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Molecular phylogenetics as a tool for crop improvement and disease resistance: A case study of Solanaceae

Mitali Gupta & Avinash Kumar*
University Department of Botany, Vinoba Bhave University, Hazaribag, Jharkhand, India

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Abstract- The nightshade family Solanaceae is a highly diverse family encompassing several major crop species like *Solanum tuberosum*, *S. lycopersicum, S. melongena, Capsicum*, *Datura*, *Atropa*, *Withania*, *Nicotiana* etc. that are essential for human nutrition, medicine and industry but these crop species are susceptible to pests, pathogens and abiotic stresses. And continued domestication of some crops often leads to decrease in crop yields due to loss of genetic diversity. For the improvement of these crops, molecular phylogenetic analysis act as a road map for finding the genes associated with the productivity, disease resistance and valuable traits. Our present study reviewed various research applications where phylogenetic analysis help in identifying beneficial genes from wild relatives of domesticated economically important Solanaceae species, in exploring the evolutionary origin of genes associated with the biosynthesis of secondary metabolites, in finding genes in the family with similar functions and in CRISPR-CAS genome editing techniques and molecular breeding. All these approaches ultimately enhance crop breeding strategies for the development of disease and pest resistant, stress resilient and high yielding solanaceae crops.

Keywords: Solanaceae, Phylogenetic analysis, Crop improvement, Evolutionary relationships, Disease resistant, CRISPR-CAS

INTRODUCTION

The word phylogenetics comes from Greek words “Phylon” meaning tribe and “Genesis” meaning origin. It is the study of evolutionary relationship among organisms based on genetic, morphological and biochemical data.[1] Molecular phylogenetics emerged as a powerful tool that combines genomics, evolution and bioinformatics in order to improve our knowledge of biological world. Precision in phylogenetic studies and resolving specific evolutionary relationships demand the careful selection of accurate molecular markers. As different markers can yield different phylogenetic result for the same group.[2] Based on genomic origin, the genetic markers can be of three types – nuclear markers (located in nuclear genome, highly variable and biparental), chloroplast markers (located in chloroplast genome, lesser variability and mostly maternally inherited) and mitochondrial markers (located in mitochondrial genome, lowest mutation rate and uniparental). The selection of genetic markers is based on the taxonomic level of study. For resolving the evolutionary relationships at family or order level, slowly evolving markers are selected, whereas the phylogenetic study at genus or species level, rapidly evolving markers are more suitable.[3]

The Angiospermic family Solanaceae, which is commonly known as nightshade family belonging to order Solanales comprises of about 100 genera and more than 2500 species[4] which include highly diverse, agronomically,

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*Corresponding author :
Phone : 9470186567
E-mail : mitaligupta226@gmail.com,
avinashkmr2412@gmail.com

economically and ethnobotanically important plant species encompassing major crop plants like Potato (*Solanum tuberosum*), Tomato (*Solanum lycopersicum*), Eggplants (*Solanum melongena*), Chilies and Bell Peppers (*Capsicum* spp.), medicinal and psychoactive plants (*Withania somnifera, Atropa belladonia, Nicotiana tabacum, Datura stramonium*) and ornamental plant species like (*Petunia* and *Brugmansia*).[5,6] However, these plants are highly prone to biotic and abiotic stresses including the deadly bacterial wilt by *Ralstonia solanacearum*[7], range of viral[8] and fungal[9] pathogens, drought and heat stress[10], etc. and there are several wild species in this family which are not yet explored for their potential traits[11].

Molecular phylogenetic analysis is recognized as a strategy for constructing evolutionary relationships between species, in understanding the diversity, inheritance of valuable traits and evolutionary origin and source of disease and stress resistant traits, enabling the development of improved crops through hybridization and biotechnological approaches.

