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DIFFERENTIAL EXPRESSION OF INDOLE ALKALOID PATHWAY GENES ACROSS *CATHARANTHUS ROSEUS* PLANT ORGANS

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Abstract

Vinblastine and vincristine are two indole alkaloids of the medicinal plant *Catharanthus roseu*s, widely used in cancer chemotherapy such as leukemia, Hodgkin's disease and solid tumors. Transcriptomic data of *C. roseus* across different organs (stem, hairy root, flower, immature and mature leaves) was collected and *de novo* assembled then differential expression analysis was achieved. The target of this study was the detection of components of terpenoid indole alkaloid pathway based on gene expression analysis. The *in-silico* analysis was focused on six genes (*SLS, sgd, STR1, OMT*, *DAT* and *G8H*) of the TIA pathway that were expressed in different plant organs. The results indicated posability of formed enzyme in organ and transfer to other organs to work and restore the TIA pathway. The highest expressions level of most TIA key genes took places in root, flowers and mature leaves.

Key words: Transcriptome analysis, vinblastine, vincristine, *de novo*, *in silico*.

Introduction

Plants are attractive sources of pharmaceutical compounds used for therapeutic purposes, especially anti-cancer compounds, albeit being naturally synthesized in plants for the defence against pests and diseases (Ziegler & Facchini, 2008; Roepke *et al.,* 2010; Perveen *et al.,* 2020). Several other important pharmaceutical compounds exist in higher plants such as baccharin, ellipticine, homoharringtonine, tripdiolide, bruceantin, indicine-N-oxide, thalicarpine and maytansine (Ziegler & Facchini, 2008; Guirimand *et al.,* 2010). Among plant products that are marketed as anti-cancer drugs are vinblastine, vincristine, etoposide and camptothecin. Some of these pharmaceutical compounds, e.g., vinblastine and vincristine, are produced in terpenoid indole alkaloid (TIA) pathway of *Catharanthus roseu*s and are widely used in cancer chemotherapy against various leukemia, Hodgkin's disease, solid tumors, and depression (Contin *et al.,* 1998; Dogru *et al.,* 2000; Lange *et al.,* 2001; Burlat *et al.,* 2004; Hamid *et al.,* 2017).

Apocynaceae is a family of medicinal plants including about 550 genera that are considered as a rich resource of TIA. *Catharanthus roseus* is a member of this family and comprises about 130 TIA; 25 of them are dimeric indole alkaloids. TIA biosynthesis pathway has received considerable attention; however, the full characterization of alkaloids pathways is not completely accomplished (Zhu *et al.,* 2015). TIAs are metabolites that have antitumor features such as vincristine and vinblastine that are utilized to treat many types of cancer (Sofowora *et al.,* 2013; Greenwell and Rahman, 2015; Nejat *et al.,* 2015; Soares *et al.,* 2016). The general mechanisms of these metabolites are destroying cancer cells through inhibiting tubulin polymerization; and attacking enzymes involved in DNA duplication and transcription such as topoisomerases I and II (Chi *et al.,* 2015; Chan *et al.,* 2015; Montecucco *et al.,* 2015).

Pharmaceutical compounds synthesized in *C. roseus* plant are extracted at low concentrations. The concentrations of vinblastine and vincristine did not exceed 0.0005% of the dry matter (Neumann *et al.,* 2009). Earlier efforts to produce these useful compounds more efficiently relied on plant tissue culture and recombinant DNA technologies (Carew, 1966). Recently, several studies have used next-generation sequencing (NGS) tools to get a new vision on the eukaryotic transcriptome size and complexity (Wang *et al.,* 2009). RNA-Seq has emerged as a powerful platform for providing an enormous and accurate millions of transcriptome sequence data. Researchers utilized NGS to illustrate the function of novel genes at a high-throughput basis (Oliver, 2002; Yegnasubramanian, 2013). The main advantages of RNA data analysis are the accuracy, low cost, high coverage, and great resolution compared to traditional methods (Kukurba & Montgomery, 2015; Park *et al.,* 2016). RNA-seq analysis of gene expression have played a critical role in defining TIA encoded genes in *C. roseus* (El-Domyatiet al., 2017). This study was designed to detect spatial and temporal transcriptome profiling in *C. roseus* with emphasis on TIA pathway across different organs.

Materials and Methods

RNA-Seq data collection: Transcriptome sequences of *C. roseus* (Little Bright Eyes cultivar) were obtained from SRA (NCBI) with accession numbers of SRR122239 (flower), SRR122243 (immature leaf), SRR122251 (mature leaf), SRR122253 (stem), SRR122254 (root), and SRR122257 (hairy root). Data quality was measured using fastQC program after trimming adapter sequences. Read length of ≤ 50 bp and low qualities reads (≥ 20 for all bases) were removed.

Sequence assembly, genes annotation and clusters analysis: Recovered RNA-Seq reads were *de novo* assembled at *kmer* value = 50 and analyzed using Trinity RNA-Seq assembly (version r2013-2-25) and the tuxedo suite software packages. The relative abundance of the resulted transcripts was estimated using RSEM (v1.1.6). For differential expression analysis, gene clusters formed for each organ were analyzed via EdgeR software (version 3.0.0, R version 2.1.5). Blast-2-GO software (version 2.3.5) was used to detect differentially expressed (DE) transcripts. Clusters with functional enrichment were identified by setting a significant Pearson's correlation through permutation analysis (Brown *et al.,* 2006).

