MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

DEFINING THE ANTI-FUNGAL POTENTIAL OF PLANT THAUMATIN-LIKE PROTEIN (TLP): A MINI REVIEW

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REVIEW ARTICLE

History
Received: 11th November 2020
Accepted: 24th December 2020

Keywords:

Pathogenesis-related protein, plant defense, plant-microbe interaction, host-pathogen interaction, anti-fungal property, transgenic plants

Abstract

Unrelenting threats of fungal diseases have caused huge losses to farmers around the world. Despite efforts and progress made in strengthening the conventional breeding strategies, a sustainable solution is yet to be discovered. Thus, the development of transgenic crops using anti-fungal genes such as thaumatin-like protein (TLP), a member of class 5 pathogenesis-related protein (PR-5), is deemed as a viable solution to achieve better resistance trait. However, the plant genome usually has multiple TLP copies with varying signature domains and motifs. It is possible that not all of the copies exert anti-fungal activity. In addition, the biological roles of TLPs underlying their ability to improve the plants' tolerance are still inconclusive. This mini review will discuss the unique characteristics of TLPs, which might contribute to their anti-fungal property as well as the various biological roles the proteins may play in the plants' defense mechanism against fungal infection. Several TLPs that have been proven to possess anti-fungal activity will also be discussed. Moreover, the impact of transgenic plants overexpressing TLPs in combating fungal diseases will also be elaborated via several successful transgenic researches. The information presented in this mini-review will greatly highlight the potential of TLPs as an anti-fungal agent, especially in the generation of transgenic plants with improved tolerance against fungal diseases.

INTRODUCTION

Plant defense mechanism is one of the most complex mechanisms, which involves the intricate interaction between host and pathogen. This interaction leads to activation of various defense responses such as cell wall modification, induction of pathogenesis-related (PR) proteins, production of reactive oxygen species (ROS) and secondary metabolites as well as activation of diverse signal transduction pathways [1]. Of these, big family classes of pathogenesis-related (PR) proteins are of particular interest and have been widely studied. Presently, PR proteins are classified into 17 classes which are PR1, PR2 until PR17. The classification is made based on their protein sequence similarities, enzymatic activities, molecular, biochemical, serological and other biological features [2]. Each of PR protein carries a specific role in protecting the plant. For example, PR-1 is annotated for its function against fungal infection and is also recognized as an allergen protein. PR-12, which has similar characteristics and function to the defensin gene, has been proven to possess anti-fungal properties. Other than that, chitinase, which is classified as a PR-3 family member, can degrade chitin and contribute to the production of carbon and nitrogen to the ecosystem [3,4,5]. Expression of PR proteins can also be activated by a specific signalling pathway that enables distinct recognition by defense-related signaling-molecules during pathogen induction. These include the salicylic acid (SA) pathway [6], jasmonic acid (JA) pathway [6] and ethylene (ET) pathway [7]. The activation of these pathways can be differentiated

by several factors such as type of PR protein-induced, type of plant stress, and the lifestyles of invading pathogen (biotrophs or necrotrophs) [6,8]. Different types of pathogens will activate different pathways, which lead to the accumulation of distinct PR genes [8-10]. Similar to other PR proteins, pathogenesis-related 5 protein (PR-5) also has varieties of sub-families, which include thaumatin, zeamatin, and osmotin [11,12]. TLP, in particular, has demonstrated potent anti-fungal properties. However, plants usually contain more than one copy of TLPs. Variation in the motifs and signature domains may lead to distinct protein conformation as well as different biological activities among TLP members. In this mini-review, the unique characteristics of plant TLPs that may contribute to their anti-fungal property will be discussed. In addition, potential biological roles with regards to plant defense mechanisms will be elaborated. Several plant TLPs with proven anti-fungal activity and transgenic crops successfully over-expressing TLPs for fungal tolerance will also be examined. Knowledge of the mechanisms and biological roles of these plant TLPs will greatly assist in understanding the relationship between the host and pathogen as well as shedding light on the efforts for fungal-tolerant crops.

