SciMedCentral

JSM Environmental Science & Ecology

Mini Review

A Brief Outline of Next-Generation Sequencing Research on *Podophyllum* spp. – an Endangered Medicinal Herb

Soumi Biswas[1], Saptarshi Hazra[2], Sharmila Chattopadhyay[1]*

[1]*Plant Biology Laboratory, Organic and Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, India*

[2]*Molecular Biology Department, Research and Development, Nagarjuna Fertilizers and Chemicals Limited, India*

***Corresponding author**

Sharmila Chattopadhyay, Plant Biology Laboratory, Organic and Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata-700032, India, Tel: 91 33 24995868, Email: sharmila@iicb.res.in

Submitted: 04 October 2018

Accepted: 29 October 2018

Published: 31 October 2018

ISSN: 2333-7141

Keywords

- Podophyllotoxin; Transcriptome sequencing; Endangered; Secondary metabolites

Abstract

Podophyllum spp. - an endangered, perennial medicinal herb, is native to eastern North America and Himalayas. Medicinal plants are a vital source of several specialized, organic compounds called secondary metabolites. These compounds are widely used in pharmaceutical industries for their therapeutic potential. The biosynthetic pathways of these secondary metabolites are quite complex. Next-generation sequencing (NGS) has accelerated research opportunities in medicinal plants whose genome sequences are not available. In recent past, NGS technologies have generated a large number of transcriptome data in several economically important medicinal plants. NGS has also paved the way of exploring genomes of various endangered medicinal plants and this will in turn help us to develop conservation strategies to protect these valuable plants. In the present review, we have briefly discussed NGS research on various medicinal plants with special emphasis on the anticancer herb - *Podophyllum* spp. NGS methods play an important role in discovering genes that are involved in secondary metabolites biosynthesis. Identification of pathway genes is necessary for metabolic engineering of medicinal plants for the production of improved secondary metabolites.

ABBREVIATIONS

PTOX: Podophyllotoxin; **NGS:** Next-Generation Sequencing; **RNA-Seq**: RNA Sequencing; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **IUCN:** The International Union for Conservation of Nature; **miRNA:** micro Ribonucleic Acid

INTRODUCTION

Plant genomics - a recently evolved field of plant sciences, provides valuable information of structures, organizations and functions of plant genome. It also helps us to understand the molecular network of genes which are involved in the growth and development of plants. Next-generation sequencing (NGS), also known as massively parallel sequencing, can generate millions to billions of reads in a single instrument run. In comparison to Sanger sequencing [1], which is considered a first-generation DNA sequencing technology, NGS technologies has efficaciously reduced the cost and sequencing time. NGS methods have some significant advantages over the first-generation sequencing technology. First, NGS do not require arduous bacterial cloning method; here the sequencing libraries are clonally amplified *in vitro*. Second, Sanger sequencing utilizes chain termination chemistry whereas, NGS method is based on the addition of nucleotides to the complementary strand. Lastly, NGS methods perform sequencing in an immense parallel fashion. The commonly used platforms of NGS technologies are Roche 454 pyro-sequencing, Illumina, Ion Torrent, PacBio and SOLiD. Next generation RNA sequencing (RNA-seq) is a highly advanced approach to study the cellular transcriptome. RNA-seq is rapidly replacing the microarray technique because, unlike the microarray method, RNA-seq can assemble the reads *de novo* without the need of mapping to reference genome.

A brief account of high-throughput sequencing in medicinal plants

NGS has opened a new era in plant genomics by genetically improving the crop plants and identifying biosynthetic pathways of various secondary metabolites. Plants produce a variety of specialized compounds called secondary metabolites (Alkaloids, Terpenoids, and Phenolic compounds). Plant secondary metabolites are widely used as pharmaceuticals, flavourings, fragrances and industrial materials. These compounds are mostly found in medicinal plants for which genomic sequences are yet not available [2]. The *de novo* transcriptome sequencing has helped to identify the important pathway genes that are involved in the biosynthesis of secondary metabolites. *P. ginseng* is an important medicinal herb that

