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Computational Analysis for Regulation of Podophyllotoxin Biosynthesis Pathway in Podophyllum with Potential Substitute Species

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ABSTRACT

Podophyllum species, the source of important secondary metabolite, podophyllotoxin, is over-exploited for production of anti-cancer drugs endangering this genus globally. Lack of complete knowledge on podophyllotoxin biosynthesis is a major drawback in its cultivation and identification for alternative plants. The current study on secoisolariciresinol dehydrogenase, dirigent protein oxidase and pluviatolide synthase identifies their role in regulating podophyllotoxin biosynthesis. The present computational analysis of podophyllotoxin proposes a correlating interconnected network of pathways for podophyllotoxin biosynthesis besides identifying potential substitute species for the biosynthesis of podophyllotoxin and accounting for possible reason for variation in podophyllotoxin yield from different species of this genus.

Keywords: Podophyllotoxin; Secoisolariciresinol dehydrogenase; Dirigent protein oxidase; Pluviatolide synthase; Podophyllum; BLAST

1. INTRODUCTION

Podophyllum belongs to the family of Berberidacea of the order Ranunculales and is globally represented largely by three species, the P. hexandrum, P. peltatum, and P. sikkimensis. Its peculiar growth requirements is of well-drained humus rich soil and temperature of not more than 10-20 ºC, persistent largely in the temperate and sub-alpine regions, has restricted the natural occurrence of this species globally. P. hexandrum grows in the Himalayan region of the Indian subcontinent and is known as The Himalayan or Indian mayapple[1]. P.peltatum is found in North America and known as American Mayapple[2]

Podophyllotoxin (PTOX) is the most significant and medicinally important secondary metabolite isolated from this species. It is obtained abundantly from the roots and rhizome of Podophyllum species. It is enlisted under the List of Essential Medicines released by the World Health Organisation (WHO) in 2017[3] and is the most active naturally occurring cytotoxic product used for the preparation of anti-cancer drugs Etoposide, Teniposide and their derivatives. It is also reported to have anti-viral activity and protective ability against radioactivity damages[1,4-7].

The precursor to podophyllotoxin biosynthesis is the phenylpropanoid pathway[8]. The scattering of genes that govern this extensive pathway are a major limitation in understanding it[9]. Coniferyl alcohol is converted to pinoresinol which is further reduced to secoisolariciresinol involving enzymes dirigent protein oxidase and coniferyl alcohol dehydrogenase[10-15]. Secoisolariciresinol is converted to matairesinol by secoisolariciresinol dehydrogenase and further on to podophyllotoxin by a series of multi-step reactions[16], a brief overview of which is given Fig. 1.

The biosynthesis of PTOX involves the production other secondary metabolites as hinokinin and yatein which are considered to be its precursors[17-19.] This biochemical pathway is found not only in Podophyllum but also various other plant species[20-23]. Not many natural sources are available for obtaining PTOX, but of those present, the rhizomes of Podophyllum species[24] form the major source for the procurement of this important lignan. Due to extensive demand coupled with the slow growth rate of this important plant[25-26], this species is now endangered, leading to the exploration of other approaches for the chemical synthesis and in-vitro production of this compound[27-32]. But these methods have been unable to adequately supply to fulfil the demand of PTOX on commercial level due to lack of complete conclusive information on the biosynthetic pathway of this metabolite.

Figure 1 A schematic flowchart for showing the biosynthesis of podophyllotoxin and the intermediate pathways involved in the process. The texts in red are our enzymes of interest governing the different steps in the biosynthesis of podophyllotoxin. The present work focuses on the utilisation of computational data for

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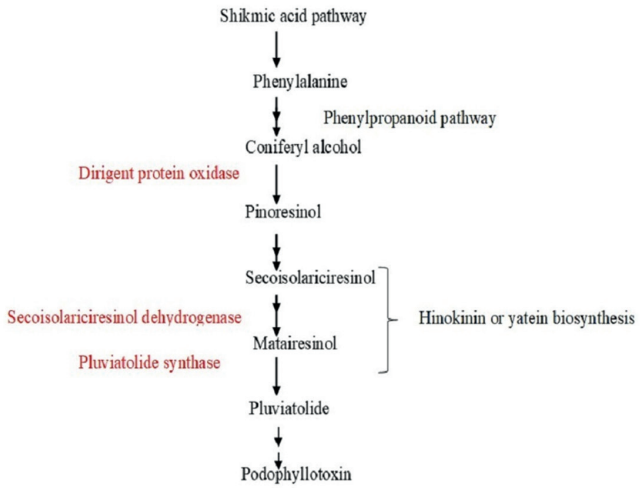