Validation of RNA-Seq datasets: Biological samples were collected from stem, hairy root, flower, root, immature and mature leaves (three months old) of *C. roseus* SunStorm™ cv. Apricot. Plants were grown at 25°C in growth chamber with 12 hours light as described (Brenchley *et al*., 2012). RNAs were isolated using Rneasy™ kit and relative expression of the *SLS*, *sgd*, *STR1*, *OMT* and *DAT* genes of *C. roseus* was detected via qRT-PCR to validate the RNA-Seq data. To synthesize cDNA, 2 µg of total RNA, oligo (dT) and SuperScript III Reverse Transcriptase (Invitrogen™ cat. no. 18080044) was used. Primer BLAST was used to design specific primers (Table 1) for qRT-PCR. Templates were amplified to recover 196 bp fragment of the *C. roseus actin* gene (accession no. EF688556). qRT-PCR was done according to Ramadan *et al.* (2019). Gene relative expression to the internal reference control was calculated using equation : $2^{-\Delta\Delta CT}$ = {(CT target gene – CT internal control) tissue A-(CT target gene – CT internal control) tissue B} (Thomas & Kenneth, 2008).

Table 1. Primer sequences along with the annealing temperatures and expected band sizes (bp) to be utilized in validating RNA-Seq dataset of *C. roseus* via qRT-PCR. The *actin* gene was used as the house-keeping gene (196 bp).

Gene	Acc. no.	Forward primer	Reverse primer	Anneal temp.
SGD	EU072423	AGCTCTTGTAGGAAGCCGTC	CGTAACCCGGAGTATCGGGA	60 ºC
OMT	EF444544	TCTACCGCCTAATGCGTGTT	AAGTTGAACAGGGTCAGCCA	60 ºC
DAT	AF053307	TGAAGGATTGGGCTGCTTCT	TTCTATGGCTTCCGGAGGGA	58ºC
SLS	KF309242	AGGACACAAAGTTAGGGCCG	CTTGGTGGCATTGGCAACTC	58 ºC
STR1	Y10182	AGCGCAGATGGTTCCTTTGT	ACCCAAAAATGGCCATCAGAA	60 ºC
Actin	EF688556	TGGTCGTCCAAGACACACTG	CTCTTCAGGGGCAACACGAA	60 ºC

Table 2. Statistics analysis of *Catharanthus roseus* RNA-Seq data. F=flower, IML=immature leaf, ML=mature leaf, S=stem, R=root, HR=hairy root.

Transcriptome data file (a)	Organ organs (b)	Total no. of reads (c)	No. of mapped reads (d)	% of mapped reads (e)	No. of unmapped reads (f)	% of unmapped reads (g)	Total no. of transcripts (h)
SRR122239.fastq	Flower	31645190	28970625	91.55%	2674565	8.45%	40344
SRR122243.fastq	Immature leaf	29918941	27580411	92.18%	2338530	7.82%	39952
SRR122251.fastq	Mature leaf	25325632	23317495	92.07%	2008137	7.93%	40336
SRR122253.fastq	Stem	28903929	26398362	91.33%	2505567	8.67%	40936
SRR122254.fastq	Root	30896527	29222863	94.58%	1673664	5.42%	41281
SRR122257.fastq	Hairy root	25316070	23404947	92.45%	1911123	7.55%	41806

[a]C. roseus transcriptome data files
[b]The organ of each transcriptome file
[c]Total number of reads recovered from C. roseus RNA-Seq data
[d]Number of de novo assembled mapped reads across each organ
[e]Percentage of de novo assembled reads
[f]Number of unmapped reads
[g]Percentage of unmapped reads
[h]Total number of transcripts generated from de novo assembled contigs

Results

Analysis of RNA-Seq datasets: RNA sequencing of *C. roseus* transcriptome data yielded between ~ 25-31 million reads (Table 2). The number of *de novo* assembled mapped reads was 158,894,703 comprising percentages of ~91-94% across organs. The total number of transcripts generated from *de novo* assembly was 244,655 transcripts comprising ~39,000-42,000 across *C. roseus* organs.

Clusters of gene expression in different organs: Differential expression (DE) of transcripts across *C. roseus* organs was detected from RNA-Seq data. To confirm the differences statistically, likelihood ratio was used to compare RPKM-derived read counts with a threshold of ≥ 2 RPKM value. Two-fold expression rate of transcripts changes and 10^{-3} of false discovery rate were specified. The number of deferentially expressed transcripts resulted from *de novo* assembly was 2,266 transcripts. Pearson's

correlation was used to analyze clusters in order to sort out expression profiles of DE transcripts and subject them to hierarchical clustering (Fig. 1). They were sorted out based on the relative abundance of transcripts across different organs. The cluster analysis of gene expression levels provided information for transcript expression patterns and pathways across different organs. In general, the number of upregulated clusters was 16 in immature leaf, while 13 in flower, 12 in mature leaf, 6 in stem, 6 in **root,** and 4 in hairy roots. Six out of these upregulated clusters (nos. 7, 22, 94, 97, 100 and 154; Fig. 2) contained the eight genes of TIA pathway.

