Establishment of High-Efficiency Agrobacterium-Mediated Transformation of Callus Derived from *Sehima nervosum*, an Important Range Grass Species

Krishna Kumar Dwivedi
Indian Grassland and Fodder Research Institute, India

Tanu Sonkar
Indian Grassland and Fodder Research Institute, India

R. Katiyar
Indian Grassland and Fodder Research Institute, India

Auji Radhakrishna
Indian Grassland and Fodder Research Institute, India

P. Kaushal
Indian Grassland and Fodder Research Institute, India

The 23rd International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection) took place in New Delhi, India from November 20 through November 24, 2015.

Proceedings Editors: M. M. Roy, D. R. Malaviya, V. K. Yadav, Tejveer Singh, R. P. Sah, D. Vijay, and A. Radhakrishna

Published by Range Management Society of India

Paper ID: 1341
Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
Sub-theme 4.1. Plant genetic resources and crop improvement

Establishment of high-efficiency Agrobacterium-mediated transformation of callus derived from *Sehima nervosum*, an important range grass species

Krishna Kumar Dwivedi*, Tanu Sonkar, R. Katiyar, A. Radhakrishna, P. Kaushal
ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India
*Corresponding author e-mail: kk_dwivedi@rediffmail.com

Keywords: *Agrobacterium*-mediated genetic transformation, Callus, GUS gene, *Sehima nervosum*,

Introduction

Sehima nervosum is one of the important rangeland grass in India, It is commonly known as Saen grass in India, white grass in Australia, and has also been reported from the Central East Africa and Sudan. It is a good forage grass and maybe utilized for grazing as well as for hay preparation. It is a perennial grass, prefers hot and dry climate and survive even in limited rainfalls. As this natural grass is found inherently rich in precursors for several industrially important biomolecules, fractionation of these precursors seems to be a promising endeavour. Production of nutraceuticals (prebiotic xylo-oligosaccharides) from the lignocellulosic biomass of this grass is promising, as this grass does not compete with food crops, and is comparatively less expensive than conventional agricultural food-stocks. However, germplasm of this grass has narrow genetic variability. Being largely apomictic in reproduction, generation of variability through hybridization approaches have been limited. Utilization of biotechnological tools is one of the potential ways for introducing variability and transfer of desirable traits. The development of an efficient genetic transformation procedure for *Sehima* could facilitate physiological and molecular biology studies as well as the production of transgenic cultivars for higher productivity and quality.

To the best of our knowledge there are no reports on *in-vitro* callus induction, regeneration and transformation in *Sehima*. Herein, for the first time, efficient *in-vitro* callus induction from mature seed explant and transformation efficiency in *Sehima* is reported. Here we standardized a reproducible, rapid and efficient *Agrobacterium* mediated transformation using *Agrobacterium* strain EHA105 harbouring binary vector pCAMBIA 1305.

Materials and Methods

Callus induction: *Sehima nervosum* (*var*. Bundel saen 1) seeds were surface sterilized with 70% ethanol for 1 minute, followed by 0.1% mercuric chloride with a drop of Tween-20 for 5 minutes and then rinsed several times with sterile distilled water. The surface sterilized seeds were inoculated on to a basal MS medium, containing different concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l) of 2,4-D (2,4-dichlorophenoxyacetic acid) for callus induction.

***A. tumefaciens* preparation:** *A. tumefaciens* strain EHA105 harboring binary vector pCAMBIA1305 were grown overnight in 25 ml of LB broth medium containing 50 mg/l kanamycin at 28°C and shaken at 200 rpm in dark. The *Agrobacterium* culture (OD_{600}: 0.6, 1.0 and 1.4) was centrifuged at 5000 rpm for 10 min at 4°C. The pellet was resuspended in the same amount of MS liquid medium of pH 5.6 containing 100µm acetosyringone.

Explant preparation and inoculation: Calli were sliced in to small pieces using sharp scalpel and incubated in *Agrobacterium* suspension for 15, 30, 45 and 60 minutes. The infected calli were blot dried with sterile filter paper. The agro infected calli were then transferred on the co-cultivation medium (MS + 3.5 mg/l 2,4-D) with approx 10 pieces/plate. Plates were placed in the dark at 25°C. After four days of co cultivation, the calli were washed with cephotaxime 250 mg/l and transferred into the selective (the same of co-cultivation medium) but containing kanamycin 50 mg/l along with cephotaxime 250 mg/l. Healthy calli were subcultured at every three weeks interval.

GUS histochemical assay: The histochemical GUS assay was performed as described by (Jefferson *et al*., 1987) to monitor GUS gene expression in putative transgenic GUS calli. GUS gene expression were observed and photograph using Leica S6D trinocular stereozoom microscope.

Results and Discussion

In the present study, effect of 2, 4-D on callus induction and embryogenesis from mature seed explant was observed. Callus induction frequency was increased with the increasing concentration of 2, 4-D (Table 1). The best callus induction frequency was at 3.5 mg/l 2, 4-D, (93%). In grasses better response of 2, 4-D for callus induction has been observed (Batra and Kumar, 2002). To standardize the conditions for *Sehima* transformation the effect of parameter that influence transformation frequency was examined. Two factors were identified enhancing T-DNA delivery and/or favoring callus recovery, the OD_{600} of bacterial suspension and the infection time. Calli were inoculated with *Agrobacterium* suspension

at OD_{600} of 0.6, 1.0 and 1.4 for 15, 30, 45 and 60 minutes. The GUS staining was carried out for the expression of GUS gene activity (Figure1). The highest percentage of transformation efficiency 90% was obtained in the calli inoculated with *Agrobacterium* for up to 30 minutes at OD_{600} of 1.0, followed by 83% and 81% at OD_{600} of 0.6 and 1.0 respectively (Table 2).

