1 promoter. Protoplast transformation with either the 35S or the ubiquitin promoter resulted in an increase in GUS activity compared to the untransformed controls; however, the extent of GUS activity was considerably higher for the ubiquitin promoter than for the 35S promoter. Our efficient protoplasts isolation and transient gene expression system provide insight into the versatility of the transient assay system in studies of gene expression in switchgrass.

Keywords: Switchgrass, *Panicum virgatum*, protoplast, transient gene expression, biofuel