Manuscript title:

Evaluation of parameters affecting switchgrass tissue culture: toward a consolidated procedure for *Agrobacterium*-mediated transformation of switchgrass (*Panicum virgatum*)

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- **Figure S7.** Vacuum-infiltration and desiccation treatment procedure for *Agrobacteria*-meditated callus transformation. (**A**) Transferred 2-day pre-culture WT calluses to the prepared *Agrobacterium* suspension. (**B**) Vacuum-infiltration the *Agrobacterium* suspension in plastic desiccator. (**C**) Desiccation treatment using 10 calluses with 100 µl sterile water in the middle. (**D**) Two days after desiccation treatment.
- **Figure S8.** The confirmation of the transgene using 3-month-old greenhouse-grown transgenic switchgrass plants by genomic DNA PCR. Primer set A (EV-F, EV-R) is specific for *Hph* gene. Primer set B (RFP-F, RFP-R) is for *pporRFP* gene. Primer set C (Gus-F, Gus-R) is for *GUSPlus* gene.
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- **Figure S11.** Detection of pporRFP expression in root tissue of empty vector (EV) and pCAMBIA-RFP (RFP) transformed switchgrass. (**A**) Bright field image. (**B**) Epi-fluorescence image under red fluorescence filter.
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Table S1. Comparison of the starting materials, target genes and transformation efficiency for the reported protocols and the protocol developed and used in this study.

	Cultivar	*Agrobacterium* Strain	Callus Origin	Callus Type	Gene	Selection Marker	Transformation Efficiency (%)	Reference
1	Alamo	AGL1	Somatic embryos* Embryogenic calluses Mature caryopses Basal part of plantlets	N/A	*GUS*	*Bar*	3.4[#]	Somleva et al., 2002; 2008
2	Alamo 2	EHA105	Inflorescences	Type II	*pporRFP*	*Hph*	4.4[#]	Burris et al., 2009
3	Alamo	EHA105* AGL1* LBA4404	Mature caryopses Inflorescence Nodes	N/A	*GUS*	*Hph*	N/A	Xi et al., 2009
4	Alamo Performer Colony	EHA105	Mature caryopses	Type II	*GFP*	*Hph*	Alamo (56.6) Colony (44.6) Performer (91.6)	Li and Qu, 2011
5	Alamo* Cave-in-Rock (CIR)	EHA105* LBA4404 GV3101	Seedlings (basal part)	N/A	*GUS*	*Bar** *Hph** *Npt*	Alamo (4.9-5.9) CIR (0.0)	Song et al., 2012
6	Alamo	AGL1	Mature caryopses	Type II	*GFP*	*Hph*	6.0	Ramamoorthy and Kumar, 2012
7	Alamo	EHA101	Mature caryopses	Type I	*GFP* *GUS*	*Bar*	2.0 -17.4	Ogawa et al., 2014
8	Alamo Performer Blackwell Dacotah	EHA105	Mature caryopses	Type II	*GUS*	*Hph*	Alamo (72.8) Dacotah (8.0)	Liu et al., 2015
9	Alamo Kanlow Blackwell CIR Trailblazer	EHA101	Mature caryopses	Type I	*GFP* *GUS*	*Bar*	Alamo (12.5-59.2) Kanlow (6.3- 20.0) Trailblazer (7.5-16.3)	Ogawa et al., 2016
10	Alamo	EHA105	Mature caryopses	Type I Type II	*pporRFP* *GUS*	*Hph*	50.0-100.0[^]	This study

GUS, β-glucuronidase; *pporRFP*, red fluorescence protein; *GFP*, green fluorescence protein

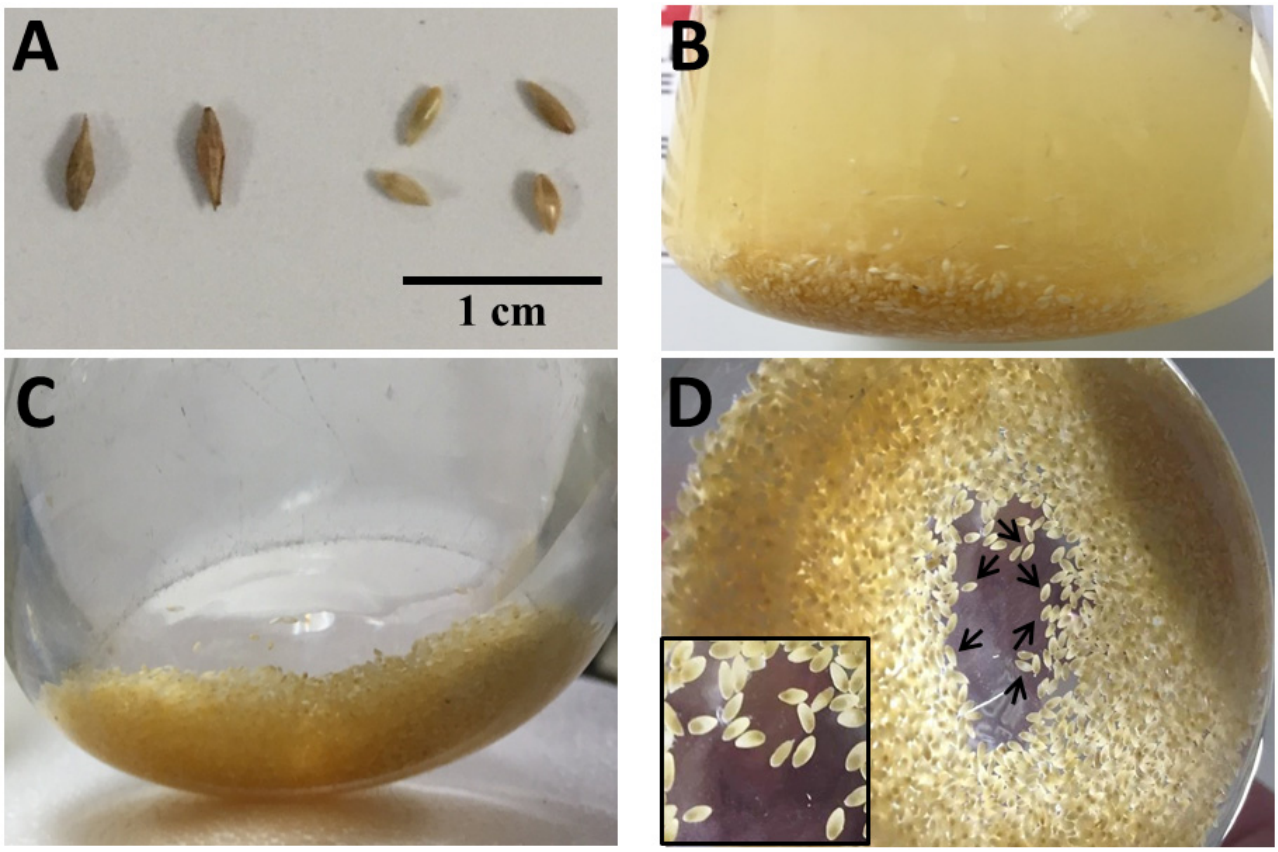
Bar, phosphinothricin acetyltransferase; *Hph*, hygromycin phosphotransferase; *Npt*, neomycin phosphotransferase

