Evolutionary Relationships and Distribution Pattern of Cytochrome P450 Enzymes Among *Melampsora* Species Using In-Silico Approaches

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Abstract

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Cytochrome P450s (CYPs) form one of the most notable protein families that are ubiquitously present in all spheres of life. They regulate fungal pathogenic virulence by neutralizing the antifungal compounds synthesized and released by hosts. This study was conducted to elucidate the diversity and abundance, evolutionary relationships and cellular localization of 224 cytochrome P450 in 7 *Melampsora* species. Eight phylogenetic groups were identified from the total number of CYPs protein sequences. A total of 14 and 13 CYPs families and clans, respectively, were recognized. Eleven cytochrome P450 families: Cyp63, Cyp5025, Cyp5139, Cyp5093, Cyp5035, Cyp53, Cyp597, Cyp526, Cyp5147, Cyp5152 and Cyp5015 were identified to be abundantly domiciled in *Melampsora,* hence, implying a vital conserved functional activity by these proteins in the *Melampsora* genus. The endoplasmic reticulum was established to be the primary location of the CYPs. The non-ribosomal peptide synthetase-like (NRPS-like), a gene cluster associated with secondary metabolic activity, was observed to be dominant across all the seven selected *Melampsora* species except in *M. allii-populina* 12AY07 v1.0 2, where polyketide synthase-like (PKS-LIKE) dominated. The proliferation of CYPs families in *Melampsora*, which is linked to the evolution of several fungal traits, including their pathogenicity, is indicated by the prevalence of several cyp clans and families. Hence, results obtained provided a solid foundation that could be explored using biotechnological tools to effectively manage *Melampsora* species causing diseases in economic tree crops worldwide.

Keywords: Cytochrome P450, in-silico, *Melampsora,* metabolites, phylogenomics.

Introduction

Melampsora spp. has several phytopathogenic members and causative agents responsible for diverse plant diseases (Nazarov *et al.,* 2020; Ratcliffe *et al.,* 2014). They are responsible for conifer-aspen leaf rust, conifer-cottonwood rust and willow rust. The infections caused by this species affect major conifers, such as the lodgepole pine, tamarack, ponderosa, western larch, douglas-fir trees and trembling aspen and poplars. They cause abundant uredinia leaf production, resulting in premature defoliation and growth reduction (Tyagi *et al.,* 2023). According to OEPP/EPPO (2009), the infection could produce trees' dieback which might eventually result into death, especially for the younger plants. A highly common disease of poplar trees called *Melampsora* rust results in significant financial losses in commercial poplar production (OEPP/EPPO, 2009). The rust disease significantly affects the production of fiber and food, as well as the flax and linseed sectors (Dean *et al.,* 2012; Moyse *et al.,* 2023). Originating in North America, *Melampsora medusae* has spread to other continents. In addition to its occurrence in the USA, Canada, and Mexico, it has spread throughout the 20th century to Europe, South America (Brazil, Bolivia, Chile), Asia (Japan), Southern part of Africa (Zimbabwe, South Africa) and Oceania (Jeger *et al.,* 2018).

The fungal pathogen can spread quickly to other regions through spore dispersal and the movement of host plants or cut branches. The varying climate is not a limitation to the pathogen's establishment (Jeger *et al.,* 2018). *M. Medusae,* which is the most prevalent and significant *Melampsora* rust in North America, causes substantial damage to conifers and *Populus* species in nurseries, plantations, and woodlands, particularly in western Canada. The fungus also causes damage in Australia and New Zealand. If aggressive isolates of *M. medusae* were introduced into the EU, it could lead to economic and environmental repercussions (Jeger *et al.,* 2018). Therefore, studies aimed at furthering the understanding the biology of this fungus is crucial to facilitate the development of strategies capable of tackling its impact on economically important plants.

Furthermore, CYP450s are present in all realms of life. It is the most well-known protein family with diverse primary and secondary metabolic functions (Bernhardt, 2006). They existed before the emergence of living forms

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that metabolize oxygen (Lewis *et al.,* 1998). The heme cofactor absorbs light at 450 nm, identifying CYPs when they oxidize various metabolic intermediates and environmental chemicals. CYPs play a significant role in numerous primary, secondary, and xenobiotic metabolic processes (Guengerich, 2008). Furthermore, CYPs play a major role in the synthesis of important metabolites in fungal pathogenesis (Siewers *et al.,* 2005). The growth and diversification of different family members of CYPs are linked to the fungal pathogenicity evolution (Soanes *et al.,* 2008). Therefore, an analysis of CYPs' functions and evolutionary history will shed more light on the functional diversity and ecological roles of various fungal taxa (Soanes *et al.,* 2007).