Figure 1. A schematic flowchart for the biosynthesis of podophyllotoxin and the intermediate pathways involved in the process of biosynthesis of podophyllotoxin.

the study of PTOX, by plant bioinformatics approach. This report might be able give an insight and a better understanding of the metabolite production at the molecular level besides indicating some closely related species, by phylogenetic analysis, which can be used for the commercial production of podophyllotoxin.

2. METHODOLOGY

Since the purpose of this study was to investigate the effect of some important enzymes known to regulate other secondary metabolites, for their potential role in regulating the podophyllotoxin biosynthesis pathway, the selections of these enzymes were made as per extensive literature survey.

2.1. Selection of Enzymes

NCBI was used to identify three major enzymes that may have significant role in podophyllotoxin biosynthesis pathway in Podophyllum peltatum and Podophyllum hexandrum as mentioned below. The selection was done in accordance with the roles of these enzymes in governing the other secondary metabolite biosynthesis pathways in these species[33-34].

1. Secoisolariciresinol dehydrogenase
2. (rhizome) Dirigent protein oxidase
3. Pluviatolide synthase

The nucleotide and protein ID for all the three enzymes was obtained from NCBI for future reference and given in Table 1.

Table 1. Nucleotide and protein sequence ID numbers corresponding our enzymes of interest obtained

Enzyme Name	Nucleotide (GenBank ID)		Protein ID	
	P.peltatum	P.hexandrum	P.peltatum	P.hexandrum
(rhizome) dirigent protein oxidase	AF352736.1	KJ595571.1	AAK38666.1	AIA24213.1
Seccoisolariciresinol dehydrogenase	KR779861.1	EF205022.1	ALD51317.1	ABN14311.1
Pluviatolide synthase CYP719A2 (3)/(4)	KC110998.1	KC110997.1	AGC29954.1	AGC29953.1

3. TOOLS AND DATABASES

BLAST was performed for all the nucleotide (BLASTn) and protein (BLASTp) sequences against the three enzymes. The Blast results were studied further to obtain distance tree results which were analysed for obtaining the most closely related species of potential importance

UniprotKB was simultaneously studied to identify the related pathways and detailed metabolic networks associated with them were obtained from BioCyc