APPLICATIONS

Role of phylogenetics in identifying wild relatives as genetic resources-

The phylogenetic review on *Solanum* sections *Lycopersicoides*, *Juglandifolia* and *Lycopersicon* by integrating the combined result of multiple genetic markers including- AFLP (Amplified fragment length polymorphism), *GBSSI* (Granule Bound Starch Synthase I or waxy gene), chloroplast DNA including *ndhF* sequences and ITS (Internal transcribed spacer region of nuclear ribosomal DNA)[12] revealed that there are total 13 species within *Solanum* subsection *Lycopersicon,* including the cultivated tomato and the clades formed, suggested that the cultivated tomato (*S. lycopersicum*) evolved from a wild tomato species- *S. pimpinellifolium*. Further genomic analysis indicated that *S. pimpinellifolium* possesses resistance to fungal (*Phytophthora infestans*), bacterial (*Ralstonia solanacearum*) and Viral (tomato yellow leaf curl virus) pathogens.[13,14] Due to its wide spectrum of resistance, this wild variety of tomato can be used as genetic resource in different breeding programs for improving disease resistance in cultivated tomato.

Phylogeny constructed for 298 genotypes from 29 accessions representing 26 tuber bearing *Solanum* species using Ry_{sto} gene, which is known for preventing viral replication[15] gave an idea that as compared to the cultivated potatoes, 2 of the wild relatives, *S. antipovizii* and *S. hougasii* exhibited a higher range of diversity of Ry_{sto} gene sequences. Thus, giving an idea that these wild species can be applicable in crop improvement programs for potato against Potato Virus Y.

Disease resistance and pathogen evolution tracking-

With an objective of comparing the genetic difference between *S. melongena* (cultivated eggplant) and *S. torvum* (turkey berry)[16], constructed phylogenetic tree with these two and 11 other species of solanaceae using the NBS-LRR resistance genes. The analysis revealed that *S. torvum* can be used to introduce stronger resistance genes into commercial eggplants against bacterial (*Ralstonia solanacearum*) and fungal (*Verticillum dahlia*) pathogens using hybridization and genetic engineering.

Reportedly a phylogenetic analysis using *NtALS1* gene, responsible for trichome based acylsugar biosynthesis in *Nicotiana*[17] for exploring the evolutionary relatedness of *NtALS1* gene within the members of Solanaceae. *NtALS1* orthologs were identified from other solanaceae members like *Solanum lycopersicum*, *Capsicum annum*, which are responsible to provide defense against insect herbivores and some environmental stresses.

The phylogenetic structural and evolutionary analysis of nucleotide binding site leucin rich repeat (*NBS-LRR*) gene family in peppers[18] revealed that these are gene family were primarily known for their role in pathogen and insect resistance. Around 252 *NBS LRR* genes in the pepper genome were identified which were highly diverse through tandem duplications and structural variations. These divergences enable the plant's ability to respond to a broad range of pathogens. This analysis aid in the development of stronger insect or pathogen resistant pepper crops by enhancing the expression of *NBS LRR* genes through breeding and biotechnological strategies.

Molecular phylogeny guides CRISPR/CAS editing-

A phylogenetic study by integrating CRISPR/CAS editing technique explores the role of *SlHyPRP1* and *SlDEA1* genes for the growth and abiotic stress tolerance of tomato.[19] The phylogeny constructed, revealed 96% sequence similarity for *SlHyPRP1* and *SlDEA1* genes between *S. lycopersicum* (cultivated tomato) and *S. pennelli* (wild tomato). This high degree of homology between these species ensures that these two genes can be easily incorporated and edited into the gene pool of cultivated tomatoes (*S. lycopersicum*) using CRISPR-CAS 9 technique for enhancing stress resistance.

Phylogenetic insight into alkaloid and steroid biosynthesis in Solanaceae-

Phylogenetic analysis helped in understanding the evolution origin and diversification of tropane alkaloids within the family Solanaceae.[20] The analysis revealed that the genes involved in tropane alkaloids (TAs) biosynthesis originated in early ancestral solanaceae species but lost over times but retained in lineages like *Atropa belladonna* and *Datura stramonium* despite their distant phylogenetic relationship. This implies the role of gene duplication and natural selection in the evolution of tropane alkaloid genes. This knowledge would be helpful in developing pest resistance in crops and production of medicinal tropane alkaloids through selective breeding and biotechnological applications.