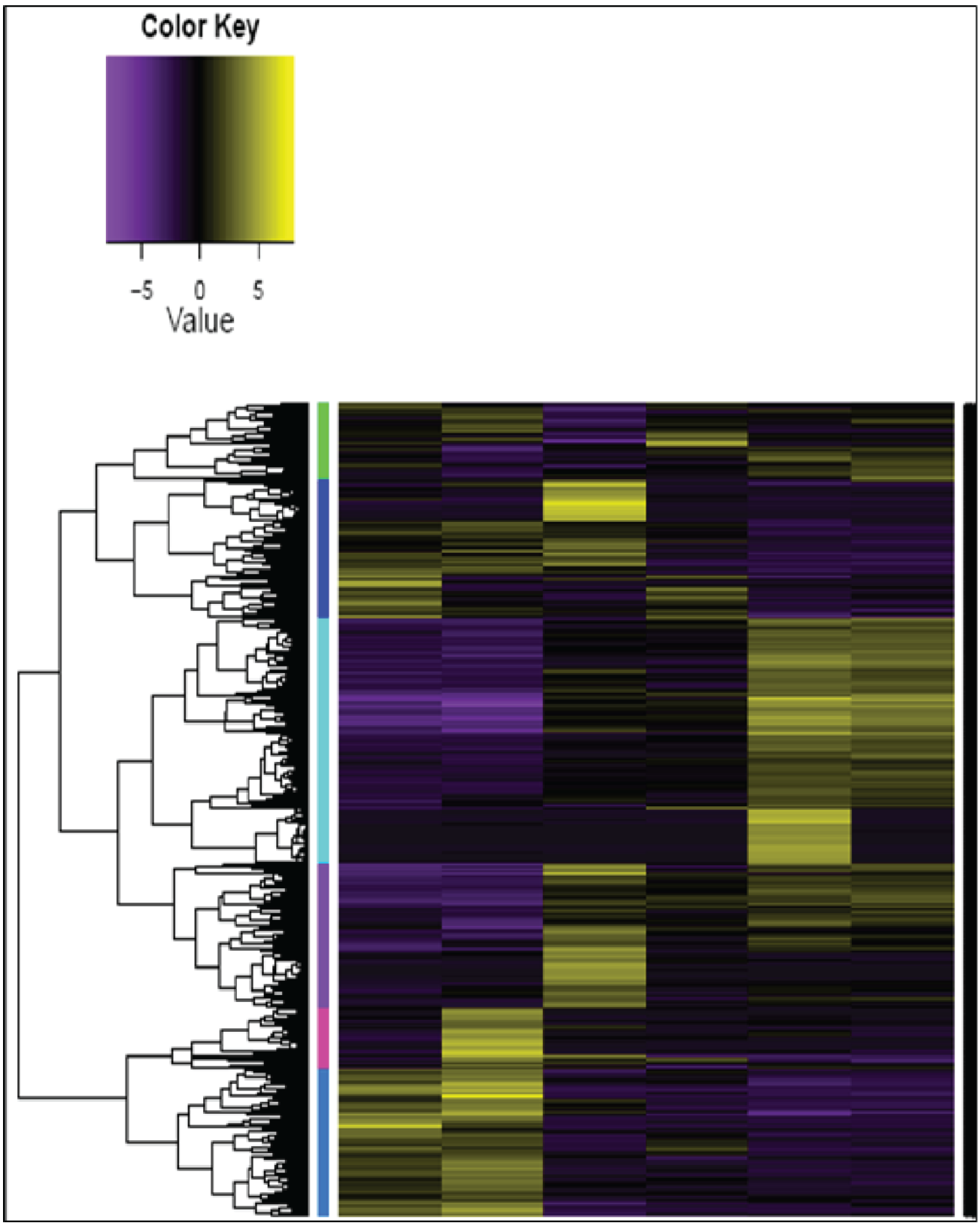

Fig. 1. Hierarchical cluster analysis of gene expression across different *C. roseus* organs.