Several researchers have validated the roles of CYPome available in numerous fungi such as *Aspergillus nidulans* (Kelly *et al.,* 2009), *Trichoderma* spp. (Chadha *et al*., 2018), *Mycosphaerella graminicola* (Newsome *et al.,* 2013), *Fusarium* spp., (Dauda *et al.,* 2021b), *Alternaria* spp., (Dauda *et al.,* 2022a), *Aspergillus* spp., (Dauda *et al.,* 2022b), *Candida tropicalis* (Dauda *et al.,* 2022c), *cryptococcus neoformans* (Dauda *et al.,* 2022d) and *Xylaria* spp. (Dauda *et al.,* 2023) have been thoroughly elucidated. However, such information is unavailable for *Melampsora*, an important agricultural fungal group which constitutes a significant economic threat across all continents of the world (Jeger *et al.,* 2018). Therefore, this has necessitated the need to catalogue and annotate the CYPome in seven *Melampsora* species of agricultural importance using *in silico* approaches which could provide further information on their pathogenesis and management.

Materials and Methods

Selection of *Melampsora* species

Seven species of *Melampsora* were selected for this study, namely *Melampsora allii-populina* 12AY07v1.0, *M. americana* R15-033-03v1.0, *M. larici-populina* v2.0, *M. lini* CH5, *M. medusae f. sp. Clatskanie* Mmd05TRE539v1.0, *M. medusae f. sp. tremuloidae* Mmt05Ida529v1.0, *Melampsora x Columbiana Clatskanie* v1.0. The selection of the species was made in light of the reported effects of the species on important agricultural crops including cotton and conifer trees (OEPP/EPPO, 2009).

Sequence Retrieval and Screening of Sequences of CYPs

The genomes for the seven *Melampsora* species were downloaded from the MycoCosm fungal genome database. The downloaded genomes were screened for CYP450 conserved domain in the National Center for Biotechnology and Information (NCBI). The results were tabulated, and the potential protein sequences belonging to the P450 superfamily were chosen for additional examination. The EXXR and CXG hallmark motifs of the P450 family, which are present in the chosen proteins, were examined (Syed & Mashele, 2014). The proteins employed in the current investigation were thought to be genuine P450s because they displayed both motifs.

Annotation of CYP450 clans and families in seven *Melampsora* species

The validated CYP sequences of the seven *Melampsora* species were blasted in http://p450.riceblast.snu.ac.kr for homology against the sequences of other fungal species whose CYP families have been determined and deposited in Fungal Cytochrome P450 Database. The sequences for each of the queried *Melampsora* species were assigned into CYP families and clans following the criteria described by Nelson *et al.* (2006). The allocation of families into clans in the database (http://p450.riceblast.snu.ac.kr) was also used to determine clans. *Melampsora* species P450s and P450-fragments are displayed in Table 1.

Phylogenetic Analysis of cytochrome P450 sequences in *Melampsora* spp.

Molecular evolutionary genetics analysis (MEGA) software was employed to run ClustalW analysis on the P450 signature domains of the chosen fungal P450s (Kumar *et al.,* 2018). MEGA-based ClustalW has the benefit of incorporating multiple and pairwise alignment as part of ClustalW. The organization of amino acids in the P450 signature motifs (CXG and EXXR) were examined in the ClustalW-aligned P450 sequences. The ClustalW algorithm from MEGA was chosen to compute the residue of amino acids in P450 signatures, and the results were tabulated in accordance with Dauda *et al.* (2021a).

Annotation of CYP450 gene clusters associated with secondary metabolic roles

A genome-wide search was conducted using the annotation menu on the JGI online server to identify cytochrome P450s linked to gene clusters for secondary metabolism such as the PKS, NRPS, NRPS-like, PKS/NRPS, and terpenecyclase clusters. These results were compiled for the *M. x Columbiana Clatskanie* v1.0 genome*, M. medusae* f. sp. *Clatskanie* Mmd05TRE539v1.0*, M. larici-populina* v2.0*, M. allii-populina*12AY07 v1.0*, M. lini* CH5, *M. medusae* f. sp. *tremuloidae* Mmt05Ida529 v1.0 *and M. americana*R15-033-03 v1.0, respectively.

Cellular localization analysis of cytochrome P450 gene sequence in *Melampsora* spp.