ROLE OF PR PROTEINS IN DISEASE RESPONSE CAUSED BY FUNGI

In general, plants can be attacked by three major groups of pathogens, which are fungus, bacteria and viruses. When infecting the plants, the fungus will produce various types of hydrolytic enzymes such as pectinase, cutinase, cellulase and protease that hydrolyze the cell wall of the plants. Meanwhile, in plants, there are numerous immune strategies to defend themselves from fungal pathogen, starting from pathogen recognition, activation of defense signal pathways and production of anti-fungal compounds like PR protein, which further restrict pathogen invasion and its replication [2,13]. During infection, pathogen-associated molecular patterns (PAMPs)/microbe-associated molecular patterns (MAMPs)/Damage-/danger-associated molecular patterns (DAMPs) released by the fungus are recognized by the extracellular membrane-embedded pathogen recognition receptors (PRR) (Figure 1).

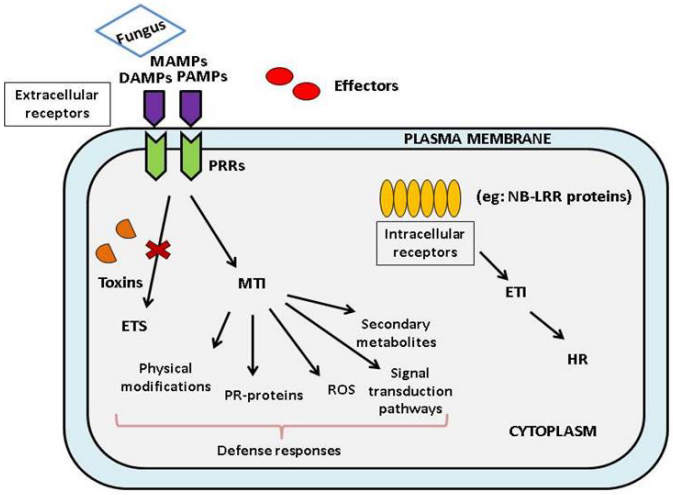

Figure 1. Elicitation of plant's defense pathways in response to fungus attack.

The recognition activates MAMP-triggered immunity (MTI), which leads to downstream defense activation responses that include physical modification of the host's cell wall, release of defense-related proteins such as PR proteins, production of ROS, various signal transduction pathways and secondary metabolites. In a successful attack, the activation of MTI can be suppressed by the pathogen's toxins/effectors leading to effector-triggered susceptibility (ETS). Inside the cytoplasm, effectors are recognized by one of the intracellular receptors (eg: NB-LRR proteins) that elicits the effector-triggered immunity (ETI), which is an upgraded version of MTI that leads to hypersensitive response (HR) at the infection site [1].

Under control conditions, the expression of PR genes is at the basal level. However, after fungal infection, the expression of PR genes drastically increases thus activating the systemic acquired resistance (SAR) pathway [14-16]. Many differential gene expression and transcriptomic studies reported the up-regulation of PR genes in a broad range of crops after being infected by a pathogenic fungus. For example, transcript levels of *PdPR5-1* in European plum (*Prunus domestica*) rapidly increased for the first three days after being infected by necrotrophic fungus *Monilinia fructicola* [17]. A transcriptomic study on apple leaves (*Malus* × *domestica* Borkh.) upon *Podosphaera leucotricha* infection demonstrated the up-regulation of

several PR genes namely PR-2 (β-1,3-glucanase), PR-10 (ribonuclease) and PR-14 (lipid-transfer protein) [18]. In addition, Taheri & Tarighi [19] reported that the expression of PR9 (peroxidase) was elevated in partially resistant (Sunny 6066) tomato (*Solanum lycopersicum*) cultivar upon the infection of *Rhizoctonia solani*. Besides, several studies exemplified the role of PR proteins in the improving the plants' resistance against pathogenic fungus. Over-expression of rice *TLP-D34* enhances the host's resistance towards sheath blight pathogen, *Rhizoctonia solani* [20] while *VpPR-10.*1 in Chinese wild grape (*Vitis pseudoreticulata*) plays a vital role in the resistance towards fungal pathogen, *Erysiphe necator* [21]. Transgenic grape over-expressing TLP, *vvtl-1* showed resistance towards different fungal pathogens such as *Uncinula necator* and *Botrytis cinerea* [22]. Various in vitro studies have discovered that PR proteins target fungal cell walls or hydrolyse them, eventually lead to cell death [8].

