Plant Tissue Cult. & Biotech. **35**(1): 129-140, 2025 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v35i1.82504
©Bangladesh Assoc. for Plant Tissue Culture & Biotechnology
PTC&B

Tissue-Specific Expression Profiling of *Strictosidine Synthase* (*str1*) in *Rauvolfia tetraphylla* at Various Developmental Stages

Rabbi Hoque, Rita Sarah Borna, M. Imdadul Hoque and R. H. Sarker*

Plant Breeding and Biotechnology Laboratory, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: *Rauvolfia tetraphylla*, Strictosidine synthase gene (*str1*), Gene expression, Monoterpenoid indole alkaloids (MIAs)

Abstract

Rauvolfia tetraphylla, one of the most medicinally important species, belongs to the Apocynaceae family. It produces monoterpenoid indole alkaloids (MIAs) with significant pharmacological value, especially for the treatment of neurological disorders and hypertension. Strictosidine synthase gene (*str1*), the crucial gene responsible for the production of monoterpene indole alkaloids (MIAs) and its presence substantiates the medicinal value of these plants. In this investigation, we successfully analyzed the tissue-specific (root, stem, and leaf) expression of the *str1* gene in different developmental stages (4- and 6-month-old plants) in *R. tetraphylla* through RT-PCR. Normalized ImageJ analysis showed high *str1* expression in roots (1.005 ± 0.005 to 1.152 ± 0.210 ratios), moderate expression in stem (0.242-0.919) and low expression in leaf that decreased with age (from 0.702 ± 0.189 at 4-month-old plants to 0.303 ± 0.384 at 6-month-old plant). Furthermore, we confirmed the target specificity of *str1* by sequencing, which showed 94.4-94.56% identity with *Rauvolfia str1* homologs. As the first report of *str1* expression in Bangladeshi *R. tetraphylla*, our findings confirm root tissue as the principal site of alkaloid biosynthesis and offer a molecular basis for optimizing cultivation tactics to increase production of its therapeutically valuable compounds.

Introduction

Rauvolfia tetraphylla L., a perennial woody shrub within the Apocynaceae family, is recognized for its evergreen foliage, compact growth habit and potential therapeutic properties. The common name of this plant is 'Be Still Tree' or 'Devil Pepper tree' while in Bangladesh it called 'Boro Chador'. Indigenous to the neotropics, its native range extends from Mexico and the West Indies through Central America to northern South

*Author for correspondence: <rhsarker2000@yahoo.co.uk>.

America, where it thrives in diverse ecological niches (POWO 2024). *R. tetraphylla* has been introduced and naturalized across several regions, including Bangladesh, India, Myanmar, Nepal, Pakistan and Sri Lanka, where it adapts to diverse environments (Ahmed et al. 2008).

R. tetraphylla holds significant medicinal value, particularly in treating hypertension, cardiovascular disorders and psychiatric conditions (Sharma et al. 1999, Faisal et al. 2005). Its roots, rich in bioactive alkaloids, serve as a substitute for *R. serpentina* in traditional medicine (Shenya et al. 2025). The plant exhibits anticonvulsant, diuretic and expectorant properties, with latex and leaves used for dropsy, malaria and snakebites (Prashith 2019). Additionally, root extracts aid in gastrointestinal ailments, uterine stimulation during labor (CSIR 1986) and CNS-related disorders (Harisaranraj et al. 2009). This plant exhibits pharmacological properties, primarily attributed to its diverse alkaloid compounds, mostly monoterpene indole alkaloids (MIAs). According to Farooqi and Sreeramu (2001) and Stöckigt et al. (2008), this plant contains reserpine, serpentine, ajmaline, vinblastine, quinine, yohimbine, ajmalicine and tetraphyllincine as major secondary metabolites which contribute to its therapeutic value in traditional and modern medicine. Among them reserpine shows multiple pharmaceutical properties, including antihypertensive, antipsychotic, and sedative effects, making it valuable for treating chronic hypertension and psychological disorders (Pullaiah 2002 and Duke 2005).