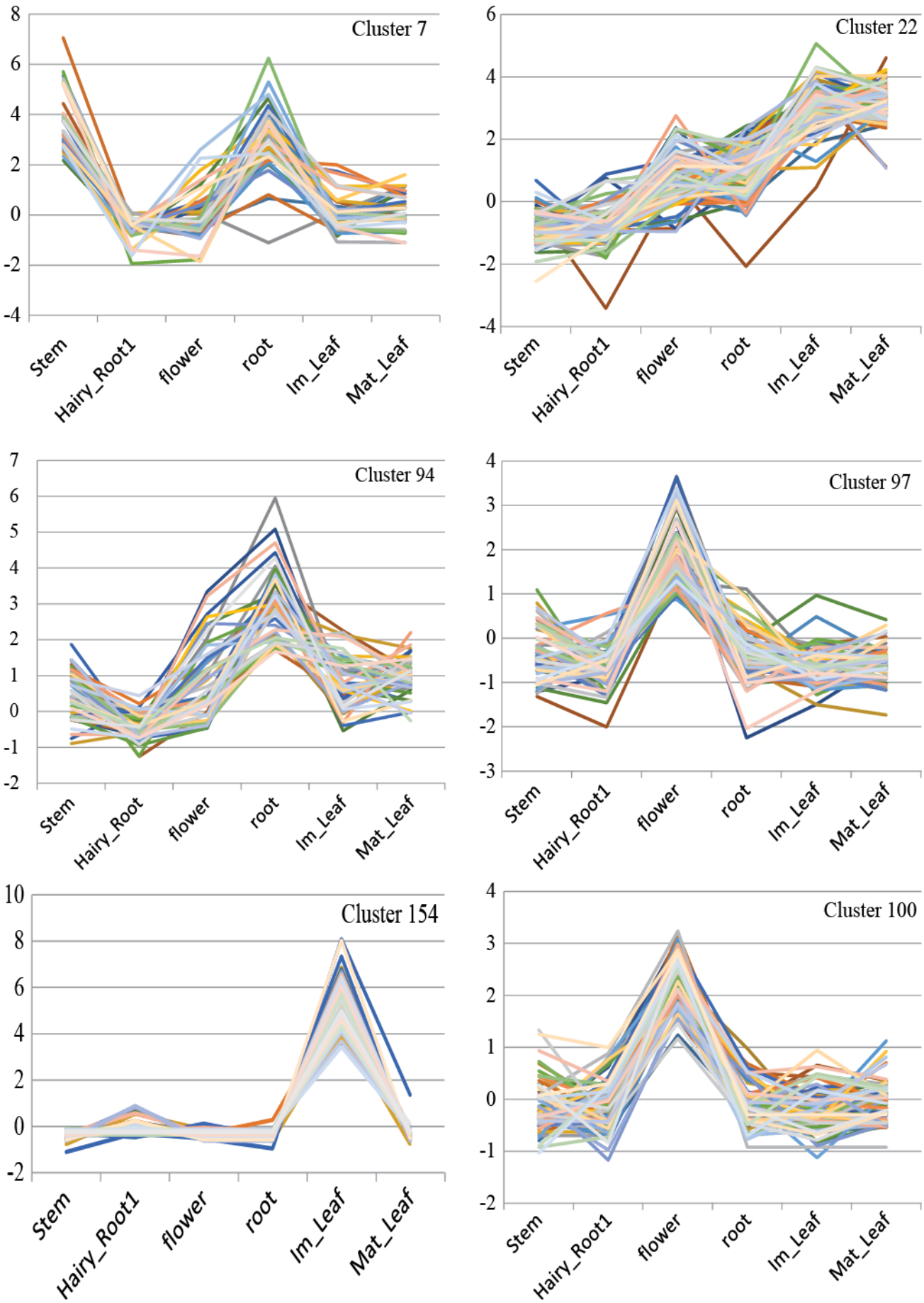

Fig. 2. Six selected clusters of DE transcripts from different *C. roseus* organs.

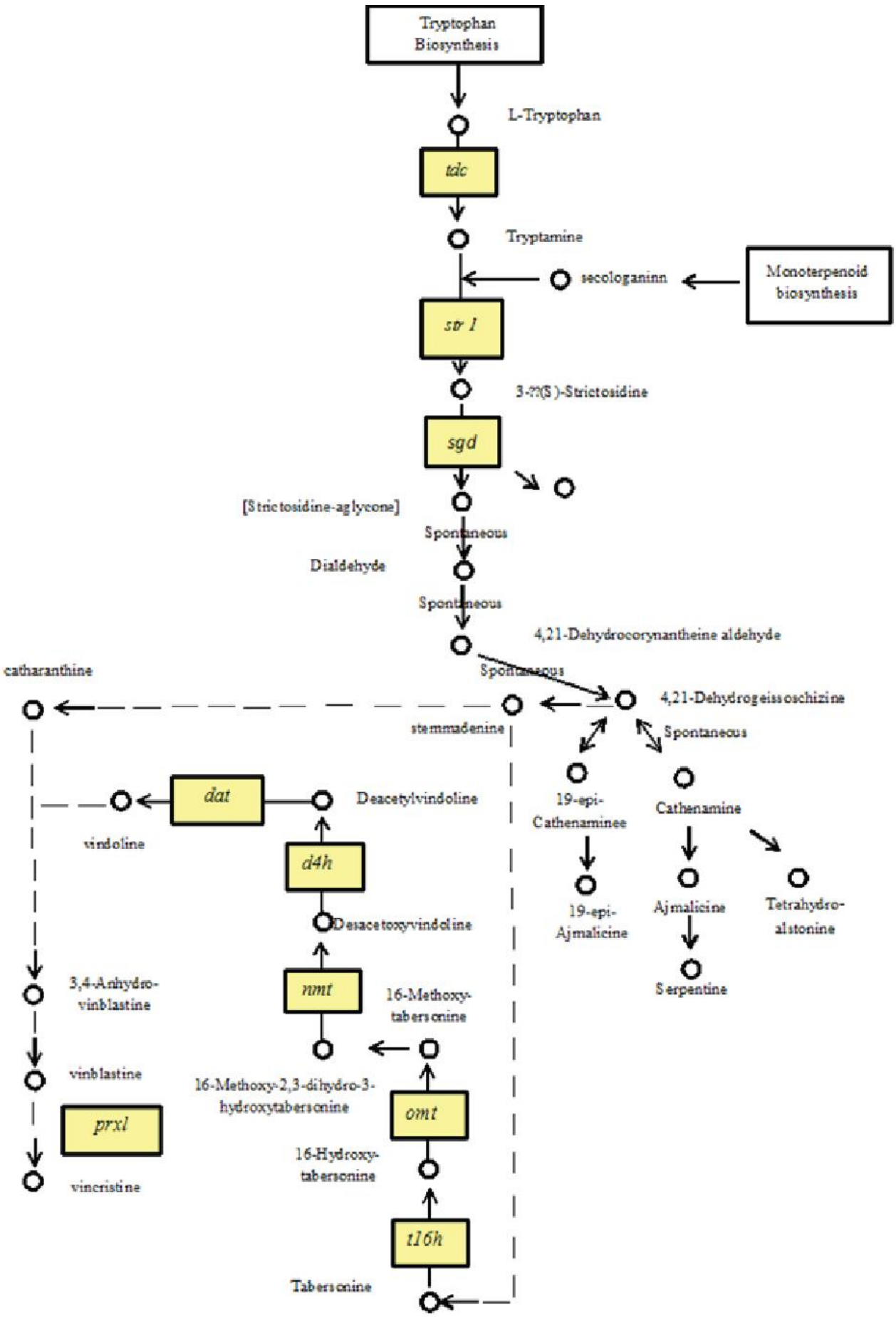

Fig. 3. Indole Alkaloid biosynthesis in TIA Pathway (El- Domyati *et al*., 2017).

Analysis of differentially expressed transcripts: According to TIA pathway (Fig. 3), upregulation was found in the three genes in the beginning of the TIA pathway in mature leaf, flower and root (Fig. 4). These genes encode enzymes secologanin synthase (SLS) (comp28933_c0), strictosidine synthase (STR1) (comp11842_c0), and strictosidine beta-glucosidase (SGD) (comp16844_c0) that exist in cluster 94.