PATHOGENESIS-RELATED (PR) PROTEIN 5 AND THAUMATIN SUPERFAMILY

PR-5 sub-families were annotated by thaumatin family domain (IPR001938) [23]. Thaumatin family domain belongs to pathogenesis-related 5 (PR-5) superfamily (PIRSF002703) [24,25]. Despite sharing the same thaumatin family domain, all these different types of proteins have their own function. Thaumatin-like protein (TLP), for example, is a highly complex gene that is involved in the host defense mechanism [26,27]. Osmotin-like protein (OLP) is responsible for tolerance towards biotic and abiotic stresses [28] while zeamatin plays role in the inhibition of fungal activity [29]. Other proteins that belong to thaumatin family domain are PR5 kinase which is known for its kinase activity [6,30] and protein P21 which involves in cell regulation [31].

CHARACTERISTICS OF THAUMATIN-LIKE PROTEINS (TLPS) WITH ANTI-FUNGAL PROPERTY

Among all the proteins that share thaumatin family domain, only some of them are involved in plant defense mechanisms against pathogen invasion, particularly fungus [32]. One of the proteins that carry anti-fungal property is thaumatin-like protein (TLP). Thaumatin-like proteins (TLPs) can be classified into two types which are L-type and S-type. A plant species may contain both L-type and S-type TLPs. L-type TLPs consist of 16 cysteine residues that form eight disulphide bonds. L-type TLPs have three domain structures. Domain I contains 12 β-sheets while Domain II is comprised of 12 α-helical. Domain III is composed of two β-sheets and is stabilized by two disulphide bonds [33-35]. On the other hand, S-type TLPs consist of only 10 cysteine residues and lack Domain II. Several L-type TLPs namely from *Piper colubrium, Vitis vinifera, Musa acuminata* and *Oryza sativa* demonstrated anti-fungal activity [33,35]. Interestingly, some S-type TLPs such as from *Hordeum vulgare* and *Oryza sativa* also possess the same anti-fungal property [6]. Since both L-type and S-type TLPs are capable of exerting anti-fungal activity, analysis on the presence of domains alone is insufficient to predict the ability of particular TLPs to function as anti-fungal protein.

TLPs depend on their binding target to lyse the fungal cell wall through several mechanisms such as acidic cleft that was identified as specific receptor binding for anti-fungal activity [6,38]. TLPs also have binding activity to β-1,3-glucans as shown by in vitro studies [6,32]. This finding suggests that carbohydrate is a common binding target for TLPs [6,29].

Substitution, insertion or deletion of some protein residues during evolution lead to the formation of highly divergent families with different potential functions. Following this phenomenon, the ancestral gene maintains its novelty while adapting to a new function [6,12]. This could have explained why only certain TLPs can function as anti-fungal proteins. Being a part of a huge gene family, more than one *TLP* copy usually exists in the plants' genome. Wild banana (*Musa acuminata spp.* malaccensis*)*, for instance, harbors 30 copies of *TLP*s (*MaTLP*s) scattered at different chromosomes location throughout the genome. Further analysis on these MaTLPs reveals that each of them is denoted by various conserved motifs of different location, length (Figure 2a) and composition (Figure 2b).

Cleft residues of TLPs have different types of crescent fold structures, which can be electropositive, electronegative or neutral [12,36,39,40]. TLPs that carry the characteristic of anti-fungal protein such as PR5a-d in *Nicotiana tabacum,* TLP in *Brassica oleracea* and TLP in *Vitis vinifera* all have acidic cleft, which is comprised of electronegative amino acid residues, Arg-Glu-Asp-Asp-Asp (R-E-D-D-D) [7,41,42] (Figure 3).