N/A, not applicable; *, more effective; [#], values are according to Ogawa et al., 2014; ^, selection efficiency

Table S2. Primer sets used in this study.

Primer	Sequence (5' to 3')	Target	Amplicon (bp)	Reference
pporRFP-F	ATCG**CAGCTG**CCACTGGAGAGGGGCACA	pporRFP expression cassette (pANIC6A)	3, 014	This study
pporRFP-R	CGATTCTAGAGTTTAATTCCCGATCTAG			
EV-F	CGTTATGTTTATCGGCACTTTGCAT	*Hygromycin B phosphotransferase* (*Hph*) (pCAMBIA-EV)	950	This study
EV-R	AGCGAAACCCTATAGGAACCCTAAT			
RFP-F	GCCTTTGCCATTTTCTATTGACATT	*pporRFP* (pCAMBIA-RFP)	836	This study
RFP-R	GTTTAATTCCCGATCTAG			
Gus-F	CAAGCACCGAGGGCCTGAGC	*GUSPlus* (pCAMBIA1305.2)	574	Muthukumar et al., 2013
Gus-R	CTCAGTCGCCGCCTCGTTGG			
RT_EV-F	CAACCAAGCTCTGATAGAGT	*Hph* (pCAMBIA-EV)	745	Ramamoorthy and Kumar 2012
RT_EV-R	GAAGAATCTCGTGCTTTCA			
RT_RFP-F	Same as RFP-F	*pporRFP* (pCAMBIA-RFP)	549	This study
RT_RFP-R	GAAGAATCTCGTGCTTTCA			
RT_ACTIN2-F	GCGAGCTTCCCTGTAGGTA	*Pv_ACTIN2* (FL724919.1)	317	Xu et al., 2011
RT_ACTIN2-R	CACACGGAGCTCGTTGTAGA			

***Pvu*II** site in bold and ***Xba*I** site underlined



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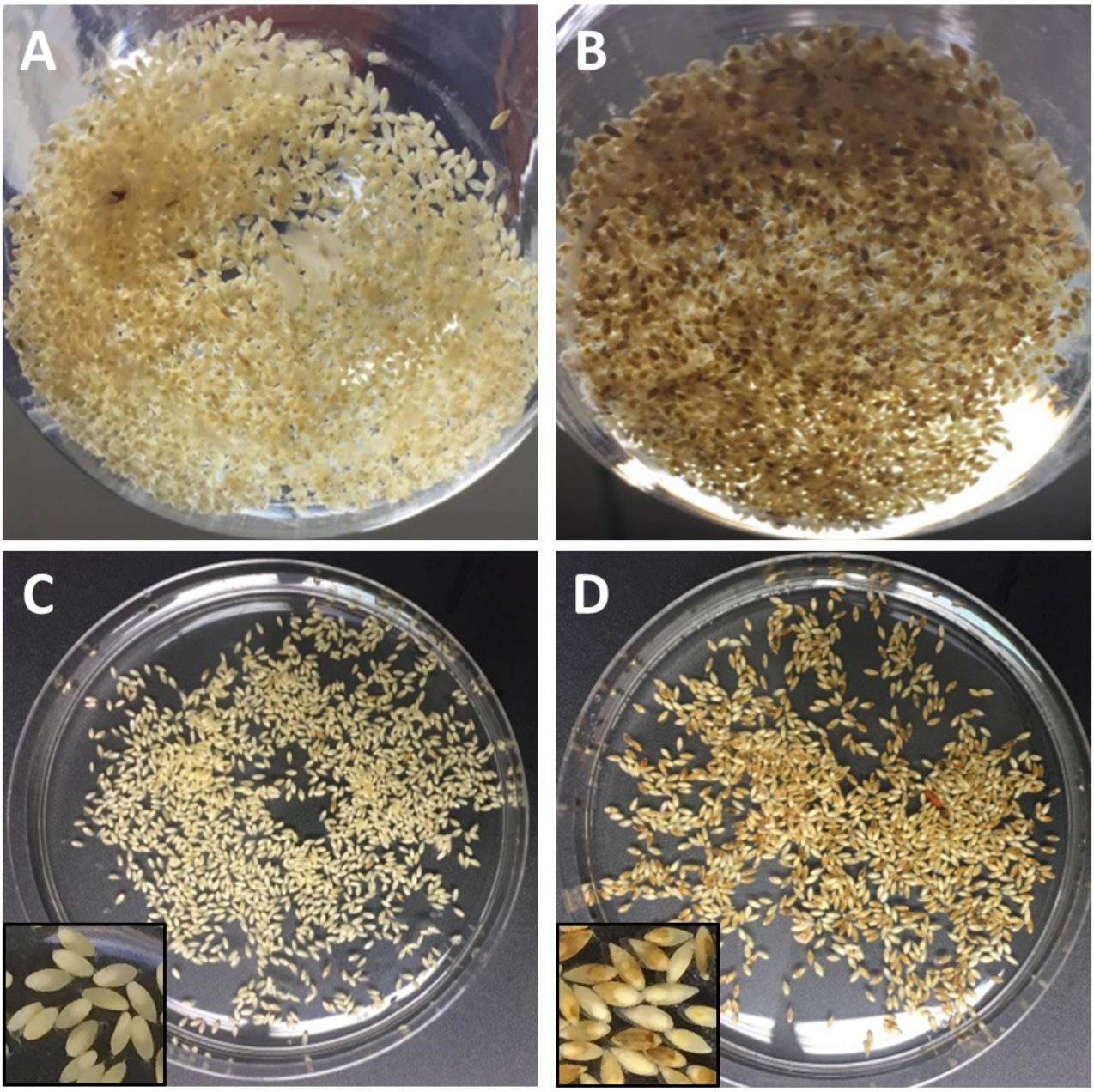