The strictosidine synthase (*str1*) gene encodes the pivotal enzyme that catalyzes the condensation of tryptamine and secologanin to form strictosidine, the universal precursor for monoterpenoid indole alkaloids (MIAs) in *Rauvolfia* species (Stockigt et al. 2008). This rate-limiting step establishes the scaffold for pharmacologically vital alkaloids like reserpine, vinblastine and quinine, making STR1 indispensable for both plant defense and medicinal applications (O'Connor and Maresh 2006). *Str1* gene was initially identified in *Catharanthus roseus* and *Rauvolfia serpentina* cell cultures, with subsequent characterization in numerous Apocynaceae and Rubiaceae species including *Vinca*, *Cinchona*, and *Ophiorrhiza* (De Waal et al. 1995, Hampp and Zenk 1988, Yamazaki et al. 2003). In 1992 Bracher and Kutchan isolated and partially characterized this gene from *R. serpentina* and *R. mannii*. The coding DNA sequence (CDS) of this gene contains no introns which fact adds to the 17% of the known plant genes which are intron-less. 100% sequence homology over 1180 base pairs (conserved region), encompassing the entire translated region, exists for *str1* between the two *Rauvolfia* species. The monomeric precursor protein with 344 amino acids exhibits 100, 79 and 58% similarities with STR1 from *R. mannii*, *C. roseus*, *Ophiorrhiza pumila*, respectively (Kutchan et al. 1993). This conserved genomic region exhibits specific binding affinity for nuclear proteins across multiple *Rauvolfia* species (*R. serpentina*, *R. mannii*, *R. verticillata*, *R. tetraphylla*, *R. chinensis*) and *Nicotiana plumbaginifolia* (Ma et al. 2006, Kutchan et al. 1993).

Gene expression analysis through Reverse Transcription PCR (RT-PCR) and band intensity quantification provides critical insights into the functional roles of genes like

str1 in plant tissues. By measuring mRNA levels, RT-PCR reveals tissue-specific expression patterns, helping identify key biosynthetic part of the plant. In *R. serpentina*, *str1* gene, are ubiquitously distributed in the plant tissue but show root-dominant accumulation, highlighting roots as the primary biosynthetic site (Kutchan et al. 1993). Differential *str1* expression across tissues (roots >stems >leaves) correlates with varying alkaloid concentrations, suggesting transcriptional regulation controls metabolic flux in MIAs pathways (Yamazaki et al. 2003). Moreover, RT-PCR analysis enables visualization of tissue-specific gene expression patterns, where band intensity on agarose gels correlates with transcript abundance-stronger bands indicate higher target gene expression levels and reflecting its functional activity in the sampled tissue (Bustin 2002, Gachon et al. 2004). Also, this semi-quantitative approach has been widely validated for studying gene expression in plant secondary metabolism (Udvardi et al. 2007).

For accurate quantification of these expression patterns, ImageJ has emerged as an essential open-source tool that enables precise measurement and normalization of RT-PCR band intensities against internal controls (e.g., housekeeping genes). Normalization is essential in gene expression studies to minimize technical variability and enable accurate biological comparisons by standardizing data against stable reference genes like 18S rRNA or actin (Livak and Schmittgen 2001). Its advanced image analysis capabilities not only facilitate reliable cross-sample comparisons but also enhance experimental reproducibility in gene expression studies (Abramoff et al. 2004), making it particularly valuable for correlating transcriptional activity with alkaloid biosynthesis potential in medicinal plants like *Rauvolfia* species.

While the *str1* gene has been comprehensively characterized in *Rauvolfia serpentina*, its expression profile and functional role in the closely related *R. tetraphylla*, an equally important producer of pharmaceutically valuable indole alkaloids, remain unexplored. This study represents the first systematic investigation of *str1* expression dynamics in Bangladesh, examining both developmental (4- vs 6-month-old plants) and tissue-specific (root, stem, leaf) patterns in *R. tetraphylla* using conserved *str1* gene sequences from *R. serpentina*, *R. mannii* and *R. verticillata*. Our findings will address critical gaps in understanding the spatiotemporal regulation of alkaloid biosynthesis in this understudied medicinal species, providing a foundation for future biotechnological applications.

Materials and Methods

R. tetraphylla plants were propagated using previously optimized tissue culture protocol (Hoque et al. 2020), followed by greenhouse acclimatization (Fig. 1). Samples were collected from roots, stems, and leaves of 4-month (juvenile) and 6-month (mature) plants for analysis. Three STR1-specific primers were designed using OligoCalc software with optimized parameters (45-65% GC content, 54-62°C Tm, 20-25 bp length, 100-200 bp amplicons) with sequences sourced from *R. serpentina* and validated against conserved

domains in *R. mannii* and *R. verticillata* through NCBI BLAST and MUSCLE alignment analyses. The primers were subsequently synthesized by Macrogen (South Korea) following stringent quality control measures. DNA isolation was carried out by using manual protocol established by Plant Breeding and Biotechnology laboratory, Department of Botany, University of Dhaka to check the primers authenticity. Also, 18S rRNA was used as a housekeeping gene. Total plant RNA was isolated from different plant samples (leaf, stem and roots of four- and six-month-old plants) using Thermo Scientific GeneJET RNA Purification Kit as per the manufacturer's protocol. Isolated RNA samples were treated with DNase I and quantified via Nanodrop (A260/A280 >1.8). First strand cDNA synthesis was carried out using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit according to the manufacturer's instructions.