Besides analysing the domains, signature motifs and cleft residues, plant TLPs with anti-fungal property can also be predicted using additional in silico analysis and bioinformatics tools. PROMALS3D, for example, may be used to perform structural alignment between the candidate genes and representatives of TLPs with reported anti-fungal property. PROMALS3D which offers both sequence- and structure-based alignment is generally more informative in the functional prediction of proteins as compared to sequence-based alignments only [43]. In a study conducted by Neeharika and Sunkar [44], PROMALS3D was used as one of the tools to predict 13 putative allergens from other Cucurbitaceae family members by comparing their structure similarity with four reported allergens, which were Cuc m 1, Cuc m 2 and Cuc m 3 found in *Cucumis melo* (Muskmelon) and Citr I 2 from *Citrullus lanatus* (watermelon). Furthermore, phylogenetics tree can also be constructed to examine the potential orthologous

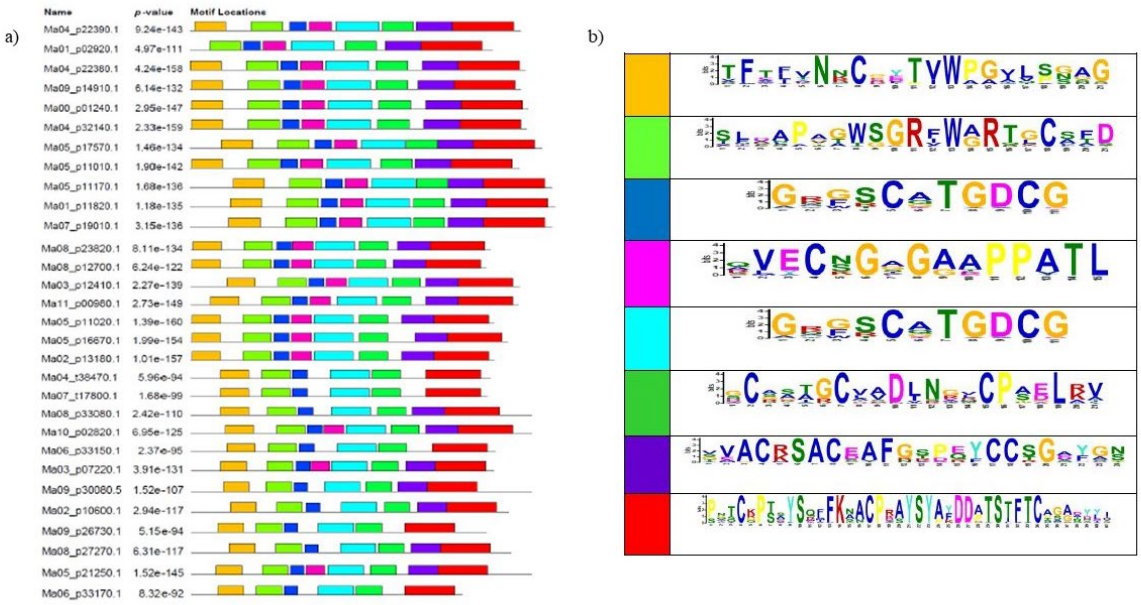

Figure 2. Analysis of conserved amino acids sequences of TLPs from wild banana (*Musa acuminata spp.* malaccensis) (MaTLPs) determined using MEME 5.1.1. a) Different motif locations of MaTLPs sharing thaumatin_2 family profile domain (PS51367) indicated using colored box; b) Composition of conserved amino acid sequences denoted by each colored box.

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PRHv-1          --------MSTSAVLFLLLAVFAAGASAATFNIKNNCGSTIWPAGIPVGGGFELGSGQTS      52
PWIR2           --------MATSPVLFLLLAVFAAGASAATFNIKNNCGFTIWPAGIPVGGGFALGSGQTS      52
pPIR2           --MASPATSSAVLVVVLVATLAAGGANAATFTITNRCSFTVWPAATPVGGGVQLSPGQTW      58
ObTLP1          --MNSLKFIS---TLLPFLLLLLHAATAIRFDIQNKCSYTIWPAVLPHGGGRRLDSGQTW      55
Ban-TLP         ----------------------------ATFEIVNRCSYTVWAAAVP-GGGRQLNQGQSW      31
OLPVv           MGLCKILSIS---SFLLT-TLFFTSSYAATFNIQNHCSYTVWAAAVP-GGGMQLGSGQSW      55
                                             : : *.*  *:* *  * ***  *  * :