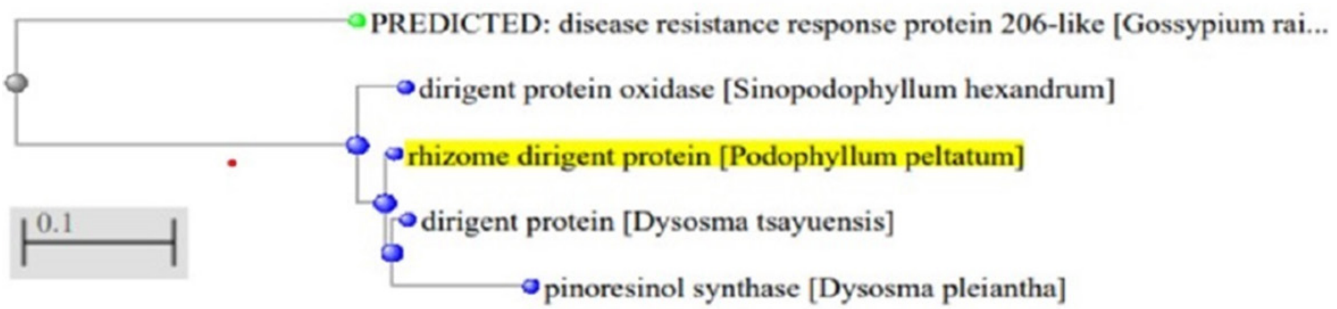

Figure 2. Distance tree obtained for protein BLAST of protein AAK38666.1

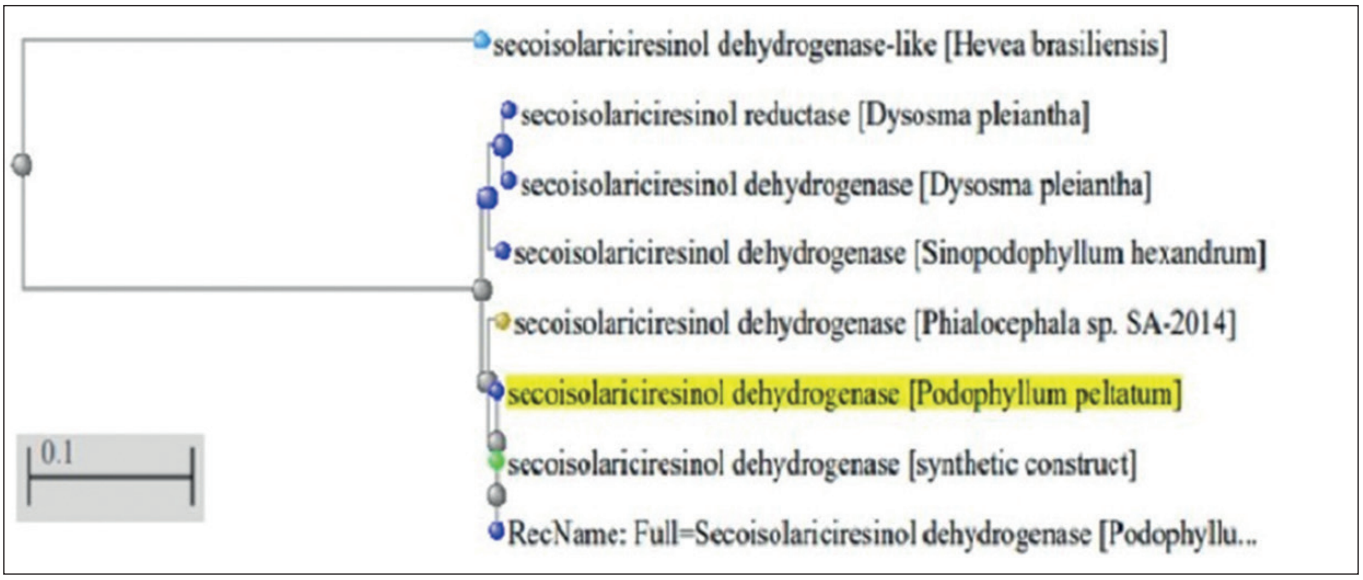

Figure 3. Distance tree obtained for protein BLAST of ALD51317.1

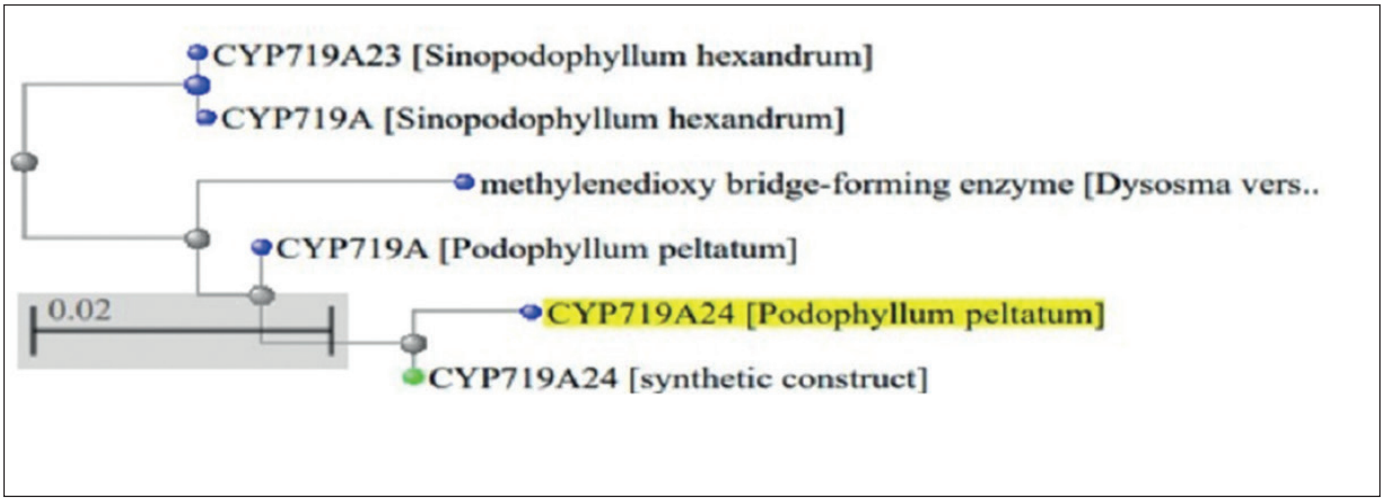

Figure 4. Distance tree obtained for protein BLAST of ID AGC29954.1

and MetaCyc databases. The metabolic networks were also studied to compute and develop a comprehensive network of interconnected pathways for the biosynthesis of podophyllotoxin in Podophyllum species.

4. RESULTS

BLAST analysis for the individual enzymes was documented as phylogenetic tree. Figures 2,3 and 4 give the distance tree results for protein BLAST (BLASTn) for these enzymes. BLAST analysis results for the three enzymes provided the phylogenetic relationship between P.hexandrum and P.peltatum.

5. DISCUSSION

The tree results revealed that Podophyllum hexandrum and Podophyllum peltatum are not essentially similar in their genomic sequences for the enzymes in question. This dissimilarity may account for the difference in the yields of PTOX with higher yields from P.hexandrum[35].The examination of the metabolic pathways governed by these enzymes, helped to identify various substrates in the biosynthesis of PTOX. This information helped develop a prospective metabolic network for PTOX biosynthesis involving the metabolic pathways for the biosynthesis of important secondary metabolites as hinokinin and yatein as shown in Fig. 5[36-37].

This network gives an overview that the biosynthesis of podophyllotoxin cannot be studied independently and must be examined with respect to the substrates involved in the biosynthesis of other secondary metabolites as well. It is also in conjunction with a study conducted by Kumar, *et.al.* who studied the pathway genes in PTOX biosynthesis. He identified 5 genes namely secoisolariciresinol dehydrogenase, p-coumarate-3-hydroxylase, cinnamyl