Fig 1. Four-month (lower pots) and six-month-old (upper pots) *R. tetraphylla* plants used as experimental material for the study.

Expression of the *str1* gene was checked by reverse transcriptase PCR (RT-PCR) as described by Sambrook and Russell 2001. For the analysis, 50 ng of cDNA template was used per reaction in a standardized 25 µL reaction volume. The programming of thermal cycler for the PCR amplification was as follows: 30 cycles consisted of initial denaturation of 95°C for 5 min then denaturation at 94°C for 1 min, annealing at 58-60°C for 30 sec., extension at 72°C for 1 min and a final extension at 72°C for 10 min. After that, PCR products were resolved on 1.5% agarose gel and bands intensity were observed.

The cDNA band intensities from RT-PCR analysis of *R. tetraphylla* were quantified using ImageJ (v1.53) following a standardized protocol. For both the target *str1* gene and the reference *18S rRNA* housekeeping gene, gel images were converted to 8-bit grayscale, contrast-enhanced (Image >Adjust >Brightness/Contrast) and inverted (Edit >Invert).

Lanes were defined using the rectangular selection tool, and intensity profiles were generated (Analyze >Gel >Plot Lanes). Band intensities were measured by selecting peaks in the lane profile plots with the wand tool, with the area under each peak recorded as relative intensity values. The *str1* expression levels were normalized against *18S rRNA* by calculating the ratio of their respective band intensities. All data was exported to Excel for comparative analysis across different ages (4- and 6-month-old plants) and tissues (root, stem, leaf).

For sequencing, amplified products were purified using ethanol precipitation by adding 2.5 volumes of ice-cold 100% ethanol, incubating at −20°C for 30 min and centrifuging at 12,000 g for 15 min. The DNA pellet was washed with 70% ethanol, air-dried and resuspended in nuclease-free water for downstream applications. Sanger sequencing was conducted at the University of Dhaka's Centre for Advanced Research in Sciences (CARS) to sequence the amplified STR1 fragment unidirectionally (forward primer). To confirm the presence of *str1* conserved region in *R. tetraphylla*, the sequence was aligned to *R. serpentina, R. mannii* and *R. verticillata str1* (NCBI Accession number: Y00756, X63431 and DQ017054 respectively) after being base-called and trimmed (Q20 threshold) by using MEGA.

Results and Discussion

Three STR1-specific primers (STR1-1, STR1-2, and STR1-3) from multiple aligned sequence of *str1* from *R. serpentina*, *R. verticillata* and *R. mannii* were designed (Table 1). Genomic DNA was successfully isolated from all tissue samples (roots, stems, and leaves) of both 4- and 6-month-old *R. tetraphylla* plants and subsequently employed as template for preliminary PCR optimization of STR1-specific primer sets. Of the three

Table 1. Designed primer sequences of *str1* for RT-PCR analysis.

Primer's name	Sequence	Amplification at (bp)
STR1_FOR1	5´-ATG GCC AAA CTT TCT GAT TCG C-3´	160
STR1_REV1	5´-CTTTGTTGGTTGAGTCGAAGGTG-3´	
STR1_FOR2	5´-CTATTATCATCTTTCTGTGGTTGG-3´	142
STR1_REV2	5´-GCTAACATCGGTGAAGTAAACAATC-3´	
STR1_FOR3	5´-GATGAGTTTGGGAACATTCTTGAAG-3´	180
STR1_REV3	5´-GAAAATTTAATGACTTGAAACAAAAGAAT-3´	

primers tested, STR1-1 and STR1-2 generated clear, reproducible amplicons of the expected sizes (160 bp and 142 bp, respectively) across all tissue types (Fig. 2a). Parallel amplification of the *18S rRNA* housekeeping gene yielded a 101 bp fragment (Fig. 2a), confirming RNA integrity and enabling expression normalization. The precise

correspondence between observed and designed amplicon lengths confirms successful primer optimization and target-specific amplification. These validated primers were subsequently used for comparative expression analysis of *str1* across developmental stages and tissue types.

Total RNA was isolated from stems, leaves, and roots of both 4- and 6-month-old *R. tetraphylla* plants and the RNA integrity was checked by 1% agarose gel electrophoresis (Fig. 2b), which revealed distinct 28S and 18S ribosomal RNA bands demonstrating suitability for downstream cDNA synthesis and RT-PCR applications. cDNA was prepared after RNA isolation by reverse transcription reaction. Then *str1* gene expression in stem, leaf and root of four- and six-month-old plants were observed by RT-PCR.