PRHv-1          SINVPAGTQAGRIWARTGCSFNGG-SGSCQTGDCGGQLSCSLSGQPPATLAEFTIGGGST      111
PWIR2           SINVPAGTQAGRIWARTGCSFNGG-SGSCQTGDCGGQLSCSLSGRPPATLAEYTIGGGST      111
pPIR2           TINVPAGTSSGRVWGRTGCSFDGSGRGSCATGDCAGALSCTLSGQKPLTLAEFTIGG--S      116
ObTLP1          TLSFQNGPKLAKVWARTNCTFDSSGKGKCLTGDCGGQLNCTTFGSPPHTKAEYGLNDFGR      115
Ban-TLP         TINVNAGTTGGRIWGRTGCSFDGSGRGRCQTGDCGGVLSCTAYGNPPNTLAEFALNQFNN      91
OLPVv           SLNVNAGTTGARVWGRTNCNFDASGNGKCETGDCGGLLQCTAYGTPPNTLAEFALNQFSN      115
                 .    *   .::*.**.*.*:.   * * ****.* * *.  *  * * **: :.

PRHv-1          QDFYDISVIDGFNLAMDFSCSTGD-----ALQCRD------------------------      141
PWIR2           QDFYDISVIDGFNLAMDFSCSTGD-----ALQCRD------------------------      141
pPIR2           QDFYDLSVIDGYNVAMSFSCSSGV-----TVTCRD------------------------      146
ObTLP1          KDYYDVSVLDGYNLPMEMTPTTNG--CTRSVKCAAEDIVANCPSQLKVDGGCQNPCTVFK      173
Ban-TLP         LDFFDISLVDGFNVPMDFSPTSGG--C-RGIRCAA-DINGQCPGALKAPGGCNNPCTVFK      147
OLPVv           LDFFDISLVDGFNVPMAFNPTSNG--CTRGISCcTA-DIVGECPAALKTTGGCNNPCTVFK      172
                 *::*:*::**:*: * :  :.        : *

PRHv-1          ----------------------PSCPPPQAYQHPNDD--VATHACSGN-NNYQITFCP--      173
PWIR2           ----------------------PSCPPPQAYQHPNDD--VATHACSGN-NNYQITFCP--      173
pPIR2           ----------------------SRCPD--AYLFPEDN-TKTHACSGN-SNYQVVFCP--      177
ObTLP1          TTEYCCHA--GECRPTDMSRFFKSRCRD--AFTYPQDDPTSTFTCPES-TSYRVVFCP--      226
Ban-TLP         TDQYCCNS--GACSPTDYSQFFKRNCPD--AYSYPKDDQTTTFTCPGG-TNYRVVFCP--      200
OLPVv           TDEYCCNS--GSCNATTYSEFFKTRCPD--AYSYPKDDQTSTFTCPAG-TNYEVIFCP--      225
                                *    *:  * *    *.:*    ..*.: ***
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Figure 3. Amino acid sequence alignment of six TLPs with proven anti-fungal property. These includes PRHv-1 isolated from *Hordeum vulgare* (CAA4144.1), PWIR2 from *Triticum aestivum* (CAA41283.1), pPIR2 from *Oryza sativa* Japonica group (X68197.1), ObTLP1 from *Ocimum basicilum* (JQ793640), Ban-TLP from *Musa acuminata* (pdb ID : 1Z3Q) and OLPVv from *Vitis vinifera* (Y10992). The TLP family signature in thaumatin (Prosite:PS00316; PFam: PF00314), G-x-[GF]-x-C-x-T-[GA]-D-C-x(1,2)-[GQ]-x(2,3)-C is underlined. The conserved residues are highlighted in yellow while the acidic amino acid residues that represent the electronegative cleft residues are highlighted in purple. Residues in bold indicate the starting sequence for mature protein.

relationship and evolutionary relatedness which could also contribute to proteins' functional prediction. Following the previous example on the identification of putative allergens from Cucurbitaceae family members, Neeharika and Sunkar [44] concluded that the candidate allergens may have functional association with the reported allergens based on their phylogenetics distance and clustering. Utilizing available fully sequenced genomes of all free-living organisms, Gregoretti, Lee and Goodson [45] performed phylogenetic analysis of all histone deacetylases (HDACs)-related proteins to improve the functional annotation of these proteins. A number of tree-based tools have also been developed to assist in finding corresponding orthologs as well as predicting the functional annotation of uncharacterized proteins. The examples of those tools have been reviewed by Kuzniar [46].