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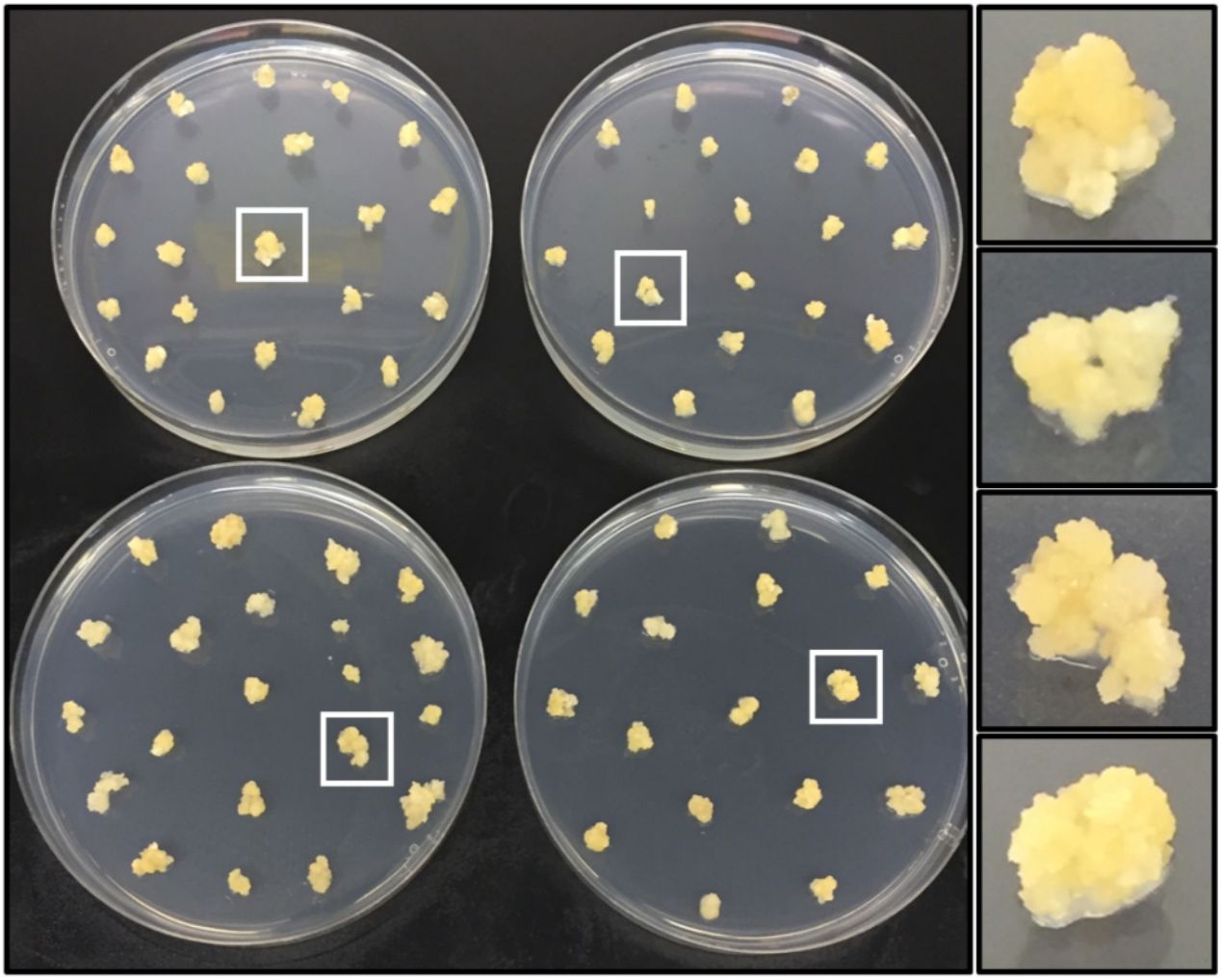