In flower, the accumulation of transcripts encoding tabersonine 16-O-methyltransferase (OMT), deacetylvindoline_4-O-acetyltransferase (DAT), hydroxytabersonine N-methyltransferase (NMT) and peroxidase n1 (PRX1) seem not to support the accumulation of vindoline, vincristine and vinblastine. Therefore, it is suggested that the TIA pathway in flower is directed towards the accumulation of other alkaloids such as ajmaline, serpentine, raucaffricine or sarpagine. However, in mature leaf, the accumulation of these transcripts was very high, indicating the possible high accumulation of vincristine, vinblastine, vindoline and possibly catharanthine. In hairy root, accumulation of previous transcripts was not observed, which indicated that vincristine, vinblastine, vindoline unlikely be accumulated in this organs. In stem, accumulation of these genes does not support accumulation of the latter alkaloids except for deacetylvindoline_4-O-acetyltransferase (DAT) (Fig. 4). In immature leaf, high accumulation of geraniol 8-hydroxylase (comp34847) was observed with no accumulation of secologanin synthase (SLS), indicating the possible accumulation of asperuloside in immature leaf (Fig. 5).

RNA-Seq data Validation: To validate RNA-Seq data, relative gene expression analysis was done via qRT-PCR. The data showed the same expression patterns of the five transcripts *SLS, sgd, STR1, OMT* and *DAT* in the RNA-Seq datasets (Fig. 6).

Discussion

The biosynthesis of *C. roseus* terpenoid indole alkaloid has been studied for decades. Although there is tremendous progress, many steps are still not deciphered. To date, *C. roseus* is the only natural resource for antitumor agents, vinblastine and vincristine. However, their contents in plants are very low. Furthermore, the chemical synthesis of these components is far from being applicable for commercial-scale production (Zhu *et al.,* 2015). Many investigators aimed to detect the accumulation of indole alkaloids in different *C. roseus* organs (Roepke *et al.,* 2010; Zhu *et al.,* 2015; Benyammi *et al.,* 2016). It was also reported that some indole alkaloids like vindoline and catharanthine in a given organ can be transferred to another organ. Accumulation of these alkaloids in *C. roseus* mainly take place in young leaves and stems, whereas synthesis of others such as ajmalicine and serpentine mainly occurs in roots (Liu *et al.,* 2016). The main aims of this study are to understand TIA biosynthetic pathways in *C. roseus* and detect the organ of which genes involved in this process are regulated. Transcriptome data of *C. rosues* collected from NCBI indicates that DE transcripts in each cluster differ in their expression levels across *C. roseus* organs. The enzymes expressed from these DE transcripts act in synthesizing TIA compounds. The six DE transcripts for TIA biosynthesis in this study were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. There are many reports that clarified that high accumulations of vindoline, vinblastine and vincristine are mainly in mature leaf whereas catharanthine was highly accumulated in root (Malik *et al.,* 2013; Srivastava *et al.,* 2014). Our findings supported the previous investigation due to the expression profile of *SLS, STR1, sgd, OMT, DAT and PRX1* genes, but we found no expression of *NMT* gene in mature leaf although it was found in root and flower. Therefore, we suggest that the NMT protein may be transferred from root to leaf as a step towards the completion of the TIA pathway. The transferred TIA proteins were proven in many reports (Yamamoto et al., 2016). Also, our finding supports previous investigation regarding the accumulation of catharanthine in root due to the high expression of *SLS, STR* and *sgd* genes in root, while no or low expression of other TIA pathway genes was observed.

Another interesting finding was that *G8H* transcript was highly accumulated in immature leaf. This outcome suggests: (1) the accumulation of asperuloside TIA product in immature leaves tissues due to the activity of *G8H* enzyme and absence of *SLS* transcript activity and (2) the transferring of G8H enzyme from immature leaves to mature leaves tissues to open TIAs pathways in the presence of *SLS* transcript activity.

Although we detected the possible location of expression of some TIA gene in organs by transcriptome analysis, indole alkaloids accumulation in plant organs is still ambiguous as the accumulation of some indole alkaloids in a given organ is not associated with the location of gene expression or with the accumulation of the encoded enzyme in the same organ. Rather, the enzymes are likely transferred from one organ to the other in order to complete the TIA pathway in the distal organ. However, many steps of Tabrsonine accumulation are still unknown.

Conclusions

Transcriptome analysis of *C. roseus* enabled us to detect the expression levels of six genes related to TIA biosynthesis pathway across different organs. Results showed that posability of transfer of formed enzyme in one organ and transfer to other organs to work and restore the TIA pathway. the highest expressions level of most TIA key genes took places in root, flower and mature leaf. These findings may be useful in future to determine the precise mechanism of TIA biosynthesis in *C. roseus* organs as well as link coexpressed genes and transcription factors involved in this process. Furthermore, it is important to investigate processes facilitating transport of enzymes from one organ to the other.