Table 1: Hormone concentration effects on callus induction from mature seeds of *S. nervosum*

2,4-D concentration (mg/l)	Callus induction frequency (%)
1.0	18.38±1.54
1.5	24.38±1.49
2.0	59.99±1.11
2.5	70.61±1.30
3.0	81.75±0.59
3.5	93.20±1.86
4.0	59.99±1.11

Table 2: Effect of different infection time and bacterial concentration on *Sehima* calli transformation efficiency

Infection time (min.)	OD_{600}								
	0.6			1.0			1.4		
	No of calli	No of GUS expressive calli	T%*	No of calli	No of GUS expressive calli	T%*	No of calli	No of GUS expressive calli	T%*
15	60	45	75.00	60	49	81.66	60	44	73.00
30	60	50	83.33	60	54	90.00	60	46	76.66
45	60	41	68.33	60	42	70.00	60	38	63.33
60	60	36	60.00	60	37	61.00	60	35	58.33

***T%: Transformation efficiency %

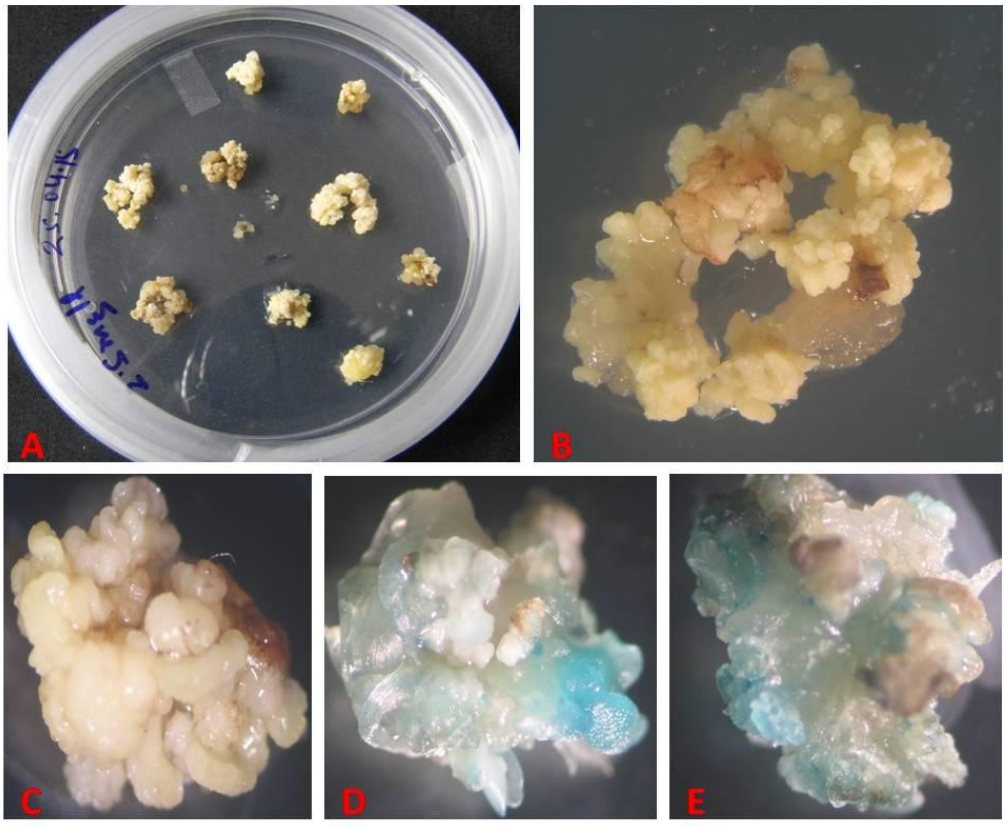

Fig. 1: *In-vitro* callus induction and *Agrobacterium* mediated genetic transformation of Sehima. **A.** Callus induction; **B.** Embryogenic callus; **C.** Non transgenic calli; **D & E.** transgenic calli showing GUS gene expression.

Conclusion

We have standardized successfully the callus induction and transformation in *Sehima nervosum*, for the first time. A highly efficient media combination and transformation protocol has been optimized to achieve as high as 90% efficiency. This information may be important in view of generating transgenics in this important crop. Furthermore, this exceptionally high response to callus and transformation in this crop showed its potential as a model crop to undertake biotechnological approaches for perennial range grass improvement.

References

R.A. Jefferson, T. A. Kavanagh and M.W. Bevan. 1987. GUS fusion: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 13:3901-3907.

Batra S. and Kumar S. 2002. *In vitro* high frequency plant regeneration in buffel grass (*Cenchrus ciliaris* L.). *J. Plant Biol*. 29:191-194.

Acknowledgement

This work was supported by Department of Biotechnology (DBT) to Indian Grassland and Fodder Research Institute, Jhansi in form of Rapid Grant for Young Investigators, Sanction No. BT/PR6008/GBD27/384/2012.