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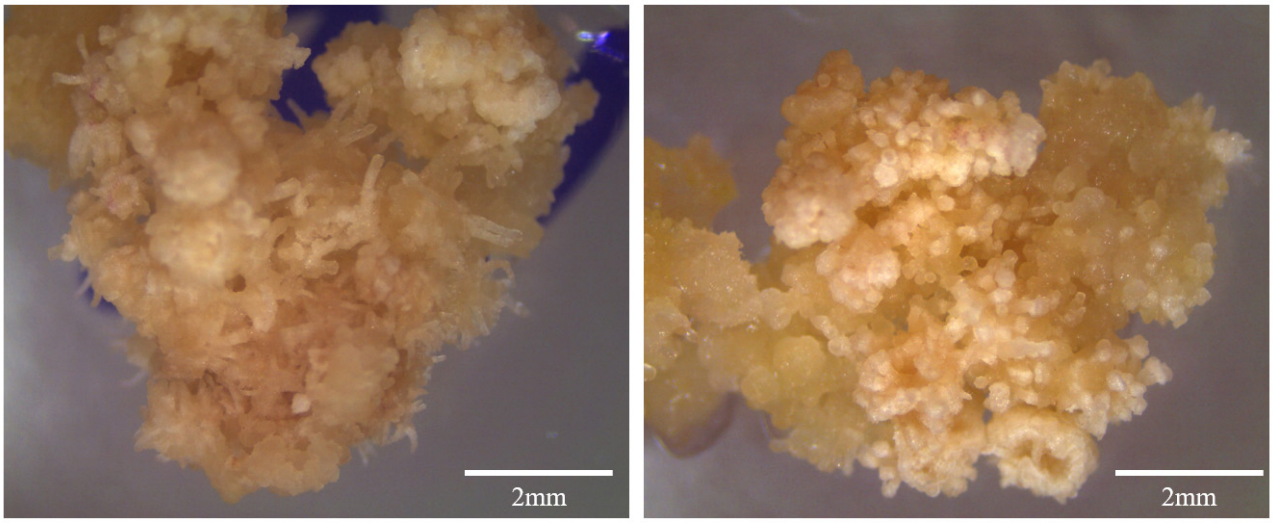


Figure S4. Lack of regeneration observed in samples of WT switchgrass callus raised on NB-based regeneration (REG) medium supplemented with 0.2 mg l^{-1} NAA + 1 mg l^{-1} BAP + 0.5 mg l^{-1} GA_3.

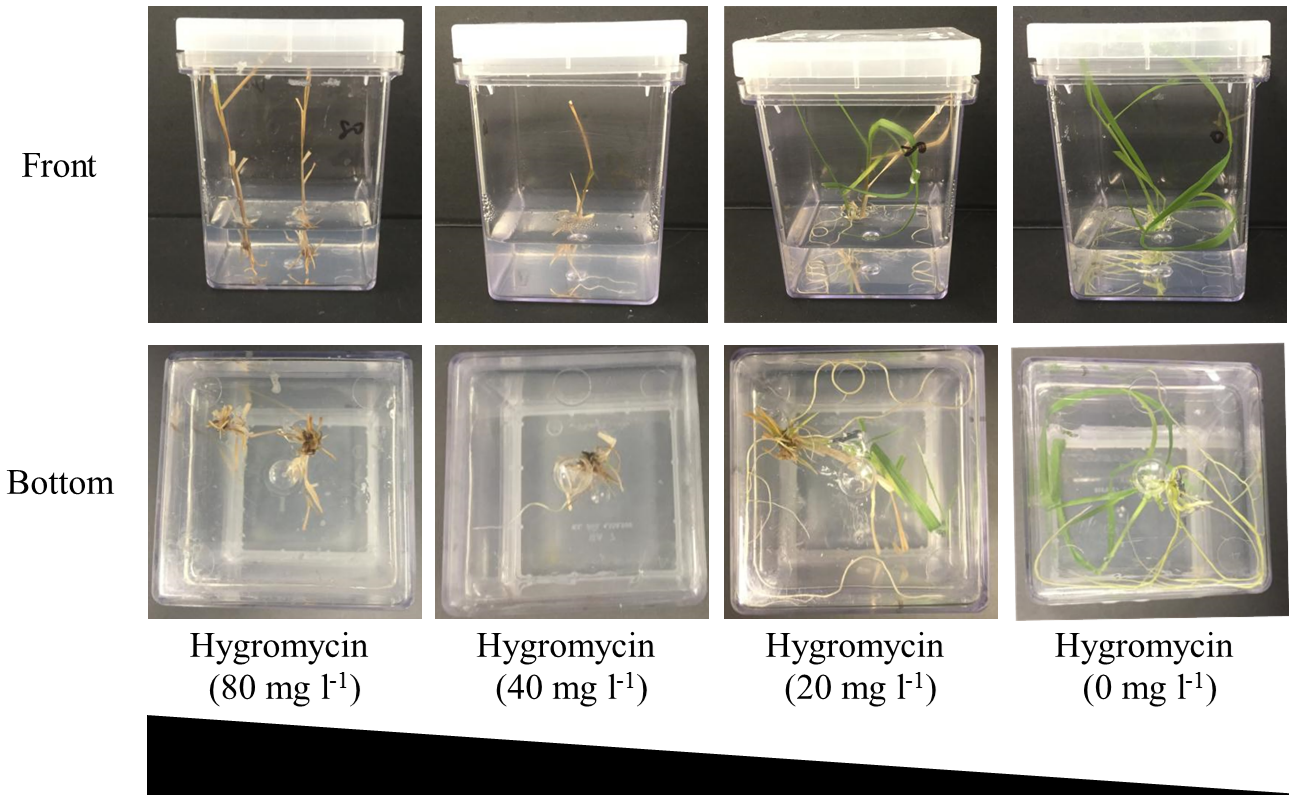


Figure S5. Impact of hygromycin B concentration on WT rooting (killing curve). From left to right, the concentration is reduced from 80 mg l^{-1} to 0 mgl^{-1} in the RM. Rooting was drastically reduced when hygromycin B concentration reached 40 mg l^{-1} or above in the RM and the growth of WT switchgrass was totally inhibited at 80 mg l^{-1} hygromycin B.

