Biotechnology Reports 6 (2015) 41–44

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Short communication

A simple and highly efficient *Agrobacterium*-mediated transformation protocol for *Setaria viridis*☆

Polyana Kelly Martins, Ana Paula Ribeiro, Bárbara Andrade Dias Brito da Cunha, Adilson Kenji Kobayashi, Hugo Bruno Correa Molinari *

Genetics and Biotechnology Laboratory, Embrapa Agroenergy, Brasília, DF, Brazil

ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form 11 February 2015
Accepted 16 February 2015
Available online 18 February 2015

Keywords:
Model system
C4 metabolism
Bioenergy
Sugarcane
Green millet

ABSTRACT

The production and use of sugarcane in Brazil is very important for bioenergy production and is recognized as one of the most efficient in the world. In our laboratory, *Setaria viridis* is being tested as a model plant for sugarcane. *S. viridis* has biological attributes (rapid life cycle, small genome, diploid, short stature and simple growth requirements) that make it suitable for use as a model system. We report a highly efficient protocol for *Agrobacterium*-mediated genetic transformation of *S. viridis*. The optimization of several steps in tissue culture allowed the rapid regeneration of plants and increased the rate of transformation up to 29%. This protocol could become a powerful tool for functional genomics in sugarcane.

Sugarcane (*Saccharum* spp.) is an important biofuel feedstock because of its ability to accumulate high quantities of biomass and sucrose and is one of the most photosynthetically efficient C4 plants [1]. Breeding programs has been the main approach towards sugarcane improvement. However, the time required for a new variety (13 years) and the complexity of the genome of modern sugarcane varieties are major constraints [2]. Since the early 1990s, much progress was achieved in the biotechnological manipulation of sugarcane [3]. Nevertheless, stable transformation and plant regeneration for genomics studies is a time consuming for this crop. Model plants for genetic transformation like *Arabidopsis thaliana* are used in the proof of concept for many traits in various important crops. Therefore, there is still a need for additional model plants to decode and translate traits that are absent in these species [4]. In order to assist this endeavor, *Setaria viridis* was recently describe as a new monocotyledonous model species for C4 photosynthesis research and genetic transformation [5]. *S. viridis* belongs to the Poaceae family, subfamily Panicoideae that is one of the most agronomically important grass, including sugarcane. *S. viridis* has a number of characteristics that makes it interesting as a model plant [4]. Therefore, there is a need for a simple and highly efficient protocol for *S. viridis* genetic transformation. Here we report a simple protocol using *Agrobacterium*-mediated transformation with reporter genes *gus* (β-glucuronidase) and *gfp* (green fluorescent protein).

Seeds of *S. viridis* (accession A10.1) were planted in soil and grown in phytotron chambers under 16 h photoperiod, 26 ± 2 °C, 65% relative humidity and light intensity of 400 μmol m^{-2} s^{-1}. Mature seeds were used for embryogenic callus induction. Seeds were disinfested after removal the lemmas and paleas with a solution of 10% sodium hypochlorite and 0.1% Tween 20® for 3 min followed by 5 rinses in sterile distilled water. After blotted on sterile filter paper the dehulled mature seeds were placed on callus induction medium (CIM) that consisted of MS salts [6], 1 mg/L d-biotin, 0.5 mg/L pyridoxine HCl, 0.5 mg/L nicotinic acid, 100 mg/L myo-inositol, 0.1 mg/L thiamine-HCl, 0.6 mg/L $CuSO_4$, 30 g/L sucrose, 2 mg/L 2,4-dichlorophenoxyacetic acid, 0.5 mg/L kinetin and 4 g/L Phytagel™. The pH of the medium was adjusted to 5.8. After 3 to 5 weeks of incubation in the dark at 25 ± 2 °C, the callus was divided into small explants and subcultured onto fresh CIM. After 4–5 days these explants are ready to transformation step.

The expression vectors used for transformation of *S. viridis* are listed in Table 1. These vectors contain the reporter genes *gus* and *gfp* both with intron (DNA Cloning Service, Germany). A primary culture of *Agrobacterium tumefaciens* (EHA 105) was prepared by inoculating a single colony from a freshly streaked YEB agar plate in 5 mL of autoclaved YEB containing 100 mg/L spectinomycin and

* Corresponding author. Tel.: +55 61 34482307; fax: +55 61 34481589.
E-mail address: hugo.molinari@embrapa.br (H.B.C. Molinari).

http://dx.doi.org/10.1016/j.btre.2015.02.002

Table 1
Summary of transgenic plants production by embryogenic calli.