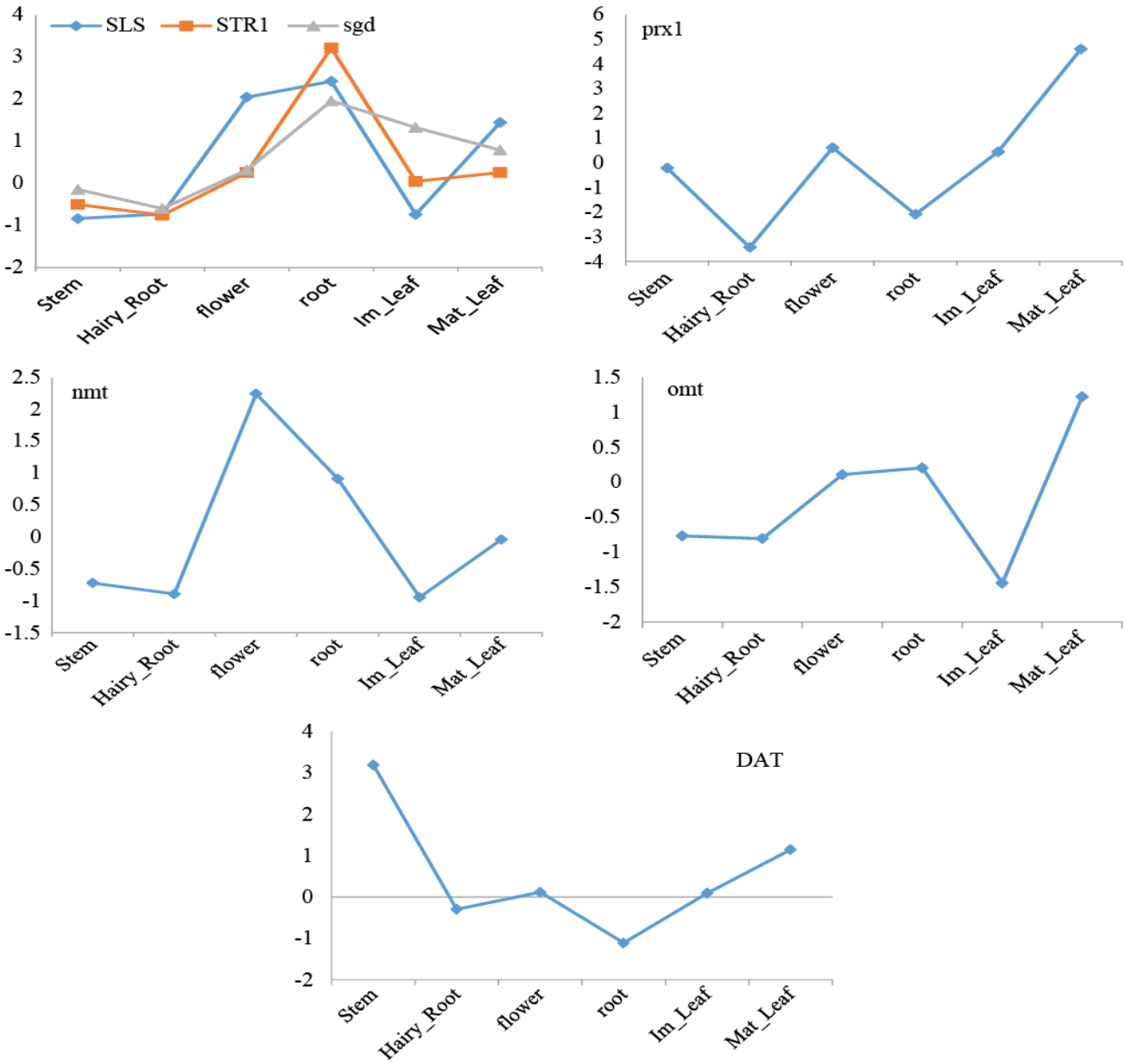

Fig. 4. Expression of transcripts related to the TIA biosynthetic pathway in *C. roseus* organs. *SLS* = secologanin synthase, *STR1* = strictosidine synthase1, *sgd* = strictosidine beta-glucosidase, *PRX1*= peroxidase 1, *NMT* = hydroxytabersonine N-methyltransferase, *OMT*= tabersonine 16-O-methyltransferase, *DAT* = deacetylvindoline_4-O-acetyltransferase.

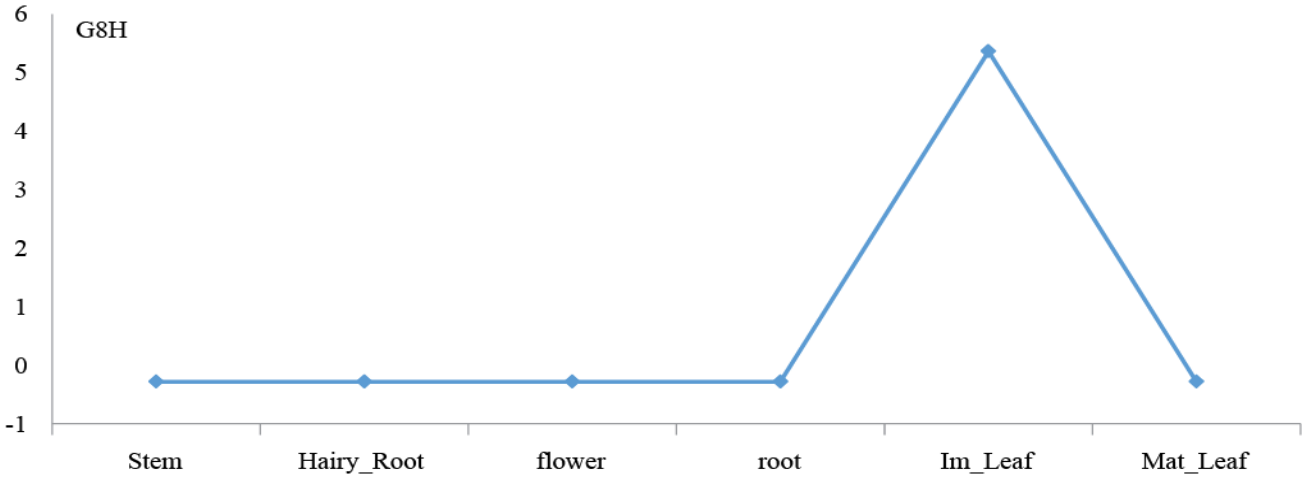

Fig. 5. Expression of geraniol 8-hydroxylase (*G8H*) transcripts related to the Monoterpenoid biosynthesis in *C. roseus* organs.