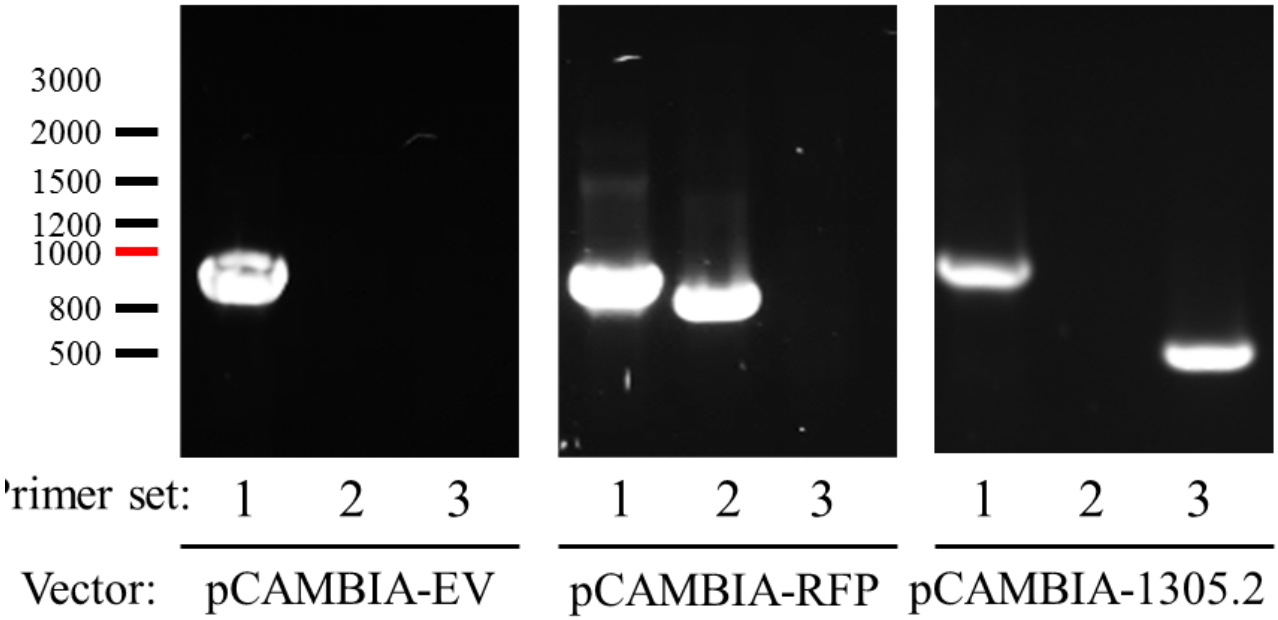
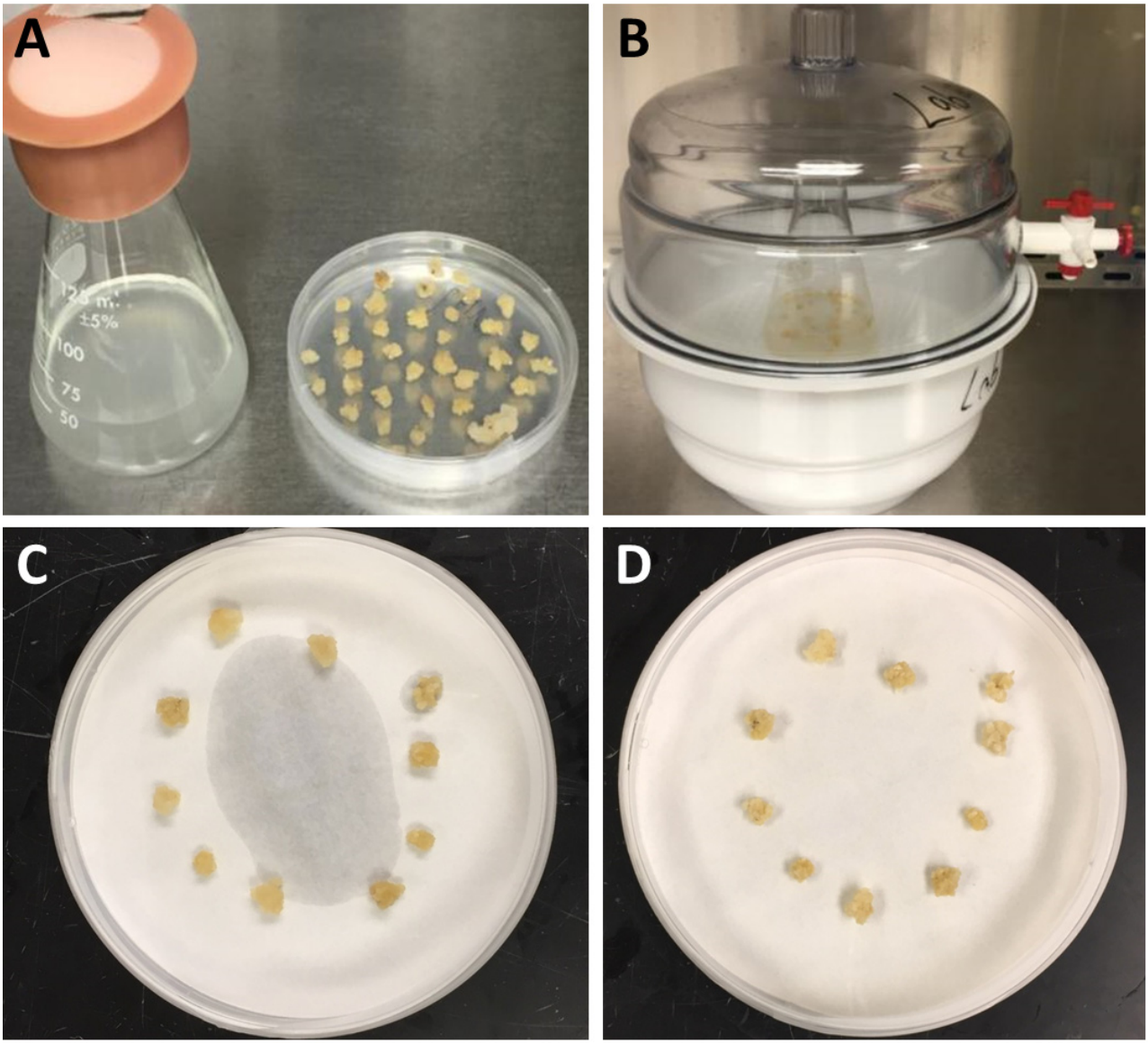


