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Original article

Amplification, sequencing and characterization of pectin methyl esterase inhibitor 51 gene in *Tectona grandis* L.f.

Nuzhat Bano [a], Shamim Akhtar Ansari [a], Abeer Hashem [b,c], Elsayed Fathi Abd_Allah [d], Mohammad Israil Ansari [e,*]

[a] Institute of Forest Productivity, PO; Lalgutwa, NH-23, Ranchi 835303, India
[b] Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
[c] Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt
[d] Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
[e] Department of Botany, University of Lucknow, Lucknow 226 007, India

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ABSTRACT

Tectona grandis L.f. (Teak), a very important source of incomparable timber, withstands a wide range of tropical deciduous conditions. We achieved partial amplification of pectin methylesterase inhibitor 51 (*PMEI*) gene in teak by *E. pilularis* cinnamoyl Co-A reductase (*CCR*) gene specific primer. The amplified teak gene was of 750 bp, 79% identity and 97% query cover with *PMEI* of *Sesamum indicum*. The phylogenetic tree clustered the amplified gene with *PMEI* of database plant species, *Erythranthe guttata* and *Sesamum indicum* (87% bootstrap value). On conversion to amino acid sequence, the obtained protein comprised 237 amino acids. However, PMEI region spanned from 24 to 171 amino acids, 15.94 kDa molecular weight, 8.97 pI value and $C_{697}H_{1117}N_{199}O_{211}S_9$ molecular formula with four conserved cysteine residues as disulfide bridges. 25.9 % protein residues were hydrophilic, 42.7% hydrophobic and 31.2% neutral. Teak 3D PMEI protein structure corresponded well with *Arabidopsis thaliana* and *Actinidia deliciosa* PMEIs. The gene maintains integrity of pectin component of middle lamella of primary cell wall and confers tolerance against various kinds of stresses. Teak conferred with overexpression of *PMEI* may secure a wide adaptability as well as luxuriant timber productivity and quality in adverse/ fluctuating/ scarce climatic and environmental conditions of tropical forests.

1. Introduction

Tectona grandis L.f. (Teak); Family -Lamiaceae) is a paragon tropical forest tree, providing one of the best quality timbers of the world (Gill et al., 1983). The demand for its quality timber is unsurmountable, for the wood is used to make high-grade furniture, plywood, boat decks, indoor flooring, door, decorative and various wood-based products (Williams et al., 2001). The high demand for teak timber is attributed to its wood strength, durability, dimensional stability, beautiful grains and ray pattern and golden colour. The timber also exhibits comparatively high resistance to rot and decay (Sumthong et al., 2008); possesses antifungal (Rachana et al., 2009) and anti-termite (Dungani et al., 2012) properties. However, the species has received very little attention related to amplification and characterization of genes involved in biosynthesis of cell wall components, particularly pectin and lignin, conferring resistance to pest and abiotic stress and strength to the timber. To some extent, teak genes involved in lignin biosynthesis, viz., CAD family of genes (Galeano et al., 2018), MYB family of transcription factors (Galeano et al., 2015), genome-wide NAC gene family of transcription factors (Hurtado et al., 2019) have been investigated. Teak draft genome has been published only recently (Yasodha et al., 2018).

Forest trees in their native range experience various biotic and abiotic stresses which influence their persistence and productivity (Hurley et al., 2016; Naidoo et al., 2019). In recent years, forests have been exposed to an exponential rise in pests and pathogen populations combined with the threat of climate change which

* Corresponding author.
E-mail addresses: shamimansari_1@yahoo.co.uk (S.A. Ansari), habeer@ksu.edu.sa (A. Hashem), eabdallah@ksu.edu.sa (E.F. Abd_Allah), ansari_mi@lkouniv.ac.in (M.I. Ansari).
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makes forest plantation vulnerable to pest attack and curtailment in productivity (Santini et al., 2013; Wingfield et al., 2015; Hurley et al., 2016). Genotypes that are resistant to various stress threats are expected to minimize losses in forestry plantation (Naidoo et al., 2019). Plant cell wall is the frontier physical barrier where plant–microbe and environment interaction occur. Cell wall is the rigid outermost layer of plant cells, serving as a shield against the biotic and abiotic stresses (McCann and Roberts, 1991). Of these, the outermost primary cell wall is made up of cellulose, hemicellulose and pectin (Juge, 2006; Willats et al., 2001). The cellulose sheet and hemicellulose component combine to forms cellulose-hemicellulose network that is embedded in the pectin matrix (McCann and Roberts, 1991). The pectin amount in the primary cell wall is estimated to be 35% in dicots and monocots, except family-Poaceae, 2–10% in grasses and 5% in wood tissues (O'Neill et al., 1990; Ridley et al., 2001) and 50% in leaves of *Arabidopsis* (Zablackis et al., 1995).