BIOLOGICAL ACTIVITIES OF TLPS

When it comes to molecular mechanisms, many possibilities require extensive researches as TLPs are diverse in their functional motifs, domains, functions and possibly their molecular phylogenetic as well as evolution [12]. Studies have shown that TLPs might be involved in β-1,3-glucan binding and degradation [25,32,47]. In the study of TLP from *Musa acuminata spp.* (banana), *Malus domestica* (apple) and *Prunus avium* (cherry), banana TLPs showed inhibition towards hyphal growth but recorded no activity of β-1,3-glucanase. In contrast, apple TLP and cherry TLP demonstrated β-1,3-glucanase activity but no inhibition towards fungal growth [39]. Thus, the association between β-1,3-glucanase and anti-fungal activity for TLPs from these three plant species cannot be concluded.

In another study, Grenier et. al. [48] analysed the β-1,3-glucanase activity of 13 different plant TLPs based on the enzyme activity on carboxymethyl (CM)-pachyman. A total of 6 TLPs exhibited hydrolytic activity, which were barley IFW19, tomato AP24 and NP24, cherry CHTL as well as tobacco SE22 and SE39b. Of these, tobacco SE22, tobacco SE39b and cherry CHTL showed active hydrolysis activity in CH-Pachyman solution while less active activity was demonstrated by tomato AP24, tomato NP24 and barley IFW19. Accordingly, cherry CHTL, tobacco SE22 and tobacco SE39b showed lysis effect on the complex fungal β-1,3-glucan as demonstrated by the decreased turbidity of *Saccharomyces cerevisiae* crude wall suspension.

Xylanase (Endo-β-1,4-xyanase, EC 3.2.1.8) is necessary for some filamentous fungus such as *Botrytis cinerea* to infect plants [49] as this enzyme can produce higher levels of extracellular enzymes [50]. Xylanase has a linear backbone of β-D-1,4–linked xylopyranoside that will be substituted with glucuronosyl, acetyl and arabinosyl side chains depending on the type of plants [51,52]. Fierens et. al. [53] discovered that wheat (*Triticum aestivum)* has a novel type of xylanase inhibitor (TLX1) that was categorized under TLP family. Wheat TLX1 showed inhibitory activity against *Trichoderma longibrachiatum* xylanase [53].

Zeamatin that was isolated from *Zea Mays* demonstrated anti-fungal activity towards several types of fungus and induced ruptured of fungus cell wall [54]. Similar activities were also observed in TLP of oat [37] and wheat [53]. Osmotin is involved in the membrane destruction of fungal hyphae [55]. The presence of this protein will increase permeability of the fungal plasma membrane to protons such as Ca^{2+} and the inability to sustain its pH gradient [27,32,56]. TLP that was isolated from Basrai Banana showed high binding attraction towards the plasma membrane ergosterol of *Aspergillus fumigatus*. It also has the potential to induce destruction in the cell membrane of pathogenic fungus [57].

