Micropropagation and DNA Barcoding Studies in the Genus *Scutellaria*

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ABSTRACT

Genus *Scutellaria*, commonly known as 'skullcaps', is a member of the Lamiaceae (mint) family of which some of species serve as an excellent source for secondary metabolites or phytochemicals. Increasing the production of medicinally and economically important plant species through micropropagation promotes *in situ* conservation by reducing effects of wild cropping, produces an ongoing supply of genetically identical plants, simplifies reproduction of recalcitrant species, promotes conservation of endangered species, and promotes plant improvement through genetic manipulation. *Agrobacterium*-mediated transformation of selected *Scutellaria* species was used to introduce AtMYB12, a transcription factor involved in the phenylpropanoid pathway. Upregulation of this pathway results in higher production of phytochemicals, thus yielding the selected species more valuable medicinally and economically. Because some of the species are rare, threatened or endangered, DNA barcoding has been used in this study for species identification based on nucleotide diversity of short DNA segments. With the DNA extracted from the germplasm collection of 21 species at Fort Valley State University, four candidate DNA barcode genes: the ribosomal RNA maturase gene (matK), the ribulose-1,4-bisphospate carboxylase/oxygenase gene (rbcL), the chloroplast intergenic spacer (psbA-trnH) and the ribosomal intergenic spacer (ITS) were amplified to discriminate between the *Scutellaria* species via polymerase chain reaction (PCR). The primers used to ensure proper identification were: rbcL, rbcL2, rbcL1-99, matK2.1-5-r, matK-3f1, psbA-trnH and ITS2. The amplicons verified by agarose gel electrophoresis were sent for sequencing and DNA sequences were uploaded to the Barcode of Life Data System (BOLD) and Basic Local Alignment Search Tool (BLAST) for analysis.

INTRODUCTION

Many *Scutellaria* species are known to have bioactive compounds with therapeutic potential. Some of the well known compounds are wogonin, baicalin, baicalein, apigenin, luteolin etc. Wogonin and baicalein have been studied extensively for anti-tumor properties. *S. intergrifolia* and *S. racemosa* are two species with medicinal potential. This research is focused on developing a callus induction and shoot regeneration protocol for the two species. Callus induction leading to suspension culture could be a desirable pathway to introduce somaclonal variation with high secondary metabolite containing callus lines or if regenerated- high yielding plant varieties.

MATERIALS AND METHODS

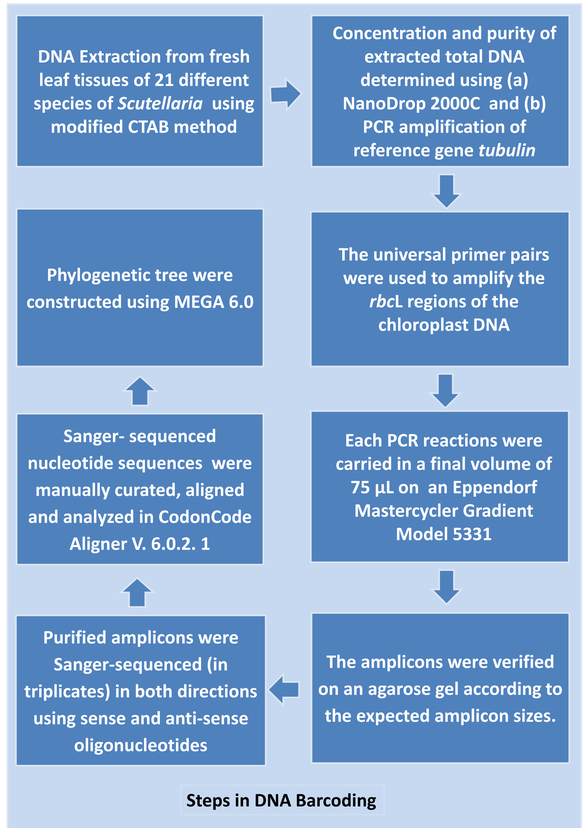
Callus induction:

MS (Murashige and Skoog, 1962) with varying levels of plant growth regulators were used (Table 1) to study callus induction (Figure 1). The pH of the medium was adjusted to 5.8 prior to the addition of 8.0 g L^{-1} T.C. agar. Media treatments were autoclaved at 121 °C for 20 min and 25 mL of medium was dispensed in 100 x 15 mm Petri dishes.

