Optimizing Tissue Culture Protocols in Warm-Season Turfgrass Species for Genetic Engineering Applications

Esdras M. Carbajal Melgar and Susana R. Milla-Lewis

Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC

INTRODUCTION

Turfgrasses are widely grown in landscapes, sports fields, and recreational areas across the United States. They contribute to the aesthetic appeal, functionality, and ecological balance of outdoor environments.

Tissue culture enables the growth and maintenance of cells or tissues under controlled laboratory conditions. It provides researchers with a powerful tool used for micropropagation, production of pathogen-free material, somaclonal variation, and embryo rescue.

Tissue culture holds significant importance for genetic engineering applications, which can aid in the development of new cultivars with unique traits.

Objective:

Evaluate the effect of different concentrations of auxins and cytokinins for callus formation, callus culture, and shoot and root development in zoysiagrass tissue culture.

MATERIALS & METHODS

Plant Materials

Seed of zoysiagrass commercial cultivar 'Zenith' was used

Explant Sterilization

- 50 grams of 'Zenith' seeds were placed in separate beakers
- Seeds were rinsed with:
  - sterile water for 5 minutes
  - ethyl alcohol solution 70% for 1 minute
  - sterile water twice for 3 minutes
  - sodium hypochlorite 4.5% for 10 mins
  - sterile water (3 min × 3 times)
- Dried on a plate for 5 min

Table 1. Effects of plant regulators in tissue culture

Auxin	Cytokinin	Response
High	High	Callus
High	Low	Roots
Low	High	Shoots

Media preparation

MS Media (1L)	MS 0.5 strength (1L)
30 gram sucrose	15 gram sucrose
4.33 grams M519	4.33 grams M524
4 grams G3251	M533
Phytohormones	4 grams G3251
Food dye	Food dye
Ph 5.8	PH 5.8

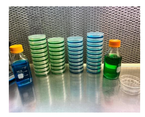


Table 2. Initial treatments for callus formation

Auxin (1)	Cytokinin (1)			
	B1	B2	B2	B2
C1	T1=C1,B1	T2=C1,B2	T3=C1,B3	T4=C1,B4
C2	T5=C2,B1	T6=C2,B2	T7=C2,B3	T8=C2,B4
C3	T9=C3,B1	T10=C3,B2	T11=C3,B3	T12=C3,B4
C4	T13=C4,B1	T14=C4,B2	T15=C4,B3	T16=C4,B4

Three reps with six explants per petri dish were evaluated

Table 3. Treatments for shoot regeneration

Treat	Media	Expl/petri
ZRT1	Cytokinin (2) +Auxin (2) + GA1	4
ZRT2	Cytokinin (2) +Auxin (2) + GA2	4
ZRT3	Cytokinin (2) +Auxin (2) + GA3	4

Seven reps were evaluated

Table 4. Root development

M524	Sugar	Gelzan	PH
4.33	15 g	3g	5.8

RESULTS

Table 5. LSD for Callus formation in Zoysiagrass seeds

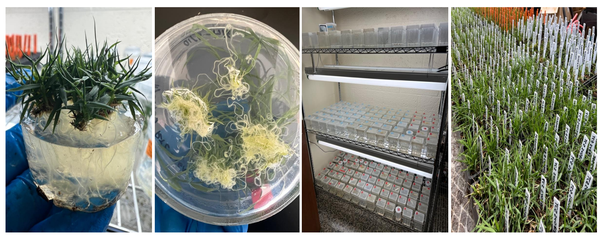
ID	Treatment	Success %	Groups
T7	C2,B3	67	a
T6	C2,B2	56	ab
T13	C4,B1	44	abc
T14	C4,B2	44	abc
T15	C4,B3	44	abc
T8	C2,B4	44	abc
T16	C4,B4	39	abcd
T1	C1,B1	33	bcd
T3	C1,B3	33	bcd
T5	C2,B1	28	bcd
T10	C3,B2	22	cd
T11	C3,B3	22	cd
T12	C3,B4	22	cd
T2	C1,B2	22	cd
T9	C3,B1	17	cd
T4	C1,B4	11	d

Table 6. Zoysiagrass Plant Regeneration

Treatment	Callus	# Plants	Plant / Callus
ZRT1	12	453	38
ZRT2	24	383	16
ZRT3	12	201	17
Total	48	1037	22
Average	16	346	22

- Treatment T7=C2,B3 provided the highest success rate of callus formation with 67% of the explants producing callus (Table 5).
- Different media for shoot regeneration from callus were evaluated (Table 3). No statistical differences among media treatments were observed (Table 5). However, treatment ZRT1 produced the highest number of plants regenerated per callus.

Root Development



- High levels of callus response ranging from 75-100% were obtained (Table 6).
- The root induction treatment resulted in regeneration of 826 new plants.
- Regenerated plants were transferred from MS media to soil substrate and placed under greenhouse conditions for future evaluation.

CONCLUSIONS

- The correct combination of auxin and cytokinin was fundamental for the development of callus.
- Although treatment was not a significant factor on shoot regeneration in zoysiagrass, ZRT1 produced the highest average number of plants regenerated per callus.
- Half-strength MS media was successful to induce root development.

ONGOING WORK

- Flow cytometry analysis will be performed to infer ploidy levels of the newly generated plants.
- New materials will be evaluated under field conditions to assess possible somaclonal variation.
- The most successful treatments will be used in efforts to improve warm-season grasses through plant transformation.

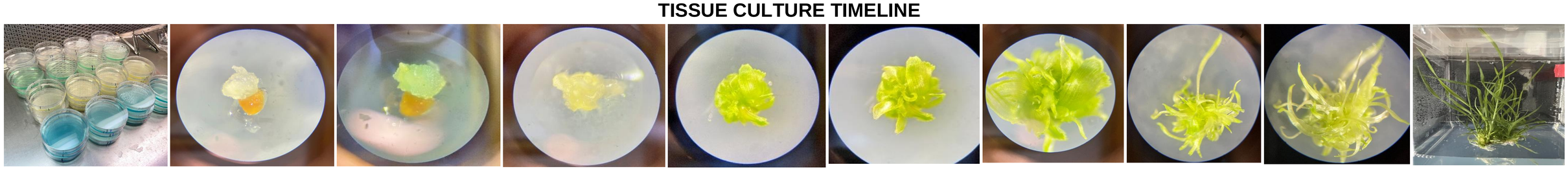
References

•Murashige, T. & Skoog, z. 1962. A revised medium for the rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15: 473–497.

•Li, R. Bruneau, A.H .Qu, R. 2006.Improved plant regeneration and in vitro somatic embryogenesis of St Augustine grass (Stenotaphrum secundatum (Walt.) Kunze). Plant Breeding, 125 (2006), pp. 52-56. DOI: https://onlinelibrary-wiley-com.prox.lib.ncsu.edu/doi/full/10.1111/j.1439-0523.2006.01193.x

•Kuo, Y. J., and M. A. L. Smith, 1993: Plant regenerating from St. Augustinegrass immature embryo-derived callus. Crop Sci. 33, 1394— 1396.

TISSUE CULTURE TIMELINE



July 2nd | July 14th | July 20th | July 25th | August 2nd | August 10th | August 20th | Sept 10th | Sept 19th | Nov 27th

Questions? emcarbaj@ncsu.edu