The subcellular localization prediction of cytochrome P450 gene sequences in seven *Melampsora* species (*M. x Columbiana Clatskanie* v1.0 genome, *M. medusae* f. sp. *Clatskanie* Mmd05TRE539 v1.0, *M. larici-populina* v2.0, *M. allii-populina12AY07* v1.0, *M. liniCH5, M. medusae* f. sp. *Tremuloidae* Mmt05Ida529 v1.0 and M. americanaR15-033-03 v1.0) CYP450 proteins was performed using an online server. The server predicts the multiple cell localization of proteins from of eukaryotic origin using a Neural Networks at its core, algorithm relying only on sequence information and trained on Uniprot proteins (Wang *et al.,* 2021).

Results

CYP450 proteins in *Melampsora* spp.

The results obtained showed that 347 cytochrome P450 proteins were discovered in seven different *Melampsora* species. Table 1 entails the lists of the total 224 Cyp proteins

screened for the in-depth investigation, while entries with missing CYP domains were excluded for further analysis. Analysis showed that *Melampsora x Columbiana Clatskanie* v1.0 genome contains the highest number of Cyps (51), then *Melampsoraallii-populina* 12AY07 v1.0 (35), *Melampsoralarici-populina*v2.0 (30), *Melampsora medusae* f. sp. *tremuloidae* Mmt05Ida529 v1.0 (29), *Melampsora medusae* f. sp. *deltoidae* Mmd05TRE539v1.0 (29), *Melampsora americana* R15-033-03 v1.0 (28) and *Melampsora lini* CH5 (22). Nine Cyp proteins without a family match were found across the seven *Melampsora* species. Moreover, it was observed that eleven Cytochrome P450 families (Cyp63, Cyp5025, Cyp5139, Cyp5093, Cyp5035, Cyp53, Cyp597, Cyp526, Cyp5147, Cyp5152 and Cyp5015) were dominant in *Melampsora* species which indicates a conserved function for these proteins.

Plethora and diverseness of CYP450 families and clans

During this study, cytochrome P450 proteins classified into 14 families and 13 clans were detected (Table 2). The variety of annotated gene families among *Melampsora* species was evident (Table 2). There were 21 (*Melampsora lini CH5*) to 49 annotated gene families among the *Melampsora* species (*M. x Columbiana Clatskanie v1.0*). *Melampsora*'s annotated CYP clans were likewise diverse (Table 2). *Melampsora larici-populinav 2.0* (13) and *M. x columbiana Clatskanie v1.0* (13) both contained the highest proportions of CYP clans (Table 1). Clans CYP63, CYP5025, and CYP5139 had the highest protein entries (55, 31, and 28 respectively) (Table 2). Among *Melampsora* species, the most prevalent clan CYP63 contained 3 to 15 proteins (Table 2). Clan CYP560 containing 6 members was present in all the species except *M. americana R15*-033-*03* v1.0 and *Melampsora lini* CH5. Whereas, clan CYP5150 was identified in *M. x columbianaclatskanie* v1.0 and *M. larici-populina* v2.0 containing 1 member in each species, respectively, whereas in the other five species it was found absent. Clans Cyp63, Cyp5025, Cyp5139, Cyp5093, Cyp5035, Cyp53, Cyp597, Cyp526, Cyp5147, Cyp5152 and Cyp5015 proteins were common to all the seven *Melampsora* species. CYP51 protein was absent in *Melampsora lini* CH5 and was found in all other species (Table 2).

Table 1. Taxonomic spread of CYPs in seven *Melampsora* species.

***Melampsora* species**	**Size of genome (bp)**	**Number of predicted genes**	**Total Cyp proteins sequences**	**Protein with complete sequences**	**Family type**	**Clan type**	**Families with no FCPD matches**
Melampsoralarici-populina v2.0	109,877,997	19,550	54	30	14	13	1
*Melampsoraallii-populina*12AY07 v1.0	335,730,080	23,089	59	35	13	12	1
Melampsoralini CH5	189,516,653	16,335	37	22	11	10	1
Melampsora x columbiana Clatskanie v1.0	184,873,583	37,633	70	51	14	13	2
Melampsoramedusae f. sp. *tremuloidae* Mmt05Ida529 v1.0	145,187,885	22,850	43	29	13	12	2
Melampsoramedusae f. sp. *deltoidae* Mmd05TRE539 v1.0	139,726,837	20,491	42	29	13	12	1
Melampsoraamericana R15-033-03 v1.0	112,350,849	15,984	42	28	12	11	1
Total		**155,932**	**347**	**224**			

bp= base pair, FCPD= Fungal cytochrome P450 database.

Table 2. Clan and family distribution of putative CYPs in seven *Melampsora* species.