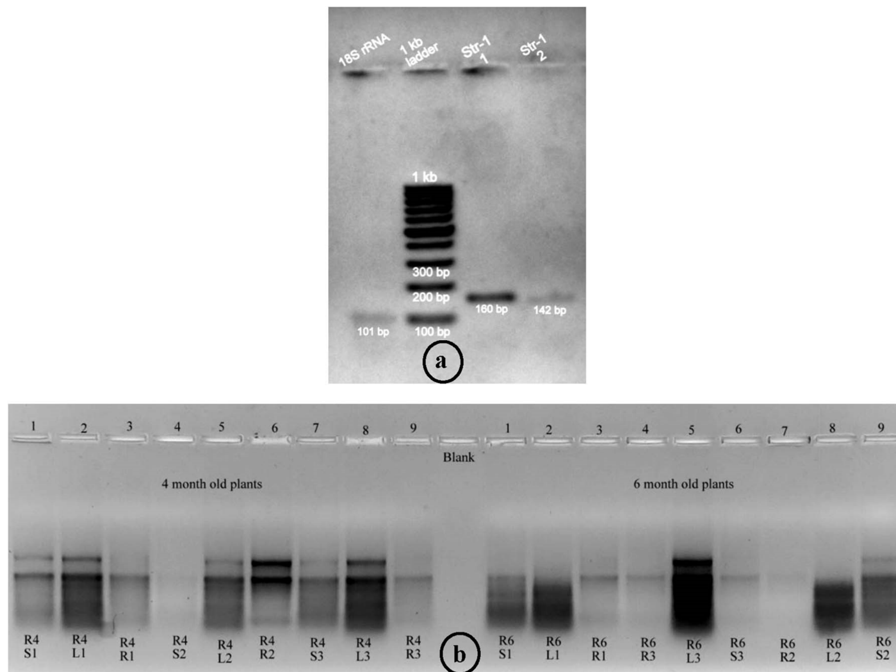

Fig. 2. Gel electrophoresis analysis. (a) Check PCR bands of primers and housekeeping gene; first lane indicate *18S rRNA* (housekeeping gene), second lane = 1 kb ladder, third and fourth lane = *str1* primers; (b) isolated RNA bands; lane 1-9 (left) indicates four-month-old plants (R4), lane 1-9 (right) indicates six-month-old plants (R6); S=Stem, L=Leaf, R=Root and, each stage with 3 replicates.

RT-PCR analysis of *R. tetraphylla* revealed distinct expression patterns for the *str1* gene across tissues and developmental stages (Fig. 3a-b). In both 4- and 6-month-old plants, root samples consistently showed the strongest amplification bands for both STR1-1 and STR1-2 primers, indicating high *str1* expression. Stem tissues displayed variable band intensities, with some replicates exhibiting noticeably low amplification. Leaf samples produced the weakest bands in most cases, except for trace amplification in a few

replicates. The STR1-1 primer generally yielded more consistent banding patterns across replicates compared to STR1-2, which showed greater variability in stem and leaf samples. The *18S rRNA* amplification patterns confirmed RNA integrity across all samples. Strong, consistent bands of equal intensity were observed in all root, stem, and leaf samples from both 4- and 6-month-old plants (Fig. 3a-b). These qualitative observations from agarose gel electrophoresis suggest that *str1* expression is predominantly localized to root tissues, with limited activity in stems and minimal detection in leaves.