Figure S6. Colony PCR results from the *Agrobacteria* transformants with different vectors (pCAMBIA-EV, pCAMBIA-RFP, and pCAMBIA-1305.2). Primer set 1 (EV-F, EV-R) is specific for *Hph* gene. Primer set 2 (RFP-F, RFP-R) is for *pporRFP* gene. Primer set 3 (Gus-F, Gus-R) is for *GUSPlus* gene.



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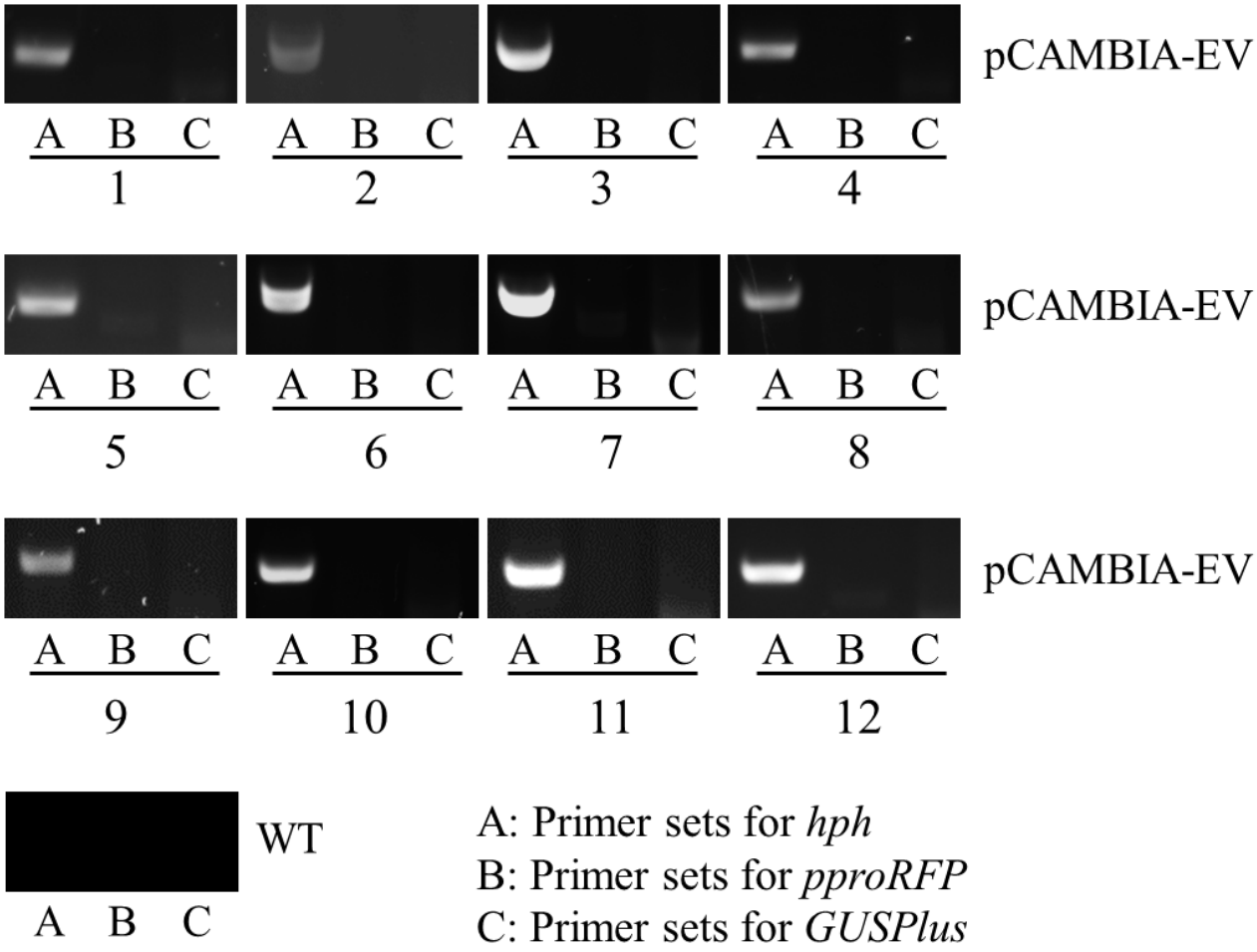