Clan	**CYP proteins in Clan**	**Families/ Species**	***M. allii-populina* 12AY07 v1.0**	***M. americana* R15-033-03 v1.0**	***M. larici-populina* v2.0**	***M. lini* CH5**	***M. medusae* f. sp. *deltoidae* Mmd05TRE539 v1.0**	***M. medusae* f. sp. *tremuloidae* Mmt05Ida529 v1.0**	***M. x columbiana Clatskanie* v1.0**	**Total**
CYP51	9	Cyp51	1	3	1	-	1	2	1	9
CYP53	9	Cyp53	2	1	1	1	1	1	2	9
CYP54	6	Cyp560	1	-	1	-	1	1	2	6
CYP63	55	Cyp63	11	7	8	4	7	3	15	55
CYP68	9	Cyp597	1	2	1	1	1	1	2	9
CYP526	9	Cyp526	1	1	1	1	2	1	2	9
CYP530	18	Cyp5093	3	2	2	3	2	2	4	18
CYP533	17	Cyp5147	1	1	1	1	1	1	3	9
		Cyp5152	1	1	1	1	1	2	1	8
CYP5014	8	Cyp5015	1	1	1	1	1	1	2	8
CYP5025	31	Cyp5025	4	4	4	3	4	5	7	31
CYP5035	14	Cyp5035	2	2	2	1	1	2	4	14
CYP5139	28	Cyp5139	5	2	4	4	5	5	3	28
CYP5150	2	Cyp5150	-	-	1	-	-	-	1	2
		Total	**35**	**28**	**30**	**22**	**29**	**29**	**51**	**224**

Phylogenetic distribution of CYP families and clans in *Melampsora* species

In 7 different species of Melampsora, the phylogenetic analysis of 224 matched Cyp protein sequences revealed the diversity and historical relationships among the cytochrome P450 groups (Figure 1). The use of P450 similarities and annotations across the complete genome in this study helps to further clarify the association between Cyp families in various *Melampsora* species. Figure 1 shows the evolutionary ties as revealed in the phylogenetic tree between the cytochrome P450 proteins of *Melampsora*. The results in Table 3 present the distribution of cyp genes into 8 phylogenetic groups, Cyp families and clans along with their presumed functions.

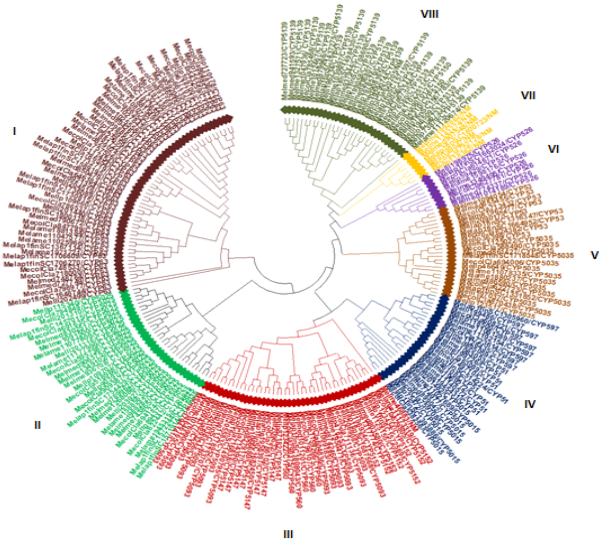

Figure 1. Phylogenetic grouping of cyp protein gene sequences from seven *Melampsora* species showing distinct classification in 8 clades.

A total of 8 phylogenetic groupings were formed from 224 cytochrome P450 proteins of 7 species of *Melampsora* spp. (Figure 1). Phylogenetic group 1 emerged as the largest group consisting of 55 Cyp proteins from clans CYP63 and predicted to engage in xenobiotic and secondary metabolism. There were 31 cyp protein genes in group 2 that belonged to the CYP5025 clan. Group 3 consisted of 42 cyp protein members belonging to clans CYP530, CYP533, CYP54 and one belonging to an unidentified clan. The cyp entries of group 3 were anticipated to play a role in secondary metabolic and xenobiotic processes. Group 4 consisted of 26 cyp protein members belonging to clans CYP5014, CYP51 and CYP68. Clan CYP68 consists of the Cyp597 family. Clan CYP5014 in FCPD comprises two Cyp families, but only one (Cyp5015) of these two families was present in *Melampsora* species. It was hypothesized that the primary role of the cyp group's members would be primary metabolism. Group 5 consisted of 23 cyp proteins from two clans (CYP5035 and CYP53) performing xenobiotic metabolism as their major role. Group 6 consisted of 9 proteins from clan CYP526 and was annotated to perform xenobiotic metabolism. Group 7 was the smallest phylogenetic group containing 7 Cyp proteins belonging to an unidentified clan. Group 8 consisted of 31 of the cyp proteins from the clans CYP5139, CYP5150 and a member belonging to an unidentified clan.