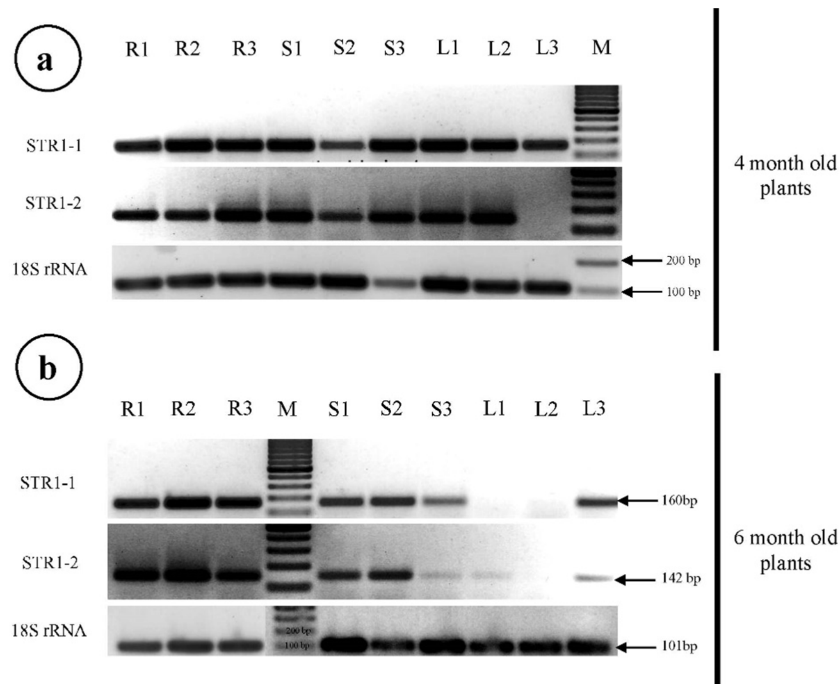

Fig. 3. Gel electrophoresis analysis of *str1* expression in different parts of *R. tetraphylla*. (a) *str1* expression in different parts of four-month-old *R. tetraphylla*. b) *str1* expression in six-month-old *R. tetraphylla* plants in different tissues. Gel pictures show RT-PCR bands of 2 set primers (STR1-1 and STR1-2) and housekeeping gene *18S rRNA* amplified with *R. tetraphylla*. Lane R1-R3 indicate roots samples, S1-S3 indicate stem samples and lane L1-L3 indicate leaf samples, lane M is 100 bp ladder; arrow indicates the expected band size.

Moreover, *str1* expression was assessed via semi-quantitative RT-PCR, with band intensities quantified using ImageJ and normalized to *18S rRNA*. In this approach, plot lane profile of the gel pictures were created to analyze the intensity of the peaks of the bands. The area under the peak represents the band intensity (Fig. 4a-c). The data of band intensity and normalized ratio was presented in Table 2 and the result showed distinct tissue-specific and age-dependent patterns across root, stem, and leaf tissues at 4 and 6 months of age.

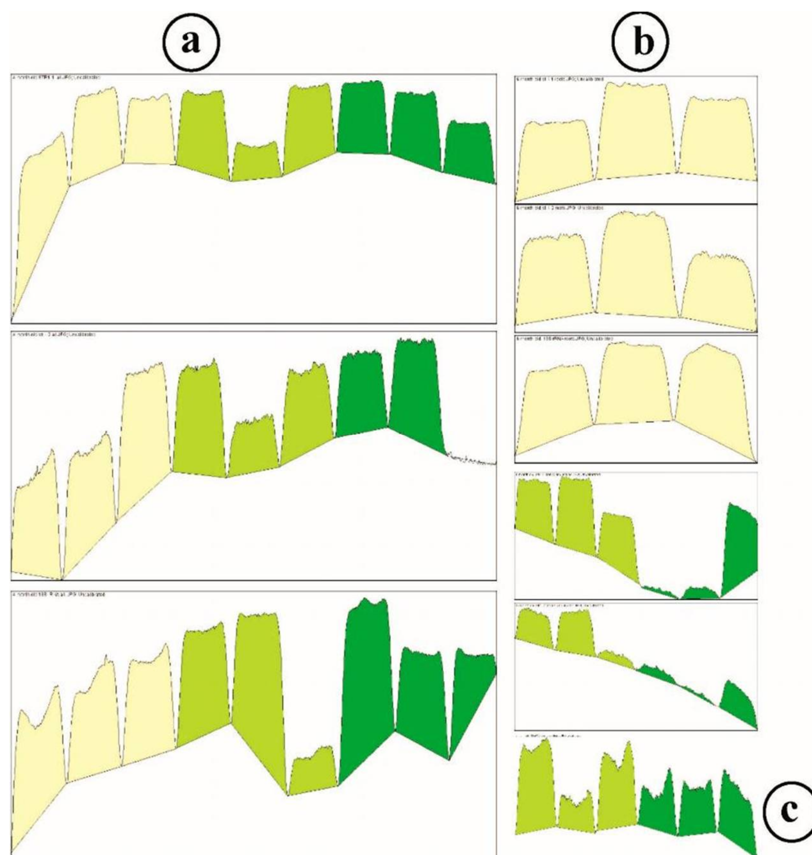

Fig. 4. Plot lane analysis of *str1* expression in different tissue of *R. tetraphylla* using the RT-PCR gel image: (a) Band area intensity peak of *str1* in different part of four-month-old *R. tetraphylla*, (b) Band area intensity peak of *str1* in roots of six-month-old *R. tetraphylla*, (c) Band area intensity peak of *str1* in stems and leaves of six-month-old *R. tetraphylla*. Every panel shows peaks: Top: STR1-1 primer, Middle: STR1-2 primer and bottom: 18S rRNA. Every band is represented by a peak. (Light yellow peak= root samples, lime green peak= stem samples and dark green mark= leaf samples).