DETERMINATION OF ANTI-FUNGAL PROPERTY OF PLANT TLPS

Anti-fungal property of plant TLPs is commonly determined using anti-fungal assay and hyphal inhibition assay (Table 1). Prior to the analysis, the gene can be purified as recombinant protein from selected expression systems (eg: bacteria, yeast, plant system including transgenic plants) or directly extracted from the tissue of interest. İn the production of recombinant TLP using bacterial expression system, the open reading frame of *Ocimum basilicum* TLP (ObTLP1) was cloned into pET-28a(+) vector and expressed in *Escherichia coli* (*E. coli*) BL21-CodonPlus (DE3)-RIPL. The recombinant protein was then purified and showed positive fungal inhibition against *Scleretonia sclerotiorum* and *Botrytis cinerea* [25]. Similarly, Sun et al. [58] expressed TLP from poplar cultivar (*Populus deltoides* × *P. Euramericana* 'Nanlin895') (*Pe-TLP*) in *E. coli* and used the recombinant protein for anti-fungal assay against Mucor sp. In the same study, total protein extracted from leaf tissues of transgenic poplar over-expressing *Pe-TLP* was used in the anti-fungal experiment. In comparison with the purified recombinant protein and total protein extracted from infected and non-infected wild poplar, only the total protein of transgenic poplar showed a significant zone of inhibition against the pathogen. However, a direct relationship between anti-fungal acitvity and Pe-TLP expression cannot be established due to inconsistent results obtained from other analyses (level of protein expression and transcript expression in transgenic poplar, non-infected wild poplar and infected wild poplar) [58]. In the study conducted by Wang et al. [59], the anti-fungal activity of extracted proteins from *Cynanchum komarovii* seeds was first tested against *Valsa mali* before the protein obtained from one of the fractions was identified as TLP (CkTLP). The inhibitory effect of plant TLPs is possibly pathogen-specific. French bean TLPs (*Phaseolus vulgaris* cv Kentucky), for instance, exhibited potent inhibitory effect against *Fusarium oxysporum*, *Pleurotus ostreatus*, and *Coprinus comatus* but not against *Rhizoctonia solani*. Though it is interesting to note that this French bean TLP lacks an N-terminal of 20 or

more residues in comparison to other TLPs, osmotins and osmotin-like proteins, the analysis on the sequences alone is deemed insufficient to explain the anti-fungal selectivity property of the protein [60].

Table 1. Proven anti-fungal TLP by anti-fungal assay (analysis zone of inhibition)

Name of protein	Sources of plant	Fungi induced	Result (Inhibition, +) (no inhibiton, -)	References
Zlp	*Zea Mays*	*Candida albicans*	+	[29]
ZLP	*Zea mays*	*Aspergillus niger*	+	[62]
ObTLP	*Ocimum basilicum*	*Sclerotinia sclerotiorum* and *Botrytis cyneria*	+	[25]
Pe-TLP	*Populus deltoides* × *P. euramericana* 'Nanlin895' (poplar)	*Mucor spp.*	+	[58]
CkTLP	*Cynanchum komarovii*	*Valsa mali*	+	[59]
rMal d 2	*Malus domestica*	*Fusarium oxysporum* and *Penicillim expansum*	+	[63]
SN-TLPf	*Sambucus nigra* L.	*Fusarium culmorum*	-	[64]
BanTLP	*Musa acuminata*	*Verticilium albo-artrum*	+	[39]
Castanopsis TLP	*Castanopsis chinensis*	*Fusarium oxysporum*	+	[65]
CkTLP	*Cynanchum komarovii*	*Verticum dahliae, Fusarium oxysporum, Rhizoctonia solani and Botrytis cineria*	+	[59]
French bean TLP	*Phaseolus vulgasris* cv. *Kentucky wonder*	*Pleurotus ostreatus, Fusarium oxysporum and Coprinus comatus*	+	[60]
		Rhizotonia solani	-	
Basrai TLP	*Basrai banana*	*Aspergillus fumigatus*	+	[57]

DEVELOPMENT OF TRANSGENIC PLANTS OVER-EXPRESSING PLANT TLPS FOR IMPROVED ANTI-FUNGAL TRAIT

Several studies reported that over-expression of TLP in plants leads to improved tolerance against various fungal pathogens (Table 2). Ojola et al. [61] tested the anti-fungal activity of transgenic cassava (*Manihot esculenta* Crantz) overexpressing rice TLP (*OsTLP*) against *Colletotrichum gloeosporioides*. Using leaves and stem cutting bioassays, severe necrotic symptoms were observed in the non-transgenic control as early as 2 days post-inoculation. In contrast, transgenic cassava displayed delayed disease symptoms which appeared 6 days-post-inoculation [61].

After confirming the anti-fungal activity of *Ocimum basilicum* TLP (ObTLP1) against necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea,* Misra et al. [25] further challenged the transgenic *Arabidopsis thaliana* over-expressing *ObTLP* against both pathogens. Constitutive expression of *ObTLP* slowed down the necrotic lesion progression on the leaves of T5 homozygous transgenic lines, TH1 and TH2 following *Sclerotinia sclerotiorum* and *Botrytis cinerea* inoculation [25].