produces ginsenoside. Research shows that ginsenoside have anticancer, antioxidative, vasorelaxation and anti-inflammatory properties. 454 pyrosequencing of *P. ginseng* roots discovered putative genes that are involved in ginsenoside biosynthesis [3,4]. Another valuable plant is *Withania somnifera*. It synthesizes withanolides which have high medicinal value. Transcriptome sequencing using 454-GS FLX platform detected genes which are involved in withanolide biosynthetic pathway [5]. NGS has also been successfully used to unravel the roles of pathway genes in *Glycyrrhiza uralensis*, *Ginkgo biloba* and *Papaver somniferum* [6,7,8]. Illumina Miseq platform was used to sequence *Phyllanthus amarus* leaf transcriptome and the study revealed the role of various pathway genes in secondary metabolites biosynthesis [9]. Recently, deep sequencing has aided the identification of genes involved in secoiridoid biosynthesis in *Swertia mussotii*, a Tibetan medicinal plant [10].

Glimpses of NGS research in Podophyllum spp. - an endangered medicinal herb

Podophyllum species are sources of an aryl tetralin lignan called podophyllotoxin. Podophyllotoxin is effective against many diseases, and is widely used as a starting compound for the semi-synthesis of anticancer drugs i.e. etoposide (VP-16-213), teniposide (VM-26) and etopophos [11,12]. The ever-growing demand has lead to habitat destruction and overexploitation of this medicinal plant and it is currently in the IUCN endangered list. Next generation high throughput sequencing has paved the way of identifying the genes that are involved in the podophyllotoxin biosynthesis. Recently, many works has been done in order to characterize PTOX metabolic pathway. Illumina transcriptome sequencing identified two cytochrome P450 enzymes, CYP719A23 from *Podophyllum hexandrum* and CYP719A24 from *Podophyllum peltatum*. Both of these enzymes can covert matairesinol into pluviatolide, ultimately leading to podophyllotoxin biosynthesis [13]. Additional studies using multi-omics approach (transcriptomics, metabolomics and bioinformatics) aided the discovery of aporphine alkaloid pathway in *Podophyllum* species and suggested evolutionary linkages between both lignan and alkaloid biosynthetic pathways [14]. 454 pyrosequencing of *P. hexandrum* cell culture transcriptome unravelled the roles of pathways genes in PTOX biosynthesis [15]. *De novo* transcriptome sequencing of *Sinopodophyllum hexandrum* at two temperatures (15°C and 25°C) was done using Illumina platform. This study showed up-regulation of phenylpropanoid pathway genes, ABA biosynthesis, ethylene & jasmonic acid at 15°C and the genes involved in stress tolerance were expressed at 25°C [16]. Lately, six novel enzymes have been identified in *P. hexandrum*. Among these enzymes, O-methyltransferases3 (OMT3) catalyzes methylation of pluviatolide to generate (–)-5′-desmethoxy-yatein. This (–)-5′-desmethoxy-yatein is then further converted to (–)-4′-desmethylepipodophyllotoxin (the etoposide aglycone), that is the immediate precursor of etoposide [17], but the final steps for podophyllotoxin biosynthesis are yet to be characterized. Our group used 454 GS-FLX titanium pyrosequencing to identify the major transcripts of PTOX biosynthesis from MeJA treated *P. hexandrum* cell suspension culture. This study showed that PTOX biosynthetic pathway gene like PAL was minutely affected by MeJA treatment, whereas CCR, HCT, 4CL, CAD, cytochrome P450 and SAM-dependent methyltransferase were notably up-regulated in treated condition [18]. A recent transcriptomic study provides deeper understanding of the mechanism that enhances plant growth and PTOX accumulation in *S. hexandrum* under higher elevation conditions. This study identified differentially expressed genes (DEGs) involved in PTOX biosynthesis, including phenylpropanoid enzymes: phenylalanine ammonia lyase (PAL), cinnamyl alcohol-dehydrogenase (CAD), dirigent protein (DIR), pinoresinol-lariciresinol reductase (PLR), CYP719A23, CYP71CU1, and 2-oxoglutarate/Fe(II)-dependent dioxygenase (2-ODD) which directly participate in PTOX biosynthetic pathway [19].