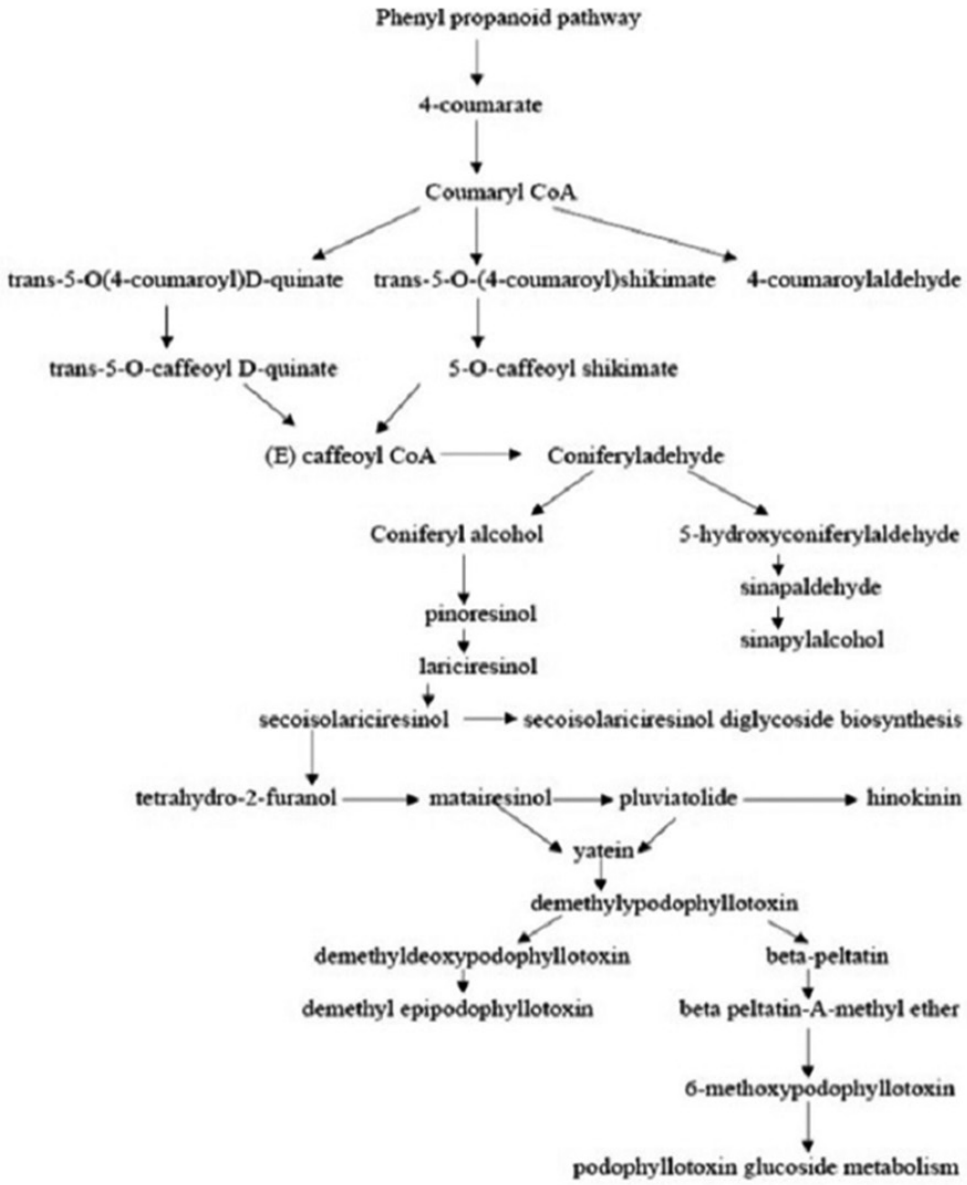

Figure 5. The interlinking pathway for podophyllotoxin biosynthesis as developed after analysis of the metabolic pathways governed by the three enzymes of interest.

alcohol dehydrogenase, cinnamoyl CoA reductase and cinnamate 4-hydroxylase, to be in abundance in the roots and rhizome of P.hexandrum, thereby confirming the role of these genes in the biosynthesis of podophyllotoxin[38]. The distance tree results also helped us to identify the other plant species that can potentially be used for the production of podophyllotoxin. The Dysosma and Linum species were found to be close relatives of Podophyllum in evolutionary context and possessed nearly similar sequences for the enzymes under investigation, Dysosma species was particularly closely related. These species are currently being studied as substitute for the production of podophyllotoxin and also for their role in promoting the growth of Podophyllum species under *in-vitro* conditions.

6. CONCLUSION

The study helped to identify three important enzymes for their indirect roles in biosynthesis of podophyllotoxin, besides studying the phylogenetic relationship between P.hexandrum and P.peltatum which gave an overview for the possible reason for the difference in yields of podophyllotoxin. It also helped identify closely related alternative plant species for Podophyllum for podophyllotoxin biosynthesis. The interconnected network of pathway developed, gives a comprehensive overview of the different substrates and the secondary metabolites produced. It also helps to establish factors influencing these individual pathways that may also regulate the biosynthesis of podophyllotoxin. Further *in-vitro* studies will help to confirm the results obtained.

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CONTRIBUTOR

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The current research work has been performed solely by her under the guidance of her supervisor, Dr. Hemant Sood.

Dr Hemant Sood is focused on various aspects of plant tissue culture, be it development of micropropagation technologies for different plant species, selection of genetically superior cell lines for various traits, optimisation of tissue culture protocols for use in genetic transformation, development of cell culture technologies for production of phytopharmaceuticals in high value medicinal plants.
She gave her guidance and input in analysing the results of the current work. The current study has been performed under her guidance and support.