Similarly, Yan et al. [7] integrated another TLP gene, *TLP29*, from wild grape *Vitis quinquangularis* cv. 'Shang-24' (*VqRLP29*) in *Arabidopsis thaliana*. A significantly lower amount of spores were eluted from the transgenic lines 7-days after the plants were infected with *Golovinomyces cichoracearum* UCSC1 phytopathogen causing powdery mildew. In contrast to the study conducted by Misra et al. [25], overexpression of *VqRLP29* seems to weaken the plant's defense ability against *Botrytis cinerea.* More severe

lesions were observed in the leaves of transgenic lines compared to that of wild type control plants.

Taken together, the constitutive expression of plant *TLP*s enhances the plants' tolerance against phytopathogens. However, this positive interaction could be gene-, host- and pathogen-specific. In this case, bioassay experiments or disease severity scoring should be conducted to analyse the true potential of a certain TLP copy.

Table 2. Transgenic research over-expressing plant TLPs for improved tolerance against fungal infection.

Name of Gene	Sources	Transgenic plant	Response against fungal pathogen	References
Tlp D34	*Oryza sativa* (rice)	*Nicotiana tabacum* (tobacco)	Resistance towards *Alternaria alternata*	[32]
tlp	*Oryza sativa* (rice)	*Triticum aestivum* cv. 'Bobwhite'	Resistance towards *Fusarium graminearum* Schw.	[66]
Tlp D34	*Oryza sativa* (rice)	*Oryza sativa* L. (rice)	Resistance towards *Rhizoctonia solani*	[32]
PR5K	*Arabidopsis thaliana*	Creeping bentgrass cv. Crenshaw	Resistance towards *Sclerotinia homeocarpa*	[67]
tlp	*Oryza sativa (rice)*	*Brassica napus* (Canola)	Resistance towards *Sclerotinia sclerotiorum*	[68]
VaTLP	Wild *Vitis amurensis* Rupr	*V. vinifera 'Thompson Seedlees'*	Improved resistance against *Plasmopara viticola*	[69]
GbTLP1	*Gossypium barbadense* L.)	*Nicotiana tabacum* L. (tobacco)	Improved resistance against *Verticillium dahliae* and *Fusarium oxysporum*	[70]
Ostlp	*Oryza sativa* (rice)	*Manihot esculenta* Crantz (cassava)	Enhanced tolerance againts *Colletotrichum gloeosporioides* f. sp. manihotis	[61]
PeTLP	*Populus deltoides* × *P. euramericana* 'Nanlin895' (poplar)	*Populus deltoides* × *P. euramericana* 'Nanlin895' (poplar)	Enhanced resistance against *Marssonina brunnea*	[58]
AdTLP	*Arachis diogoi* (wild peanut)	*Nicotiana tabacum* var Xanthi	Enhanced resistance to *Rhizoctonia solani*	[71]
PpTLP	*Pyrus pyrifolia* Nakai cv Huobali	*Nicotiana tabacum* L. cv Xanthi	Different degree of inhibition was observed when the crude protein extract from the transgenic tobacco was tested againts *Sclerotinia sclerotiorum*, *Phomopsis* sp., *Phytophthora parasitica* var. nicotianae, and *Alternaria* sp. In different degrees.	[27]
VqTLP29	*Vitis quinquangularis* cv. 'Shang-24' (wild grape)	*Arabidopsis thaliana*	Enhanced resistance to *Golovinomyces cichoracearum* UCSC1 but decreased resistance to *Botrytis cinerea*	[7]
ObTLP1	*Ocimum basilicum*	*Arabidopsis thaliana*	Enhanced tolerance to *Sclerotinia sclerotiorum* and *Botrytis cinerea*	[25]

CONCLUSION

Plant TLPs have great potential to be promoted as an anti-fungal agent and improves the plant breeding landscape concerning fungal infection. Various bioinformatics tools can be employed to narrow down the TLP candidates with true anti-fungal potential. Utilization of these in silico analysis aided by structural, phylogenetics, molecular, anti-

fungal and bioassay analysis allow wise candidate selection for bio-control applications in agriculture.

ACKNOWLEDGMENT

This work was supported by the Fundamental Research Grant Scheme (FRGS) (FRGS/1/2019/STG05/UPM/02/8) financed by the Ministry of Education Malaysia.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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