Vectors[a]	Number of explants	Selectable marker	Number of PCR positive plants	Transformation efficiency (%)[b]
p6i	225	*hpt*	32	14.22
p6mD#1	218	*hpt*	30	13.76
p6mD#2	55	*hpt*	16	29.09
p6	77	*hpt*	6	7.79
p7U	50	*bar*	3	6.0
p6mD#3	83	*hpt*	11	13.25
p6mD#4	33	*hpt*	4	12.12

hpt – hygromycin phosphotransferase gene with an intron, *bar* – phosphinothricin acetyl transferase gene with an intron.
[a] Vectors were purchased from DNA Cloning Service (www.dna-cloning.com). p6mD vector contains different genes of interest.
[b] For each construct, the transformation efficiency was calculated as the total number of PCR positive plants/total number of inoculated callus ×100.

50 mg/L rifampicin. The bacterial culture was incubated for 16 h in an orbital shaker at 180 rpm in dark at 28 °C. Secondary culture was initiated by inoculating 50 μL of primary culture into 25 mL YEB supplemented with 200 μM of acetosyringone with the same antibiotics and grown under the same conditions. Bacterial suspension was centrifuged and the pellet was resuspended in a liquid CIM medium without $CuSO_4$ to OD_{600} = 0.6. Approximately 50 calli were incubated for 5 min in *Agrobacterium* suspensions that contained 200 μM of acetosyringone and 10 μL of a 10% Synperonic® PE/F68 (Sigma–Aldrich) solution per 1 mL of the

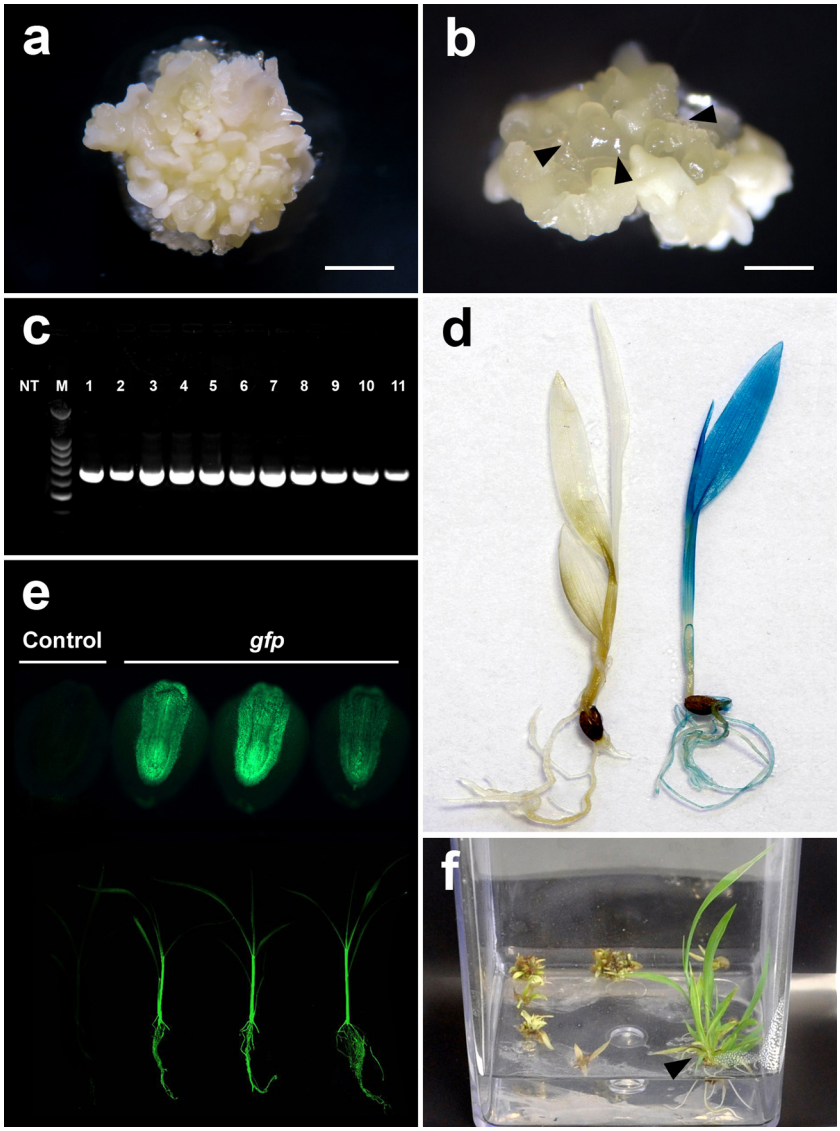

Fig. 1. *Agrobacterium tumefaciens*-mediated transformation of *Setaria viridis*. (a) embryogenic callus after 5 weeks in CIM medium (bar = 2 mm). (b) translucent embryogenic callus most suitable for transformation (arrow heads, bar = 0.5 mm). (c) PCR analysis of the transgenic plants with *gfp* specific primer (NT: non-transgenic plant, lanes 1–11 transgenic plants, M: molecular weight marker – 100 bp DNA Ladder). (d) Transgenic plant expressing GUS (right) and non-transgenic (left). (e) Transgenic T1 seeds expressing GFP (top) and T_1 seedlings expressing GFP (bottom). (f) Regenerated transgenic plantlet in hygromicin-containing selective MS medium (arrow head).