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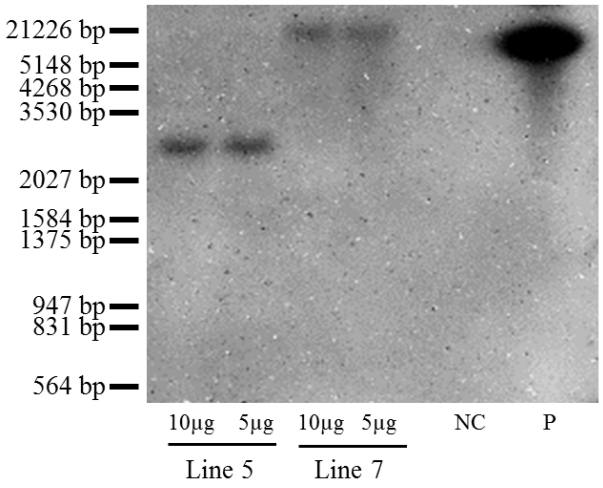

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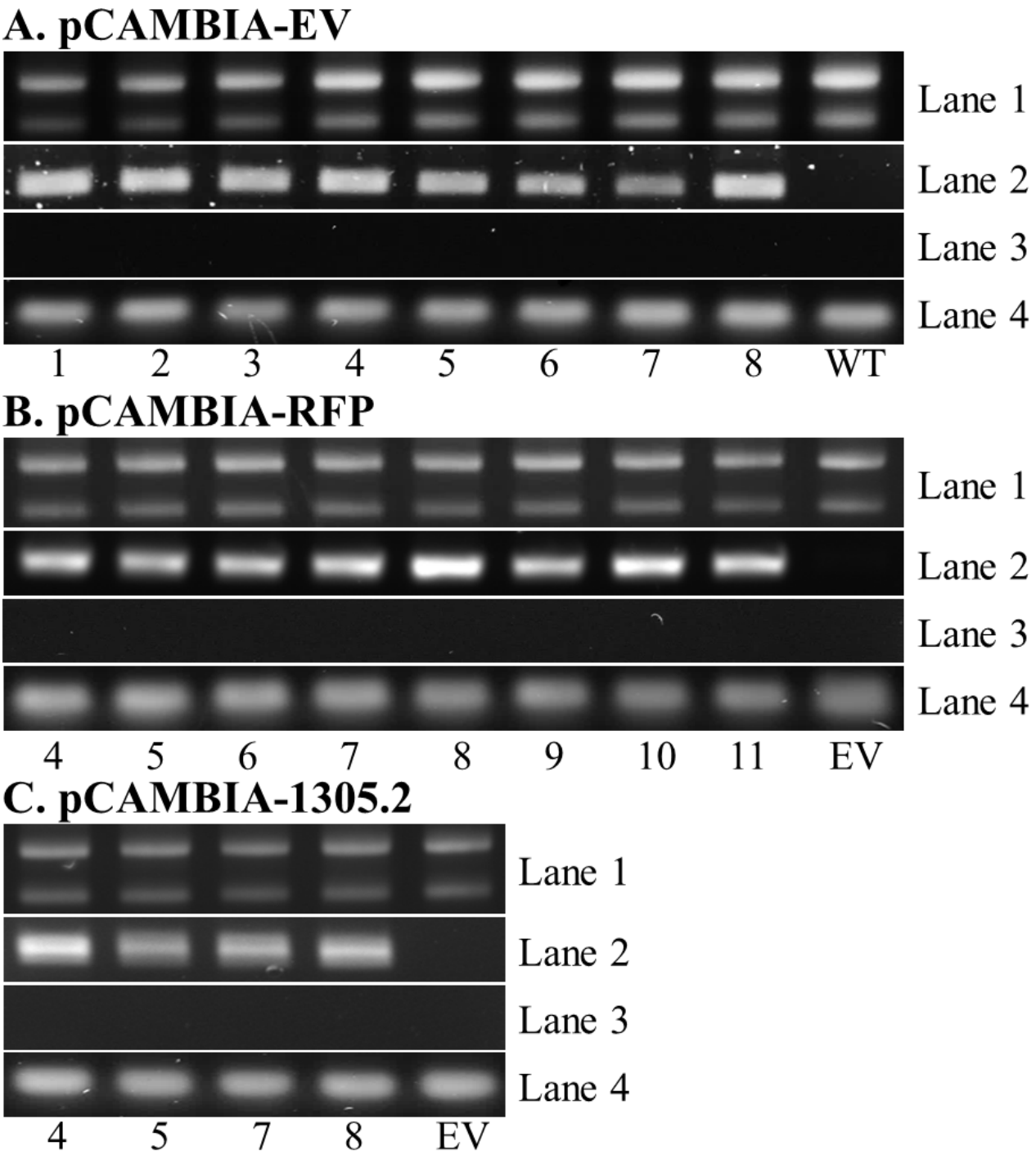
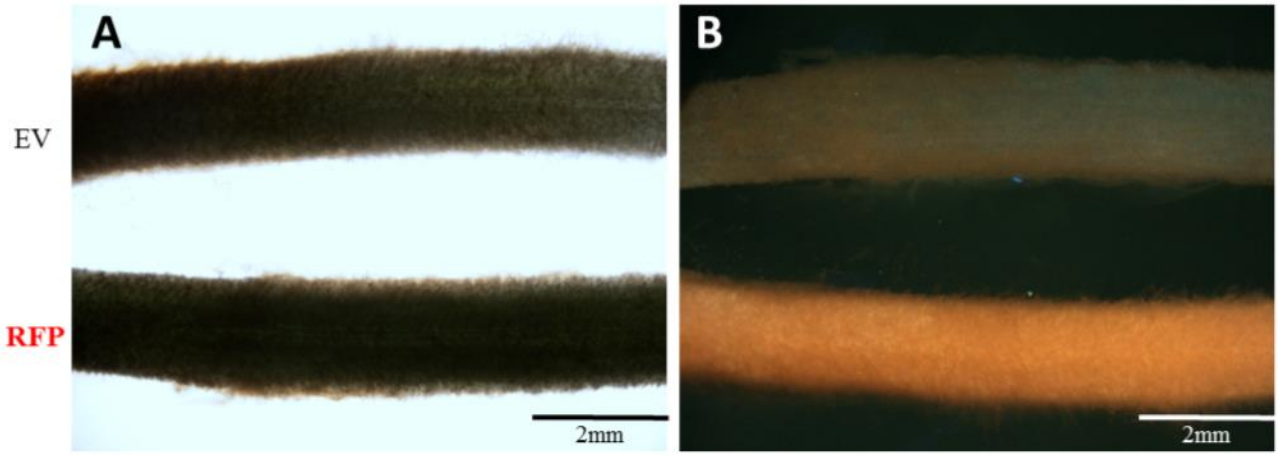