Secondary metabolism-related gene clusters and associated cytochrome P450s

This study revealed that out of 155 cytochrome P450 genes existing in the genome out of the seven *Melampsora* spp. investigated, there were 39 genes which are connected to three putative gene clusters that are involved in secondary metabolism: NRPS PKS, NRPS-LIKE, and PKS-LIKE clusters (Figure 2). In *M. americana* R15-033-03 v1.0 three genes were associated with NRPS-LIKE gene clusters, and one gene was associated with NRPS and PKS gene clusters, respectively. In the same vein, two genes were associated with NRPS-LIKE gene clusters and one gene was linked with gene clusters for NRPS and PKS, respectively in *M. lini*CH5 *and M. larici-populina*v2.0. In *M. allii-populina*12AY07 v1.0, two genes were linked with gene clusters NRPS-LIKE and PKS-LIKE and one gene was found to be associated with NRPS and PKS gene clusters, respectively. In *M. medusae f. sp. tremuloidae*Mmt05Ida529 v1.0 and *M. medusae* f. sp. *clatskanie* Mmd05TRE539 v1.0, three genes were associated with NRPS-LIKE and one gene with PKS, respectively; *M. medusae* f. sp. *tremuloidae*Mmt05Ida529 v1.0 still has one gene belonging to the PKS-LIKE gene cluster. *M. x Columbiana Clatskanie* v1.0 has the highest frequency of genes associated with NRPS-LIKE (6) and PKS (2). It also contains one gene associated with the NRPS gene cluster. NRPS-LIKE and PKS gene clusters have been identified in all seven *Melampsora* spp. These two gene clusters were found to have the highest frequency in *M. x Columbiana clatskanie*v1.0. NRPS gene clusters were found in the seven *Melampsora* spp. except for *M. medusae* f. sp. *tremuloidae* Mmt05Ida529 v1.0 and *M. medusae* f. sp. *clatskanie* Mmd05TRE539 v1.0. PKS-LIKE gene cluster was only identified in *M. allii-populina*12AY07 v1.0 and *M. medusae* f. sp. *tremuloidae*Mmt05Ida529 v1.0.

Predicted Subcellular localization across seven *Melampsora* spp.

This study revealed that the 224Cyp proteins of the seven *Melampsora* spp., were mostly found in the endoplasmic reticulum. The Cyp proteins in *M. americana* R15-033-03 v1.0 are majorly localized in the endoplasmic reticulum except for one found in the peroxisome. *M. x Columbiana Clatskanie* v1.0 and *M. lini* CH5 have the highest number of Cyp proteins localized in the cytoplasm. *M. allii-populina*12AY07 v1.0, *M. x Columbiana Clatskanie* v1.0 and *M. medusae* f. sp. *tremuloidae* Mmt05Ida529 v1.0 has one Cyp protein located in the mitochondrion. The only Cyp protein localized in the peroxisome belongs to *M. americana* R15-033-03 v1.0 species.

Table 3. Phyletic cluster of CYP450 families and clans among seven *Melampsora* species.

Phylogenetic clade	Sequence entry	CYP Families	CYP Clans	Putative functions
I	55	Cyp63	CYP63	Xenobiotic and Secondary metabolism
II	31	Cyp5025	CYP5025	
III	42	Cyp5093, Cyp5147, Cyp560, Cyp5152, No match (1)	CYP530, CYP533, CYP54, No match (1)	Xenobiotic and Secondary metabolism
IV	26	Cyp5015, Cyp51, Cyp597	CYP5014, CYP51, CYP68	Primary metabolism
V	23	Cyp5035, Cyp53,	CYP5035, CYP53,	Xenobiotic metabolism
VI	9	Cyp526	CYP526	Xenobiotic metabolism
VII	7	No match	No match	
VIII	31	Cyp5139, Cyp5150, No match (1)	CYP5139, CYP5150, No match (1)	

Discussion

The *Melampsora* CYPome from seven different *Melampsora* species, including x *Columbiana clatskanie* v1.0 genome, *M. medusae* f. sp. *clatskanie* Mmd05TRE539 v1.0, *M. larici-populina* v2.0, *M. allii-populina*12AY07 v1.0, *M. lini* CH5, *M. medusae* f. sp. *tremuloidae* Mmt05Ida529 v1.0 and *M. americana* R15-033-03 v1.0 is annotated. The phylogenetic studies demonstrated differences in size in the distribution of cytochrome P450 among the eight identified clades. The observed differences in size can be related to the varying expansions and compressions of some CYP families throughout evolution. Certain *Melampsora* species have gained some CYP clans, which helps these species endure harsh habitat environmental circumstances. This aligns with a previous report where CYP family expansion in *Alternaria* spp. was correlated to gene duplication events through evolution, allowing these organisms to adapt to and thrive in diverse habitats (Dauda *et al.,* 2022a).