In plants, miRNAs play pivotal roles in leaf development [20,21], anther development [22] and regulation of flowering time [23]. It also regulates secondary metabolites biosynthesis [24,25-27]. Several miRNAs has been identified in different plant species, but there are only few reports available on miRNA research in *P. hexandrum*. Our group identified conserved miRNAs and their putative target genes in *P. hexandrum*. KEGG analysis of these target genes showed that they are involved in various secondary metabolite biosynthetic pathways i.e. flavonoid, isoflavonoid, terpenoid, phenylpropanoid etc [28]. In another work from our group, we found five ROS non-responsive miRNAs that were significantly down regulated after MeJA treatment in *P. hexandrum*. These five miRNAs hindered expression of target genes, namely, 4-coumarate ligage (4CL), Cytochrome P450, Caffeoyl–CoA methyltransferase, and Chalclone synthase and the decline in the level of these miRNAs may bring about an up-regulation of the target genes [29]. In a recent work, six conserved miRNAs were identified in NGS transcriptomes of *P. hexandrum*. Detailed analysis showed that the target mRNAs viz. UDP glycosyltransferase, flavonol synthase, glyceraldehyde 3-phosphate dehydrogenase, peroxidase, malate dehydrogenase, phosphoenolpyruvate carboxylase, WRKY 37 and MYBF1 transcription factors are associated with secondary metabolites biosynthesis [30].

DISCUSSION AND CONCLUSION

In the past decade, next-generation sequencing has changed the scenario of genomic research in plants. It has significantly saved the cost and time of sequencing. Furthermore, NGS is a powerful tool to discover genes/gene families that are associated with secondary metabolite biosynthetic pathways. The transcriptomic data available for medicinal plants are much less than the data available for crop and model plants. NGS methods have helped the scientists to generate biologically important data for valuable medicinal plants. *De novo* sequencing has played a vital role in discovering pathway genes without foregoing sequence knowledge.

The anticancer property of podophyllotoxin has raised its demand worldwide, but there is a scarce in raw materials and the *in vitro* production of PTOX is extremely low to meet its industrial demands. Therefore, to increase the overall yield of podophyllotoxin, alternative ways i.e. metabolic engineering needs to be explored. Since the biosynthetic pathway of PTOX is still not fully known, genetic intervention strategy is a strenuous process. Massively parallel sequencing has been successfully applied to discover the pathway genes in PTOX biosynthesis.

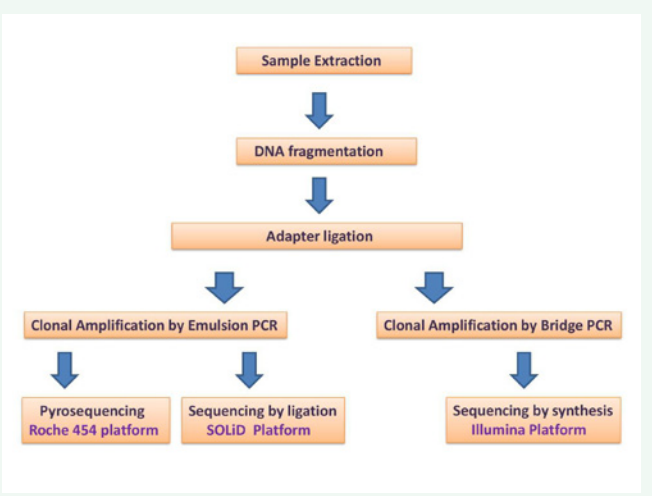

Figure 1 Workflow of Next-generation sequencing.

Figure 2 A flowering twig of *Podophyllum hexandrum* (Himalayan Mayapple) (Adapted from Bhattacharyya et al. 2012[31]). .

Till date, many works have been done to characterize the PTOX biosynthetic pathway. Our group identified potential genes and transcription factors involved in PTOX biosynthesis, thus providing valuable information for future investigation. Furthermore, we characterized PTOX specific CAD isoforms along with several candidate phenylpropanoid pathway genes which provides an insight into the molecular mechanism of PTOX biosynthesis. A recent report has also identified six novel enzymes that completes the pathway up to (–)-4′-desmethylepipodophyllotoxin, but not podophyllotoxin. Together, it can be envisaged that further in depth studies using NGS technologies would be highly beneficial to reconstitute the biosynthetic pathway up to PTOX [31].