Mean *18S rRNA* intensity (30814.1) confirms adequate cDNA input. In root tissues, both isoforms exhibited relatively high and stable expression, with STR1-1 showing consistent ratios (1.005 ± 0.005 at 4M; 1.054 ± 0.172 at 6M) and STR1-2 displaying slightly higher but more variable expression (1.152 ± 0.210 at 4M; 1.100 ± 0.300 at 6M), suggesting robust transcriptional activity in roots regardless of age. This high and stable expression of STR1-1 and STR1-2 in roots aligns with previous reports that MIA biosynthesis is root-predominant in many Apocynaceae species, including *Catharanthus roseus* (Guirimand et al. 2010), *Rauvolfia* sp. (Stockigt et al. 2008). Similarly, Bracher and Kutchan (1992) reported the expression of *str1* in *R. serpentina*. They revealed that *R. serpentina* roots show the highest strictosidine synthase (STR1) activity, while leaves and stems have minimal expression, matching the plant's alkaloid distribution pattern. According to

Court's (1983) experiment with alkaloid distribution analysis of African *Rauvolfia* species, simpler alkaloids were found in aerial parts of the plant and the more complex dihydroindole alkaloids were found mainly in the roots.

Table 2. RT-PCR analysis of *str1* expression in *R. tetraphylla*: ImageJ-derived band intensities (area values) and normalized (STR1/18S rRNA) ratios for root, stem and leaf tissues at different ages.

Tissue	Age (month old)	Replicate	Band intensity (STR1-1) area	Band intensity (STR1-2) area	Band intensity (18S rRNA) area	STR1-1/18S rRNA ratio	STR1-2/18S rRNA ratio
Root	4	R1	33540.7	31978.5	33538.5	1.000	0.953
		R2	29518.5	33143.9	29.357.6	1.006	1.129
		R3	30242.7	41176	29976.5	1.009	1.374
			Mean ± SD			**1.005 ± .005**	**1.152 ± 0.210**
Root	6	R1	34449.3	40424.5	34335.8	1.003	1.177
		R2	46881.8	51102.7	37517.4	1.250	1.362
		R3	38704	32451.3	38570.3	0.909	0.762
			Mean ± SD			**1.054 ± 0.172**	**1.100 ± 0.300**
Stem	4	S1	26210.1	30784.7	31984.5	0.819	0.962
		S2	10800.3	16342.8	44682	0.242	0.366
		S3	25277.9	26613.1	27507.3	0.919	0.967
			Mean ± SD			0.660 ± 0.389	0.765 ± 0.324
Stem	6	S1	25362	27539.3	36376.3	0.697	0.757
		S2	33761.6	33260	37250.6	0.906	0.893
		S3	25493.3	3526.55	26660.7	0.956	0.132
			Mean ±SD			0.853 ± 0.133	0.594 ± 0.418
Leaf	4	L1	23004.2	25158.1	47822.1	0.481	0.526
		L2	22426.1	21609.2	27875.5	0.805	0.775
		L3	13697.6	78.899	16724.8	0.819	.005
			Mean ± SD			0.702 ± 0.189	0.435 ± 0.434
Leaf	6	L1	1549.82	3524.94	42969	0.036	0.082
		L2	4324.85	1089.55	37419.9	0.116	0.029
		L3	31367.4	11125.7	41352.7	0.758	0.269
			Mean ± SD			0.303 ± 0.384	0.127 ± 0.130

In contrast, stem tissues demonstrated lower overall expression, particularly at 4 months, where STR1-1 ratios varied widely (0.242-0.919), possibly indicating developmental or regulatory fluctuations. By 6 months, stem expression became more stable for STR1-1 (0.853 ± 0.133), though STR1-2 showed extreme variability (e.g., 0.132 in S3 vs. ~0.8 in S1/S2), which may reflect tissue heterogeneity or experimental noise. These findings align with Yamazaki et al. (2003), who similarly reported differential *str1* expression across tissues in *R. serpentina*, supporting the concept of tissue-specific metabolic partitioning in alkaloid biosynthesis. The consistent root-dominant activity further validates the spatial regulation of alkaloid production in *R. serpentina*.

Leaf tissues displayed higher decline in *str1* expression with age, as STR1-1 dropped from 0.702 ± 0.189 at 4M to 0.303 ± 0.384 at 6M, and STR1-2 nearly diminished (0.127 ± 0.130 at 6M), with some replicates (e.g., L1, L2) approaching undetectable levels. While leaves are major sites for photosynthesis and secologanin production, they may rely on root-derived strictosidine rather than local biosynthesis. This is corroborated by studies in *C. roseus* showing that leaf STR transcripts are often undetectable unless induced by stress (Menke et al. 1999).

Our semi quantitative analysis aligns well with qualitative gel observations, where root bands were consistently intense, stem bands appeared moderate but variable, and leaf bands weakened significantly with age, particularly for STR1-2. The outliers in stem and leaf datasets (e.g., S3-STR1-2, L3-STR1-1) likely correspond to faint but detectable bands in gels, reinforcing the importance of biological and technical replicates in interpreting expression trends. Overall, the data highlight strong tissue specificity (roots >stems >leaves) and an age-dependent suppression of *str1* isoforms in leaves, suggesting potential regulatory mechanisms tied to developmental stages or environmental adaptations.