Table 1. Treatments for the callus induction studies using MS basal medium.

Treatment	IAA	2,4 – D	NAA	BAP
A	2 µM			
B		2 µM		
C			2 µM	
D	1 µM	1 µM		
E	1 µM		1 µM	
F		1 µM	1 µM	
G	1 µM			1 µM
H		1 µM		1 µM
I			1 µM	1 µM
J	5 µM			5 µM
K		5 µM		5 µM
L			5 µM	5 µM
M	10 µM			10 µM
N		10 µM		10 µM
O			10 µM	10 µM

DNA Barcoding:



Steps in DNA Barcoding

Shoot induction:

Both types of explant with intact calli were transferred to MS medium containing 3 % sucrose and 8 g L^{-1} agar for 3 weeks under 16 h photoperiod. Destructive counts were performed after 3 weeks on this shoot regeneration medium.

Genetic Transformation:

Shoot tips, nodes, internodes, and leaves were excised from *S. integrifolia*, wounded, infected with *Agrobacterium tumefaciens* strain EHA 105 harboring plasmid pq35SGR at $O.D_{600}$ = 0.05, 0.1, 0.3, and 0.6. Explants were co-cultivated for 72 h in darkness.

RESULTS

Callus induction was successful in both species. *S. integrifolia* callused best on 1 µM 2,4-D + 1 µM NAA, while *S. racemosa* callused best on 1 µM NAA + 1 µM BAP. Callus regeneration was not successful in either species. However, shoot induction was accomplished in nodal explants of both species. *S. integrifolia* produced the most shoots on 1 µM NAA + 1 µM BAP, while *S. racemosa* only produced 2 shoots, both less than 0.5 cm, with the same. Internodal explants did not produce shoots. *Agrobacterium tumefaciens* – mediated genetic transformation was more successful in *S. integrifolia* shoot tips when infected and co-cultivated with strain EHA 105 harboring pq35SGR plasmid.