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Director, CSIR-IICB, for providing infrastructural facilities. This work was financially supported by Council of Scientific and Industrial Research (CSIR), New Delhi. SB acknowledges the Department of Biotechnology (DBT), New Delhi for the fellowship.

REFERENCES

1. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 1977; 74: 5463–5467.
2. Xiao M, Zhang Y, Chen X, Lee EJ, Barber CJ, Chakrabarty R, et al. Transcriptome analysis based on next-generation sequencing of non-model plants producing specialized metabolites of biotechnological interest. J Biotechnol. 2013; 166: 122–134.
3. Chen S, Luo H, Li Y, Sun Y, Wu Q, Niu Y, et al. 454 EST analysis detects genes putatively involved in ginsenoside biosynthesis in *Panax ginseng*. Plant Cell Rep. 2011; 30: 1593–1601.
4. Li C, Zhu Y, Guo X, Sun C, Luo H, Song J, et al. Transcriptome analysis reveals ginsenosides biosynthetic genes, micro RNAs and simple sequence repeats in *Panax ginseng* C. A. Meyer. BMC Genomics. 2013; 14: 245.
5. Gupta P, Goel R, Agarwal AV, Asif MH, Sangwan NS, Sangwan RS, et al. Comparative transcriptome analysis of different chemotypes elucidates withanolide biosynthesis pathway from medicinal plant *Withania somnifera*. Sci Rep. 2015; 5: 18611.
6. Li Y, Luo HM, Sun C, Song JY, Sun YZ, Wu Q, et al. EST analysis reveals putative genes involved in glycyrrhizin biosynthesis. BMC Genomics. 2010; 11: 268.
7. Lin X, Zhang J, Li Y, Luo H, Wu Q, Sun C, et al. Functional genomics of a living fossil tree, *Ginkgo*, based on next-generation sequencing technology. Physiol Plant. 2011; 143: 207–218.
8. Pathak S, Lakhwani D, Gupta P, Mishra BK, Shukla S, Asif MH, et al. Comparative transcriptome analysis using high papaverine mutant of *Papaver somniferum* reveals pathway and uncharacterized steps of papaverine biosynthesis. PLoS ONE. 2013; 8: e65622.
9. Bose Mazumdar A, Chattopadhyay S. Sequencing, De novo Assembly, Functional Annotation and Analysis of *Phyllanthus amarus* Leaf Transcriptome Using the Illumina Platform. Front Plant Sci. 2016; 6: 1199.
10. Liu Y, Wang Y, Guo F, Zhan L, Mohr T, Cheng P, et al. Deep sequencing and transcriptome analyses to identify genes involved in secoiridoid biosynthesis in the Tibetan medicinal plant *Swertia mussotii*. Sci Rep. 2017; 7: 43108.
11. Stahelin HF, Wartburg AV. The chemical and biological route from podophyllotoxin glucoside to etoposide: ninth Cain Memorial award lecture. Cancer Res. 1991; 51: 5–15.
12. Clark PI, Slevin ML. The clinical pharmacology of etoposide and teniposide. Clin Pharmacokinet. 1987; 12: 223–252.
13. Marques JV, Kim KW, Lee C, Costa MA, May GD, Crow JA, et al. Next generation sequencing in predicting gene function in podophyllotoxin biosynthesis. J Biol Chem. 2013; 288: 466–479.
14. Marques JV, Dalisay DS, Yang H, Lee C, Davin LB, Lewis NG. A multi-omics strategy resolves the elusive nature of alkaloids in *Podophyllum* species. Mol Biosyst. 2014; 10: 2838-2849.
15. Bhattacharyya D, Sinha R, Hazra S, Datta R, Chattopadhyay S. De novo transcriptome analysis using 454 pyrosequencing of the Himalayan Mayapple, *Podophyllum hexandrum*. BMC Genomics. 2013; 14: 748.
16. Kumari A, Singh HR, Jha A, Swarnkar MK, Shankar R, Kumar S. Transcriptome sequencing of rhizome tissue of *Sinopodophyllum hexandrum* at two temperatures. BMC Genomics. 2014; 15: 871.
17. Lau W, Sattely ES. Six enzymes from mayapple that complete the biosynthetic pathway to the etoposide aglycone. Science. 2015; 349:

1224-1228.