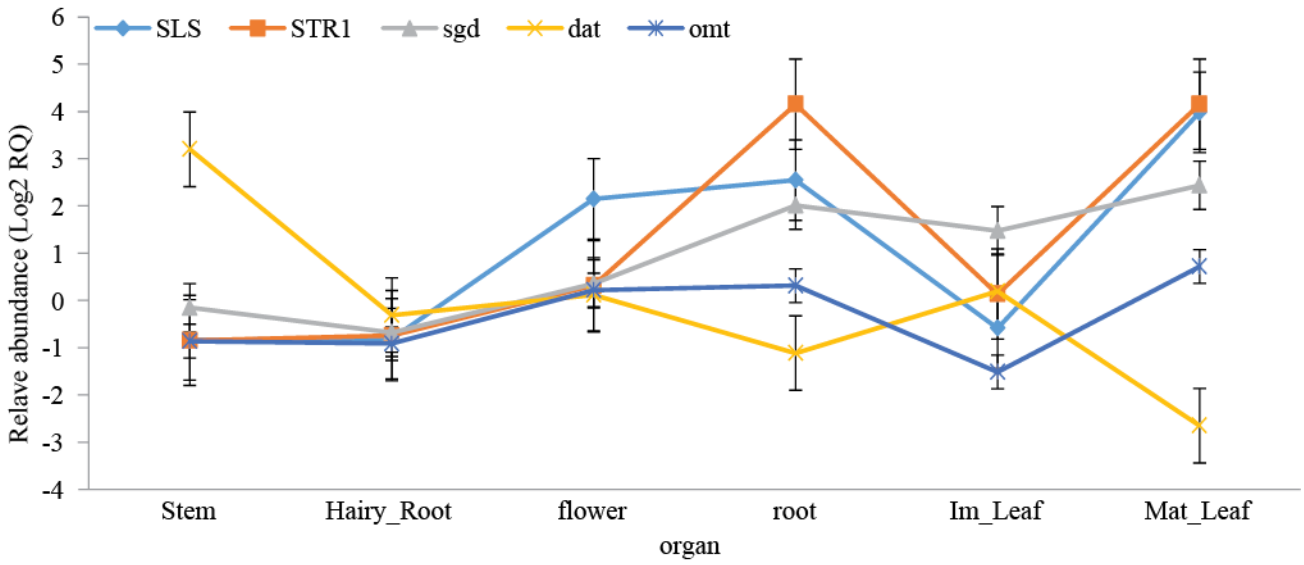

Fig. 6. Relative gene expression analysis of *SLS, sgd, STR1, OMT, and DAT* transcripts of *C. roseus* in different organs.

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References

Benyammi, R., C. Paris, M. Khelifi-Slaoui, D. Zaoui, O. Belabbassi, N. Bakiri, M. Meriem Aci, B. Harfi, S. Malik, A. Makhzoum and S. Desobry. 2016. Screening and kinetic studies of catharanthine and ajmalicine accumulation and their correlation with growth biomass in Catharanthus roseus hairy roots. *Pharm. Biol.*, 54(10): 2033-2043.

Brown, J.A., G. Sherlock, C.L. Myers, N.M. Burrows, C. Deng, H.I. Wu, K.E. McCann, O.G Troyanskaya and J.M. Brown. 2006. Global analysis of gene function in yeast by quantitative phenotypic profiling. *Mol. Sys. Biol.,* 2: doi:10.1038/msb4100043

Burlat, V., A. Oudin, M. Courtois, M. Rideau and B. St-Pierre. 2004. Co-expression of three MEP pathway genes and geraniol 10-hydroxylase in internal phloem parenchyma of Catharanthus roseus implicates multicellular translocation of intermediates during the biosynthesis of monoterpene indole alkaloids and isoprenoid-derived primary metabolites. *The Plant J.*, 38(1): 131-141.

Carew, D.P. 1966. Growth of callus tissue of Catharanthus roseus in suspension culture. *J. Pharm. Sci.*, 55(10): 1153-1154.

Chan, P. F., V. Srikannathasan, J. Huang, H. Cui, A.P. Fosberry, M. Gu, M. Hann, M. Hibbs, P. Homes, K. Ingraham, J. Pizzollo, C. Shen, A.J. Shillings, C.E. Spitzfaden, R. Tanner, A.J. Theobald, R.A. Stavenger, B.D. Bax and M. Gwynn. 2015. Structural basis of DNA gyrase inhibition by antibacterial QPT-1, anticancer drug etoposide and moxifloxacin. *Nature Comm.*, 6: 10048.

Chi, S., W. Xie, J. Zhang and S. Xu. 2015. Theoretical insight into the structural mechanism for the binding of vinblastine with tubulin. *J. Biomol. Struct. & Dynam.*, 33(10): 2234-2254.

Contin, A., R. van der Heijden, A.W. Lefeber and R. Verpoorte. 1998. The iridoid glucoside secologanin is derived from the novel triose phosphate/pyruvate pathway in a Catharanthus roseus cell culture. *FEBS Lett.*, 434(3): 413-416.

Dogru, E., H. Warzecha, F. Seibel, S. Haebel, F. Lottspeich and J. Stöckigt. 2000. The gene encoding polyneuridine aldehyde esterase of monoterpenoid indole alkaloid biosynthesis in plants is an ortholog of theα/β hydrolase super family. *The FEBS J.*, 267(5): 1397-1406.

El- Domyati, F.M., A. Atef, A.K.M. Al- Ghamdi, S. Edris, N.O. Gadallah, M.A. Al-Kordy, A.M. Ramadan, T.K. Khan, Y.M. Saad, J.S.M. Sabir, H.S. Al-Zahrani and A. Bahieldin. 2017. Identification of organ-specific regulatory framwork of C. roseus with emphasis to the TIA biosynthetic pathway. *Pak. J. Bot.*, 49(6): 2243-2254

Greenwell, M. and P. K.S.M. Rahman. 2015. Medicinal plants: their use in anticancer treatment. *Int. J. Pharm. Sci. & Res.*, 6(10): 4103.