For further confirmation, STR1-1 forward primer was sequenced, and the resulting sequence was submitted to NCBI with the accession number PV600355. A nucleotide BLAST (blastn) was performed against the NCBI database to verify its identity. The findings showed that strictosidine synthase (*str1*) genes from several *Rauvolfia* species shared a high degree of similarity (94.4-94.56% identical) including- *R. serpentina* (94.40-94.56% identity; accessions: X62334.1, Y00756.1), *R. mannii* (94.56% identity; Accession: X63431.1;), *R. verticaliata* (94.56% identity; Accessions: DQ872163.1, DQ017054.1). Thus, we confirmed that the sequenced segment was part of strictosidine synthase (*str1*) gene by 90% query coverage, nearly similar Max Scores (955-961), and E-values of 0.0 (showing high confidence) for all hits (Table 3). These findings support the primer's specificity and suitability for more research on *str1* expression in Apocynaceae.

Table 3. NCBI BLAST analysis of *str1* sequence of *Rauvolfia tetraphylla* against *Rauvolfia* homologues.

Species	Max score	Query cover	E-value	Identity percentage	Accession	Publication
R. serpentina	961	90%	0.0	94.56%	X62334.1	Bracher and Kutchan (1992)
R. serpentina	955	90%	0.0	94.40%	Y00756.1	Kutchan et al. (1988)
R. verticillata	961	90%	0.0	94.56%	DQ872163.1	Chan et al. (2008)
R. verticillata	961	90%	0.0	94.56%	DQ017054.1	Chen et al. (2008)
R. manni	961	90%	0.0	94.56%	X63431.1	Bracher and Kutchan (1992)

With its major activity in roots, less so in stems, and barely detectable in leaves, our study offers thorough evidence for tissue-specific and developmental regulation of *str1* expression in *R. tetraphylla.* This investigation covers a significant knowledge gap on the alkaloid production of *R. tetraphylla*, a medicinal plant cultivated in Bangladesh, by

offering the first comprehensive examination of *str1* expression in this plant. The trustworthiness of our experimental method is demonstrated by the successful design and validation of STR1-specific primers as well as sequence confirmation using NCBI BLAST analysis (94.4-94.56% identity with *Rauvolfia* STR1 homologs). These results support the evolutionary conservation of strictosidine synthase's root-dominant expression. Our findings set a critical foundation for further research on the secondary metabolism of *R. tetraphylla* and offer insightful information for improving cultivation techniques to increase the yield of its pharmacologically active chemical compounds.

References

Abramoff MD, **Magalhães PJ** and **Ram SJ** (2004) Image processing with ImageJ. Biophotonics In. **11(7)**: 36-42.

Ahmed ZU, **Begum ZNT**, **Hassan MA**, **Khondker M**, **Kabir SMH**, **Ahmed M**, **Ahmed ATA**, **Rahman AKA** and **Haque EU** (eds.) (2008) Encyclopedia of Flora and Fauna of Bangladesh, Angiosperms: Dicotyledons (Acanthaceae - Asteraceae). Asiatic Society of Bangladesh, Dhaka. **6**: 408.

Bracher D and **Kutchan TM** (1992) Strictosidine synthase from *Rauvolfia serpentina*: analysis of a gene involved in indole alkaloid biosynthesis. Arch. Biochem. Biophys. **294**: 717-723.

Bustin SA (2002) Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J. Mol. Endocrinol. **29**(1): 23-39.

Chen R, **Liao ZH**, **Chen M**, **Wang Q**, **Yang CX** and **Yang YJ** (2008) Molecular Cloning and Characterization of the Strictosidine Synthase Gene from *Rauwolfia verticillata.* Russ. J. Plant Physiol. **55**(5): 670-675.

Council of Scientific and Industrial Research (CSIR) (1986) The useful plants of India. Publications and Information Directorate.

Court WE (1983) Alkaloid distribution in some African *Rauvolfia* species. Planta Med. **48**(8): 228-33.

De Waal A, **Meijer AH** and **Verpoorte R** (1995) Strictosidine synthase of *Catharanthus roseus*: purification and characterization of multiple forms. Biochem. J. **306**: 571-580.

Duke JA (2005) Duke's Phytochemical and Ethnobotanical Databases. USDA.

Faisal M, **Ahmed N** and **Anis M** (2005) Shoot multiplication in *R. tetraphylla* L. using thidizuron. Plant Cell Tiss. Org. Cult. **80**: 187-190.