Figure S10. Detection of transgene gene expression using reverse transcription PCR (RT-PCR). (**A**) pCAMBIA-EV transformed transgenic switchgrass. (**B**) pCAMBIA-RFP transformed transgenic switchgrass. (**C**) *GUSPlus* gene in pCAMBIA-1305.2 transformed transgenic switchgrass. Lane 1, RNA; lane 2, PCR using cDNA as template by gene-specific primer sets for *Hph* gene (RT_EV-F + RT_EV-R in **A**), *pporRFP* gene (RT_RFP-F and RT_RFP-R in **B**) and *GUSPlus* (Gus-F + Gus-R in **C**); lane 3, PCR using RNA as template by gene-specific primer sets for *Hph* gene (RT_EV-F + RT_EV-R in **A**), *pporRFP* gene (RT_RFP-F and RT_RFP-R in **B**) and *GUSPlus* (Gus-F + Gus-R in **C**); lane 4, PCR RT-PCR using primer sets for *ACTIN* gene (RT_ACTIN2-F + RT_ACTIN2-R).



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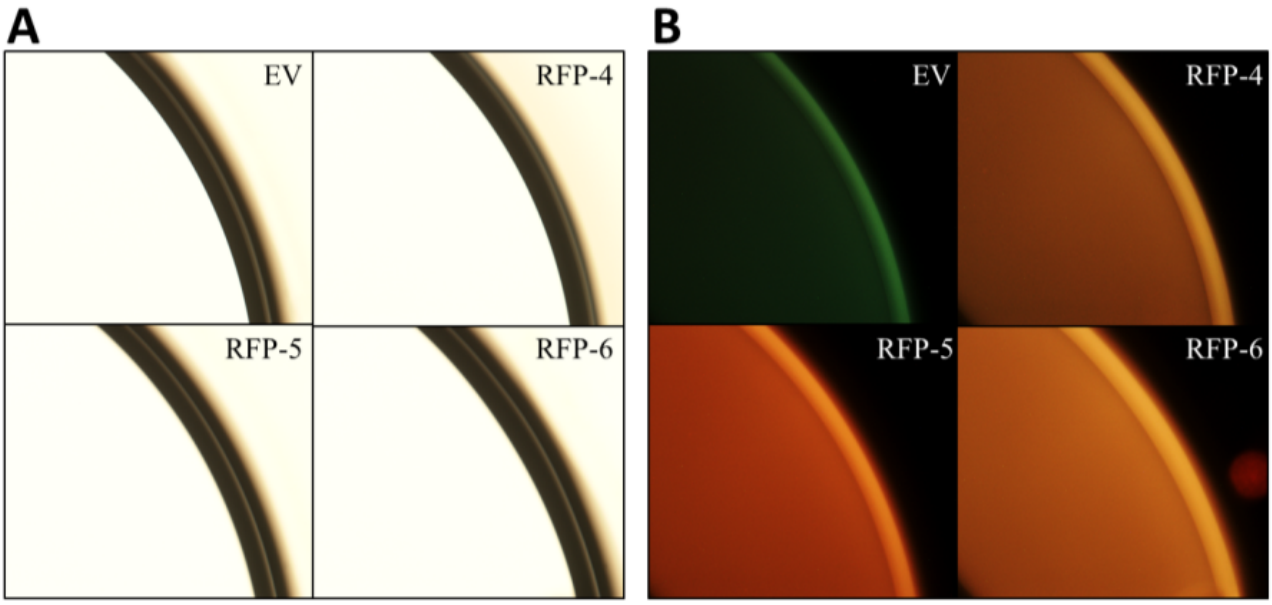

Figure S12. Detection of the pporRFP fluorescence in the total stem protein extract of pCAMBIA-RFP switchgrass transgenic lines. (**A**) Bright field image. (**B**) Epi-fluorescence image under red fluorescence filter. Each droplet has 2µg of total stem protein extract.