18. Bhattacharyya D, Hazra S, Banerjee A, Datta R, Kumar D, Chakrabarti S, et al. Transcriptome-wide identification and characterization of CAD isoforms specific for podophyllotoxin biosynthesis from *Podophyllum hexandrum*. Plant Mol Biol. 2016; 92: 1-23.

19. Li M, Sun P, Kang T, Xing H, Yang D, Zhang J, et al. Mapping podophyllotoxin biosynthesis and growth-related transcripts with high elevation in *Sinopodophyllum hexandrum*. Ind Crops Prod. 2018; 124: 510-518.

20. Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang G, Zamore PD, Barton MK, et al. MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5' region. EMBO J. 2004; 23: 3356-3364.

21. Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, et al. Control of leaf morphogenesis by microRNAs. Nature. 2003; 425: 257–263.

22. Millar AA, Gubler F. The Arabidopsis *GAMYB*-like Genes, *MYB33* and *MYB65*, Are MicroRNA-Regulated Genes That Redundantly Facilitate Anther Development. Plant Cell. 2005; 17: 705–721.

23. Aukerman MJ, Sakai H. Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. Plant Cell. 2003; 15: 2730–2741.

24. Robert-Seilaniantz A, MacLean D, Jikumaru Y, Hill L, Yamaguchi S, Kamiya Y, et al. The microRNA miR393 re-directs secondary metabolite biosynthesis away from camalexin and towards glucosinolates. Plant J. 2011; 67: 218–231.

25. Ng DW, Zhang C, Miller M, Palmer G, Whiteley M, Tholl D, et al. *cis*- and *trans*-Regulation of miR163 and target genes confers natural variation of secondary metabolites in two *Arabidopsis* species and their allopolyploids. Plant Cell. 2011; 23: 1729-1740.

26. Boke H, Ozhuner E, Turktas M, Parmaksiz I, Ozcan S, Unver T. Regulation of the alkaloid biosynthesis by miRNA in opium poppy. Plant Biotechnol J. 2015; 13: 409–420.

27. Vashisht I, Mishra P, Pal T, Chanumolu S, Singh TR, Chauhan RS. Mining NGS transcriptomes for miRNAs and dissecting their role in regulating growth, development, and secondary metabolites production in different organs of a medicinal herb, *Picrorhiza kurroa*. Planta. 2015; 241:1255–1268.

28. Biswas S, Hazra S, Chattopadhyay S. Identification of conserved miRNAs and their putative target genes in *Podophyllum hexandrum* (Himalayan Mayapple). Plant Gene. 2016; 6: 82–89.

29. Hazra S, Bhattacharyya D, Chattopadhyay S. Methyl jasmonate regulates podophyllotoxin accumulation in *Podophyllum hexandrum* by altering the ROS-responsive podophyllotoxin pathway gene expression additionally through the down regulation of few interfering miRNAs. Front Plant Sci. 2017; 8:164.

30. Kumar P, Padhan JK, Kumar A, Chauhan RS. Transcriptomes of *Podophyllum hexandrum* unravel candidate miRNAs and their association with the biosynthesis of secondary metabolites. J Plant Biochem Biotechnol. 2018; 27: 46–54.

31. Bhattacharyya D, Sinha R, Ghanta S, Chakraborty A, Hazra S, Chattopadhyay S. Proteins differentially expressed in elicited cell suspension culture of *Podophyllum hexandrum* with enhanced podophyllotoxin content. Proteome Sci. 2012; 10: 34.

Cite this article

Biswas S, Hazra S, Chattopadhyay S (2018) A Brief Outline of Next-Generation Sequencing Research on Podophyllum spp. – an Endangered Medicinal Herb. JSM Environ Sci Ecol 6(2): 1064.