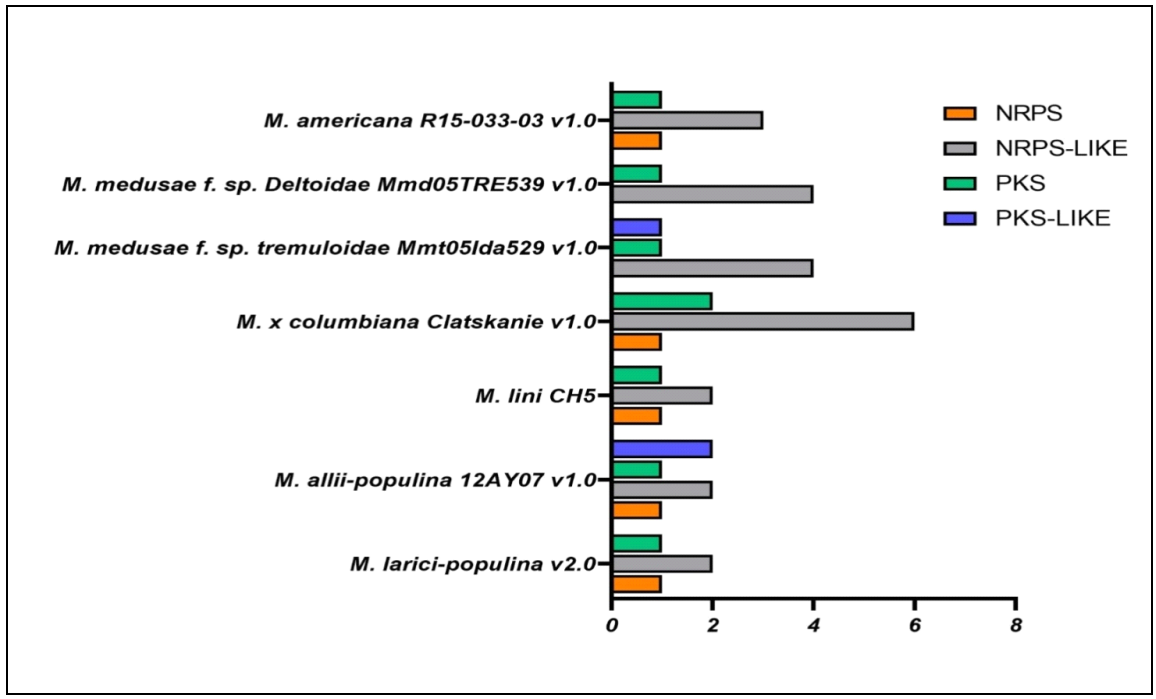

Figure 2. Spread of gene clusters associated with secondary metabolism across seven *Melampsora* species.