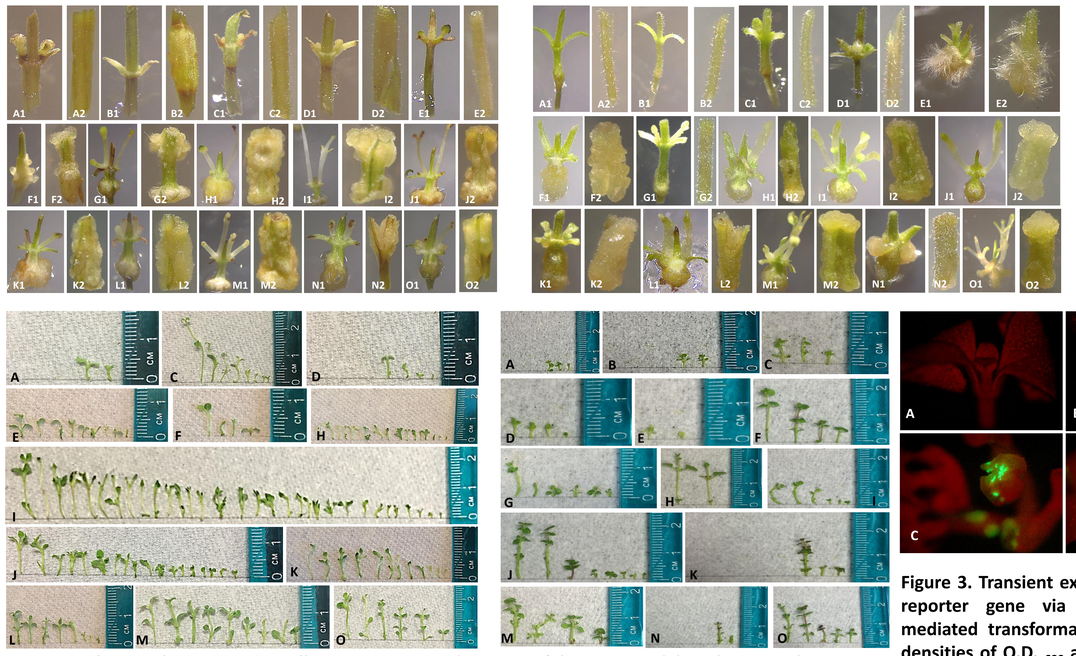


Figure 1: Callus induction in *Scutellaria racemosa* and *S. integrifolia using* nodal and internodal explants. Explants were incubated in dark for two weeks.

Figure 2: Shoot induction in *Scutellaria racemosa* and *S. integrifolia using* nodal explants. Explants were incubated under 16 h photoperiod.

Figure 3. Transient expression of EGFP reporter gene via *A. tumefaciens* mediated transformation at bacterial densities of $O.D._{600}$ at A) 0.05, B) 1.0, C) 0.3 and D) 0.6 for shoot tips.

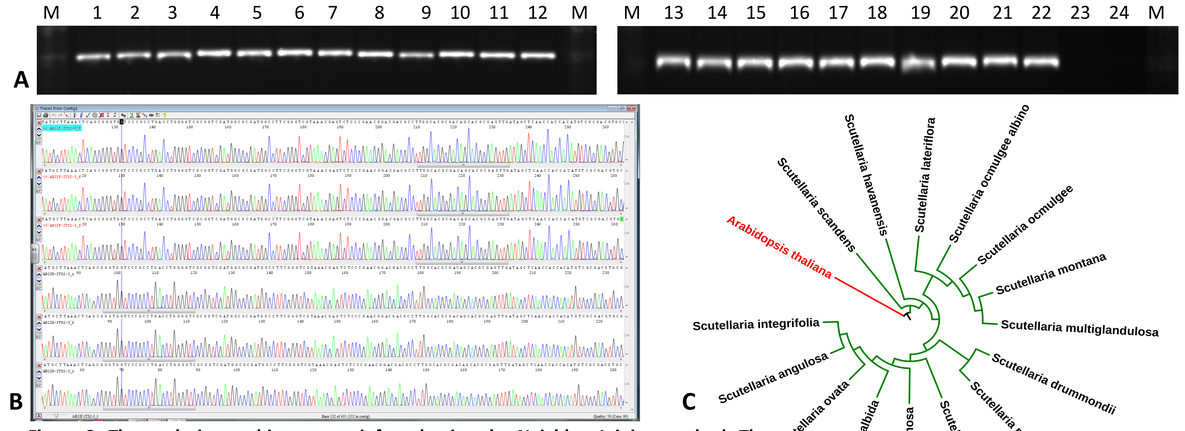


Figure 3: The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). A) PCR amplification of barcodes for primer pairs of ITS. B) DNA sequencing C) Phylogenetic tree of 15 *Scutellaria* species using ITS barcodes.

DISCUSSION and CONCLUSIONS

- Callus induction was optimized but calli could not be regenerated. Only nodal explants were useful for multiple shoot induction. Explant type, PGR combination and concentration, and genetic variability is an influencing factor in callus and shoot induction.
- There is potential for genetic transformation in *S. integrifolia*.
- Preliminary phylogenetic analysis using ITS sequences indicate a single clade of *Scutellaria* species. We are processing data generated through the use of remaining primer sets to arrive at a logical conclusion.

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