Another phylogenetic research using ITS region in Indonesia revealed that two species, *Physalis angulata* and *Physalis peruviana* is strongly related to *Withania somnifera* (Ashwagandha), which is responsible for withanone, a steroid chemical component having anticancerous properties. Since, Ashwaganda is not commonly found in Indonesia, *P. angulata* and *P. peruviana* can be seen as a potential source for the production of new variants of withanone with similar effect.[21]

An evolutionary insight for the biosynthesis of nicotine by sequencing and analyzing the genome of two wild tobacco *Nicotiana attenuata* and *N. obtusifolia*[22] revealed that the genes involved in nicotine biosynthesis like Putrescine N-methyltransferase (PMT) and quinolinate phosphoribosyl transferase (QPT) were originated from ancestral genes that were responsible for primary metabolic pathways through the process of duplication and divergence. This genome knowledge can be applied in developing tobacco varieties having desired level of nicotine catering both agricultural and pharmaceutical needs. Similarly, a phylogenetic work on *Nicotiana tabacum*[23] suggested that in order to meet the health and regulatory standards, low nicotine tobacco can be developed by manipulating the NIC 1 and NIC 2 loci.

Phylogenetic identification of desirable agronomic traits-

A recent work provided an evolutionary pattern and correlation of fruit colour and size in *Solanum* species.[24] Phylogenetic analysis gave an idea that the size of dull coloured fruits (like *S. betaceum, S. pimpinellifolium* and *S. capsicoides*). They also discussed the co-evolutionary relationships as dull coloured fruits are dispersed by mammals and bright fruits are dispersed by small birds. The knowledge of this potential link can be helpful for the breeders for selecting the suitable wild relatives for getting desired fruit size. The finding suggested that if the breeder aim to enhance the colour of fruit, they may need to consider the potential impact on fruit size, since the genes are correlated.

CONCLUSION

This review work gave an insight of phylogenetic approach in Solanaceae that facilitated the identification of Ry_{sto} genes in *Solanum* having antiviral properties *SlHyPRP1* and *SlDEA1* genes for their role of stress tolerance in cultivated and wild tomato, *NtALS1* genes and its orthologs responsible for biosynthesis of trichome based acyl sugar that provide resistance against insect herbivores in *Nicotina* and other members of Solanaceae, NIC 1 & NIC 2 loci for nicotine synthesis in *Nicotiana tabacum*, NBS-LRR gene families in wild eggplants & peppers for their ability of pathogen resistance. The molecular phylogenetic analysis identified an alternative source for Withanone production in *Physalis angulata* and *P. peruviana* apart from *Withania somnifera*, also revealed in understanding evolutionary relatedness of medicinal tropane alkaloid between *Atropa belladonna* and *Datura stramonium*. The phylogenetic analysis assisted the translation of evolutionary knowledge into practical solutions for crop reeding, in enhancing disease and pest resistance, stress tolerance and biosynthesis of secondary metabolites in economically important cultivated species of Solanaceae. But, despite these advantages, phylogenetic study is limitations and challenges due to incomplete genomic or proteomic data, in solving evolutionary complexities due to hybridization and in decoding the association of environmental factors in phenotypic expression of genes. So, to overcome these challenges, some future enhancement in research is necessary to maximize whole genome sequencing and phenotypic studies of specially the wild and unexplored Solanaceae species, to integrate phylogenetics with transcriptomics and epigenetics, to elevate CRISPR based genome editing and AI driven evolutionary modelling for precise and accurate breeding strategies. Additionally, some supportive policies will also be required to avoid overexploitation and preservation of wild varieties for sustainable agriculture and global food security.

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