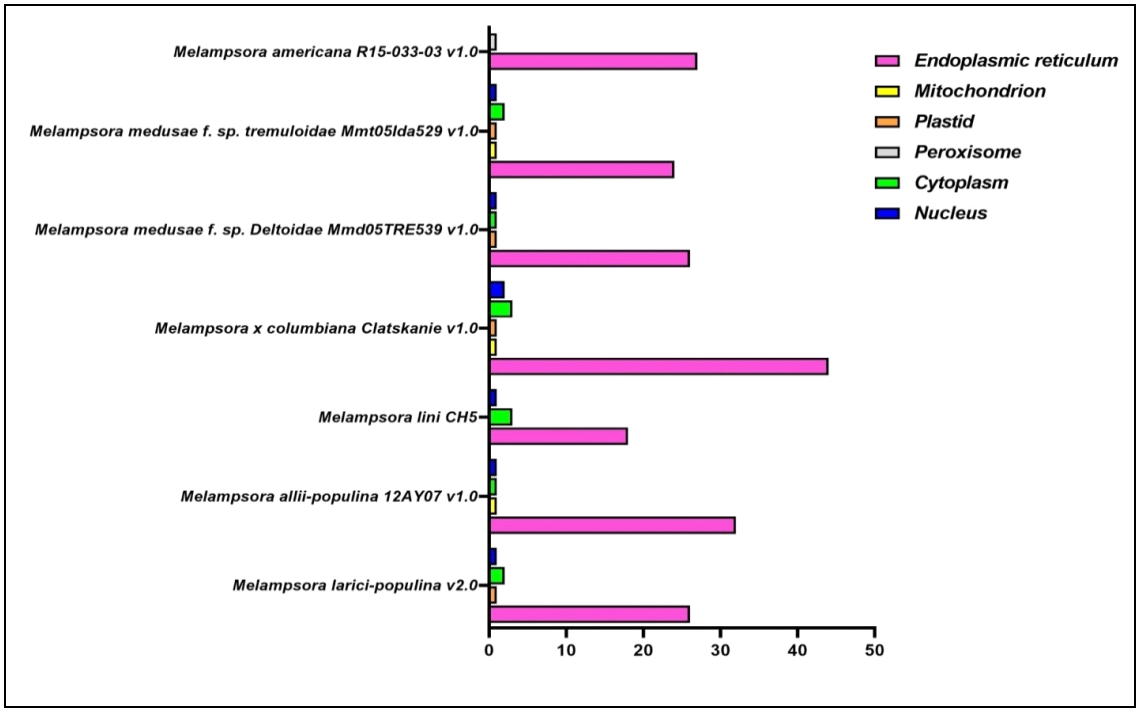

Figure 3. Predicted subcellular localization across seven *Melampsora* species.

This is also consistent with prior research by Chadha *et al*. (2018), who claimed that fungi are ubiquitous because of their ability to adapt through cytochrome P450 enzymes to various difficult ecological settings and quickly adjust to their ecological niche. As a result, these fungi are widely distributed in organically decomposing soil, living plants and animals. According to their reports, cytochrome P450 plays an important role in shielding more light on how cells function, and their role in the cells' basic metabolism (Chadha *et al.,* 2018). However, many other P450s play a vital role in xenobiotic metabolism, defense mechanisms, and secondary metabolites (Chadha *et al.,* 2018; Esteves *et al*., 2021). The innate ability of P450 to resist the antibiotics produced by the host has been linked to infections' capacity to cause disease. The cytochrome plays a significant function in the conversion of exogenous toxicants such xenobiotics, plant-derived toxins, and environmental pollutants into less dangerous ones. The proliferation of these CYPs in fungi will not be unrelated to their effective extracellular defense mechanism working along with P450-mediated approach (Minerdi *et al.,* 2020; Shin *et al.,* 2018).

Results obtained in this study showed that many CYP families of fungi have close phylogenetic connections, which indicates that gene duplications are the primary cause of the variety of CYPs in fungi, which is in support of a prior claim made by Chen *et al.* (2014) that gene duplication is the cause of the close evolutionary relationship amongst CYPs families (Chen *et al.,* 2014). The expansion of the fungus CYPs families has been connected to the evolution of many fungal characteristics, including their pathogenicity, as evidenced by the seven chosen *Melampsora* species having a large number of families and clans. The prevalence of a few distinct *Melampsora* families has also contributed to understanding the effects of the interaction between an organism's CYPs and its host and ecological niches (Qhanya *et al.,* 2015). Therefore, as previously documented by Soanes *et al.* (2008), distinct CYPs present in various *Melampsora* species could be responsible for the virulence and varying degrees of pathogenicity among different *Melampsora* species. Nelson & Strobel (1988) established that cytochrome P450 in eukaryotes are typically discovered to be attached to membranes and in most cases a short N-terminal hydrophobic region that serves as an anchor on the cytoplasmic surface of the endoplasmic reticulum (ER), however, our study demonstrated that about 95% of cyps in the seven *Melampsora* spp., were confined in the ER.

The fungal pathogenicity and the functional variation of the seven investigated *Melampsora* species could be linked to the observed growth of CYP families (Chen *et al.,* 2014; Minerdi *et al.,* 2020). Numerous diseases' pathophysiology has been linked to secondary metabolites produced by the causative agents. For instance, several unique secondary metabolites produced by *Alternaria* spp. have been linked to its virulence and pathogenicity (Dauda *et al.,* 2022a; Saha *et al.,* 2021; Soanes *et al.,* 2008; Tsuge *et al.,* 2016). There is also evidence that secondary metabolites have antibacterial, anticancer, and plant growth-promoting effects (Chadha *et al.,* 2018). Polyketides, indoleterpenes, non-ribosomal peptides, and terpenes are some secondary metabolites connected to fungi (Saha *et al.,* 2021). Methyltransferases, NRPS, P450 monooxygenases, NRPS-LIKE, reductases, DMATS, and PKS are some structural genes that contribute to the production of secondary metabolites in fungi (Saha *et al.,* 2021). The NRPS-like gene cluster, the most common among the seven *Melampsora* species for secondary metabolism, is said to be responsible for most of the secondary metabolite scaffold structure. The structural backbone enzymes such as PKS employ acyl-