Guirimand, G., V. Courdavault, A. Lanoue, S. Mahroug, A. Guihur, N. Blanc, N.G. Guivarc'h, B. St. Pierre and V. Burlat. 2010. Strictosidine activation in Apocynaceae: towards a" nuclear time bomb"?. *BMC Plant Biol.,* 10(1): 182.

Hamid, H.A., A.N. Ramli and M.M. Yusoff. 2017. Indole alkaloids from plants as potential leads for antidepressant drugs: A mini review. *Front. Pharmacol.*, 8: 96.

Kukurba, K.R. and S.B. Montgomery 2015. RNA sequencing and analysis. *Cold Spring Harbor Protocols*, 11: pdb-top084970.

Lange, B.M., R.E. Ketchum and R.B. Croteau. 2001. Isoprenoid biosynthesis. Metabolite profiling of peppermint oil gland secretory cells and application to herbicide target analysis. *Plant Physiol.,* 127(1): 305-314.

Liu, J., J. Cai, R. Wang and S. Yang. 2016. Transcriptional regulation and transport of terpenoid indole alkaloid in Catharanthus roseus: exploration of new research directions. *Int. J. Mol. Sci.*, 18(1): 53.

Malik, S., S.A. Andrade, A.C. Sawaya, A. Bottcher and P. Mazzafera. 2013. Root-zone temperature alters alkaloid synthesis and accumulation in *Catharanthus roseus* and *Nicotiana tabacum*. *Indust. Crops & Prod.,* 49: 318-325.

Montecucco, A., F. Zanetta and G. Biamonti. 2015. Molecular mechanisms of etoposide. *EXCLI J.*, 14:95.

Nejat, N., A. Valdiani, D. Cahill, Y.H. Tan, M. Maziah and R. Abiri. 2015. Ornamental exterior versus therapeutic interior of Madagascar periwinkle (*Catharanthus roseus*): The two faces of a versatile herb. *The Sci. World J.*, 2015: 982412. doi: 10.1155/2015/982412.

Neumann, K.H., A. Kumar and J. Imani. 2009. Plant cell and tissue culture-A tool in Biotechnology: Basics and Application. *Springer Science & Business Media.*

Oliver, S.G. 2002. Functional genomics: lessons from yeast. *Philosophical Transactions of the Royal Society B. Biological Sciences*, 357(1417): 17- 23.

Park, Y.J., X. Li, S.J. Noh, J.K. Kim, S.S. Lim, N.I. Park, S. Kim, Y.B. Kim, Y.O. Kim, S.W. Lee, M.V. Arasu, N.A. Al-Dhabi and S.U. Park. 2016. Transcriptome and metabolome analysis in shoot and root of *Valeriana fauriei*. *BMC Genom.,* 17(1): 303.

Perveen A., S. Ijaz and N. Ghaffar. 2020. Comparative phytochemical and physicochemical study of seeds of the genus Angelica L. from Neelum Valley Azad Kashmir, Pakistan. *Pak. J. Bot*., 52(1): 257-260.

Ramadan, A.M., A. Abdel Azeiz, S. Baabad, S. Hassanein, N.O. Gadalla, S. Hassan, M. Algandaby, S. Bakr, T. Khan, H. H. Abouseadaa, H.M. Ali, A. Al-Ghamdi, G. Osman, S. Edris, H. Eissa and A. Bahieldin. 2019. Control of β-sitosterol biosynthesis under light and watering in desert plant *Calotropis procera*. *Steroids,* 141: 1-8.

Roepke, J., V. Salim, M. Wu, A.M. Thamm, J. Murata, K. Ploss, W. Boland and V. De Luca. 2010. Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle. *Proceedings of the National Academy of Sciences*, 107(34): 15287-15292.

Soares, F.P., L.F. Cavalcante, N.R. Romero and M.A. Bandeira. 2016. *Himatanthus* Willd. Ex Schult. (Apocynaceae). *Pharmacog. Rev.*, 10(19): 6.

Sofowora, A., E. Ogunbodede and A. Onayade. 2013. The role and place of medicinal plants in the strategies for disease prevention. *Afr. J. of Trad., Complem. & Alternative Med.*, 10(5): 210-229.

Srivastava, S., R. Pandey, S. Kumar and C.S. Nautiyal. 2014. Correspondence between flowers and leaves in terpenoid indole alkaloid metabolism of the phytoplasma-infected Catharanthus roseus plants. *Protoplasma*, 251(6): 1307-1320.

Wang, Z., M. Gerstein and M. Snyder. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nature Rev. Gen.*, 10(1): 57.

Thomas, D.S. and K.J. Livak. 2008. Analyzing real-time PCR data by the comparative CT method. *Nature Protocols,* 3: 1101-1108.

Yamamoto, K., K. Takahashi, H. Mizuno, A. Anegawa, K. Ishizaki, H. Fukaki, M. Ohnishi, M. Yamazaki, T. Masujima and T. Mimura. 2016. Cell-specific localization of alkaloids in *Catharanthus roseus* stem tissue measured with imaging MS and single-cell MS. *Proc. Nat. Acad. Sci. USA.,* 113: 3891-3896.

Yegnasubramanian, S. 2013. Explanatory chapter: next generation sequencing. *Methods in Enzymol.,* 529: 201-208.

Zhu, J., M. Wang, W. Wen and R. Yu. 2015. Biosynthesis and regulation of terpenoid indole alkaloids in *Catharanthus roseus*. *Pharmacog. Rev.*, 9(17): 24.

Ziegler, J. and P.J. Facchini. 2008. Alkaloid biosynthesis: metabolism and trafficking. *Ann. Rev. Plant Biol.,* 59: 735-769.

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