Farooqi AA and **Sreeramu BS** (2001) Cultivation of medicinal and aromatic crops. India: University Press Ltd. 210-211.

Gachon C, **Mingam A** and **Charrier B** (2004) Real-time PCR: what relevance to plant studies? J. Exp. Bot. **55**(402): 1445-1454.

Guirimand G, **Courdavault V**, **Lanoue A**, **Mahroug S**, **Guihur A**, **Blanc N**, **Giglioli-Guivarc'h N**, **St-Pierre B** and **Burlat V** (2010) Strictosidine activation in Apocynaceae: towards a "nuclear time bomb"? BMC Plant Biol. **10**: 182.

Hampp N and **Zenk MH** (1988) Homogeneous strictosidine synthase from cell suspension cultures of *Rauvolfia serpentina*. Phytochem. **27**: 3811-3815.

Harisaranraj R, **Suresh K** and **Saravanababu S** (2009) Evaluation of the antibacterial and antidiarrheal effects of *Rauvolfia tetraphylla* root extracts. J. Med. Plants Res. **3**(5): 412-418.

Hoque R, **Borna RS**, **Hoque MI** and **Sarker RH** (2020) *In vitro* plant regeneration of *Rauvolfia tetraphylla* L.: A threatened medicinal plant. Plant Tiss. Cult. Biotech. **30**(1): 33-45.

Kutchan TM (1993) Strictosidine: from alkaloid to enzyme to gene. Phytochem. **32**: 493-506

Kutchan TM, **Hampp N**, **Lottspeich F**, **Beyreuther K** and **Zenk MH** (1988) The cDNA clone for strictosidine synthase from *Rauvolfia serpentina*. DNA sequence determination and expression in *Escherichia coli*. FEBS Lett. **237**(1-2): 40-44.

Livak KJ and **Schmittgen TD** (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. **25**(4): 402-8.

Ma X, **Panjikar S**, **Koepke J**, **Loris E** and **Stöckigt J** (2006) The structure of *Rauvolfia serpentina* strictosidine synthase is a novel six-bladed β-propeller fold in plant proteins. Plant Cell **18**(4): 907-920.

Menke FL, **Parchmann S**, **Mueller MJ**, **Kijne JW** and **Memelink J** (1999) Involvement of the octadecanoid pathway and protein phosphorylation in fungal elicitor-induced expression of terpenoid indole alkaloid biosynthetic genes in *Catharanthus roseus*. Plant Physiol. **119**(4): 1289-96.

O'Connor SE and **Maresh JJ** (2006) Chemistry and biology of monoterpene indole alkaloid biosynthesis. Nat. Prod. Rep. **23**(4): 532-47.

Plants of the World Online (POWO) (2024) *Rauvolfia tetraphylla* L. Royal Botanic Gardens, Kew.

Prashith Kekuda TR (2019) *Rauvolfia tetraphylla* L. (Apocynaceae) - A Comprehensive Review on Its Ethnobotanical Uses, Phytochemistry and Pharmacological Activities. Int. J. Pharm. Biol. Sci. **9**(2): 664-682

Pullaiah T (2002) Encyclopedia of World Medicinal Plants. Regency Publications.

Sambrook J and **Russell DW** (2001) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press **1-3**(3).

Sarma D, **Baruah A** and **Sarma S** (1999) Micropropagation and *in vitro* flowering of *Rauvolfia tetraphylla*: A potent source of anti-hypertension drugs. Plant. Med. **65**(3): 277-8.

Shenya S, **Dinara S**, **Sohani M** and **Niluka D** (2025) Phytochemical Composition, Medicinal Applications and Pharmacological Insights: A Comparative Review of *Rauvolfia Serpentina* and *Rauvolfia tetraphylla*. Asian Plant Res. J. **13**(1): 46-55.

Stöckigt J, **Barleben L**, **Panjikar S** and **Loris EA** (2008) 3D-Structure and function of strictosidine synthase-the key enzyme of monoterpenoid indole alkaloid biosynthesis. Plant Physiol. Biochem. **46**(3): 340-355.

Udvardi MK, **Kakar K**, **Wandrey M**, **Montanari O**, **Murray J**, **Andriankaja A**, **Zhang JY**, **Benedito V**, **Hofer JM**, **Chueng F** and **Town CD** (2007) Legume transcription factors: global regulators of plant development and response to the environment. Plant Physiol. **144**(2): 538-49.

Yamazaki Y, **Urano A**, **Sudo H**, **Kitajima M**, **Takayama H**, **Yamazaki M**, **Aimi N** and **Saito K** (2003) Metabolite profiling of alkaloids and strictosidine synthase activity in camptothecin producing plants. Phytochem. **62**(3): 461-470.

(Manuscript received on 1 June, 2025; revised on 22 June, 2025)