CoAs to form polyketides, whereas NRPS produce non-ribosomal peptides from amino acids during the polymerisation of primary metabolites (Keller, 2019). Additionally, NRPS-like biosynthetic gene clusters have been reported to possess great potential for discovering new natural products (Shi *et al.,* 2021). Generally, over 90 *Melampsora* species have been described (Kirk *et al.,* 2001). The rust disease that damages several plants, including willow and poplar, is caused by *Melampsora* spp. (Ciszewska-Marciniak *et al.,* 2011). *Melampsora* species are responsible for the most significant popular disease in the world (Steenackers *et al.,* 1995). Cytochrome P450 has been linked with the breakdown of a broad range of chemicals, whether foreign or endogenic. P450 are actively involved in the breakdown of xenobiotics due to their capacity to enhance the oxidation of large substrates under low reaction conditions (Chadha *et al.,* 2018). They also carry out primary and secondary metabolism, making them crucial for the existence of organisms (Chadha *et al.,* 2018). According to reports, fungal CYP enzymes are essential for synthesizing several secondary metabolites that are extremely significant in agriculture, industrial, and medicinal contexts (Durairaj *et al.,* 2015). Because they mediate a crucial function in bioremediation and production of organic goods in bacteria, microbes' cytochrome P450s attracted significant attention. They serve as biocatalysts and are major targets for agricultural chemicals and pharmaceuticals (Durairaj *et al.,* 2015).

It can be concluded that the present study provided more insight into the CYPome of seven *Melampsora* species of agricultural importance using *in-silico* approaches. Two hundred and twenty-four CUP protein sequences from the seven queried *Melampsora* species clustered into eight phylogenetic clades. Thirteen clans and 14 families of CYPs were discovered. Using phylogenetic research based on comparisons with the CYPomes of other creatures, it was possible to determine the distribution of CYP families and clans in various evolutionary groups as well as their most likely roles in metabolism and biosynthesis. The endoplasmic reticulum was discovered to be the site where the CYPs were primarily located. With the exception of *M. allii-populina* 12AY07 v1.0 2, where PKS-like gene cluster was equally prevalent; the NRPS-like was the dominant gene cluster associated with secondary metabolism in all seven of the examined *Melampsora* species. Numerous families and clans are indicators of the *Melampsora* CYPs family expansions, which are connected to the evolution of numerous fungal traits, including their pathogenicity. Future biological study will be greatly aided by the understanding of the physiological and pathogenic roles of P450s in *Melampsora* species provided by the findings of P450 proteins in this pathogenic fungus.

الملخص

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تشكل إنزيمات السيتوكروم P450s أكثر العائلات البروتينية شيوعاً في نواحي الحياة المختلفة. فهي تنظم شراسة الفطور الممرضة للنبات عن طريق معادلة المركبات المضادة للقطور التي ينتجها النبات العائل. هدفت هذه الدراسة إلى معرفة التباين، الكثافة ، مدى القرابة والموقع الخلوي لـ 224 بروتين سيتوكرومي (P450s) في سبعة أنواع من الجنس *Melampsora*. أمكننا تحديد تماني مجموعات متقاربة وراثياً من مجموع بروتينات الـ CYP. أمكن التعرف على 14 عائلة و 13 تجمعاً من بروتينات السيتوكروم. تمّ تحديد 11 عائلة منها: Cyp63، Cyp5025، Cyp5139، Cyp5093، Cyp5035، Cyp53، Cyp597، Cyp526، Cyp5147، Cyp5152 و Cyp5015 والتي كانت موجودة بوفرة في الجنس *Melampsora*، مما يوحي باضطلاع هذه البروتينات بدور مهم ومحافظ في الجنس *Melampsora*. من المعروف بأن الشبكة الإندوبلازمية تشكل الموقع الأساسي لوجود بروتينات الـ CYP. لوحظ وجود شبيه إنزيم سينثتاز الببتيد غير الريبوسومي، وهو جين مرافق لنشاط الأيض الثانوي، بكثافة في الأنواع السبع من الجنس *Melampsora* ما عدا *M. allii-populina* 12AY07 v1.0 2 الذي احتوى على الإنزيم PKS-LIKE. إن نشوء عائلات البروتين CYP في الجنس *Melampsora* مرتبط بتطور عدد من الصفات الفطرية بما فيها صفة الإمراضية. لذلك فإنه من نتائج هذه الدراسة إمكانية وضع أرضية علمية صلبة قابلة للتطوير بهدف مكافحة أنواع الفطر *Melampsora* المسببة لأمراض مهمة للأشجار حول العالم.

كلمات مفتاحية: التقارب الوراثي، مركبات الأيض، بروتينات السيتوكروم، In-silico.

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