Pectin is a highly heterogeneous group of polymers made up of family of galacturonic acid viz., homogalacturonan (HG), rhamnogalacturonan 1 (RG1), rhamnogalacturonan II (RGII), and xylogalacturonan (XG). Pectin contains 65% HG, 20–35% RGI and < 10% RGII and XG each (Mohnen, 2008; Zandleven et al., 2007). The primary wall of the plant cell is enriched with pectin, which is highly methyl esterified to provide mechanical strength to plants (Willats et al., 2001). Pectin synthesis occurs in Golgi bodies where about 80% of homogalacturonan residues are methyl esterified. Subsequently, the highly methyl esterified pectin are transported to the cell wall through exocytosis (Powell and Brew, 1974; Goubet and Mohnen, 1999; Sterling et al., 2001; Nunan and Scheller, 2003; Geshi et al. 2004) that confers resistance against the pathogenic attack (Herron et al. 2000, Wydra and Beri, 2006; Lionetti et al., 2012).

Pectin methylesterase (PMEs) is a cell wall-associated enzyme that catalyzes the de-methyl esterification of HG molecule (Pelloux et al., 2007). During plant development, the highly methyl esterified galacturonic acid molecule of pectin is de-esterified by PMEs (Femenia et al., 1998; Micheli, 2001). The pattern of de-methyl esterification in HG residues determines the biochemical properties of the cell wall. If methyl ester group is removed randomly from the HG residues, the random de-methyl esterified HG molecules become a substrate for pectin degrading enzymes (pectinase, pectin lyases, polygalacturonase) that cause loosening of cell wall or cell wall metabolism (Johansson et al., 2002). However, when methyl ester group is removed non-randomly from the HG residues (block-wise de-methyl esterification), it creates free carboxyl group that interacts with other HG residues via the formation of Ca^{++} bond. The formation of Ca^{++} bond facilitates the formation of pectin gels that provides rigidity to the cell wall (Ha et al., 1997).

Pectin methylesterase inhibitor (PMEI) protein regulates PME activity. PMEI covalently binds with pectin methylesterase (PME) and forms a 1:1 ratio of enzyme: protein complex that inhibits the activity of PMEs (Giovane et al., 1995; Camardella et al., 2000). Thus, PMEI plays a vital role in pectin metabolism and influences the porosity of plant cell walls (Juge, 2006; Juge and Svensson, 2006). Various investigations have revealed that PMEI provides resistance against the viral, fungal and bacterial attack to the host plant (Lionetti et al., 2017; Liu et al., 2018). During infection, a pathogen promotes host cell PME activity that elicits de-methyl esterification of HG molecules and exposes them to pectin degrading enzyme produced by the pathogens. As a result, the cell wall integrity is lost, making plant susceptible to the infection (Lionetti et al., 2007, 2017).

We partially amplified and characterized pectin methylesterase inhibitor 51 (*PMEI*) gene in teak, employing specific primer for cinnamoyl Co-A reductase (*CCR*) gene developed for *Eucalyptus pilularis* by Sexton et al. (2010). To the best of our knowledge, this the first report in the literature exhibiting amplification of a different gene, i.e. teak *PMEI* in a phylogenetically highly divergent Family-Lamiaceae (Clade: Asterids, Order : Lamiales), employing *CCR* gene specific primer of *Eucalyptus pilularis* of Family-Myrtaceae (Clade: Rosids, Order: Myrtales) (Zeng et al., 2017). The present investigation reports phylogenetic relationship of teak *PMEI* gene with *PMEI* and *CCR* genes of other plant species available in the database. Besides, teak *PMEI* nucleotide sequences have been converted into amino acid sequence and constructed 3D PMEI protein structure with validation using Ramachandran plot. The prospective role of *PMEI* for conferring abiotic/biotic stress tolerance in teak has also been discussed.

2. Materials and methods

2.1. Genomic DNA extraction and primer for PMEI gene amplification

The CTAB (cetyl trimethylammonium bromide) method of Narayanan et al. (2006) was employed to extract and purify genomic DNA from freshly collected leaves of four ramets each of three teak (*Tectona grandis* L.f.) genotypes (APKEA-2, APT-6 and APT-8) belonging to the state of Andhra Pradesh and maintained at National Germplasm Bank of Teak, Chandrapur, Maharashtra, India. *Eucalyptus pilularis* CCR gene specific primer pair, i.e. 5′GGCGGCTTCATCGCCTCC3′ (forward) and 5′AACCCCTCTCTCCTTCGCC3′ (reverse) developed by Sexton et al. (2010) was employed to partially amplify *PMEI* gene in three teak genotypes (APKEA-2, APT-6 and APT-8) in four replicates (ramets).

2.2. Gradient PCR and basic PCR

A 20 μl reaction mixture was prepared using 2x master mix (Fermentas®, #K0171), 50 ng of DNA and 0.5μM of CCR gene specific primer pair (Xcelris genomics). The reaction mixture was subjected to gradient PCR at 94°C for 5 min for initial denaturation followed by a three-step cycle of denaturing at 94°C for 45 s, annealing at 54°C to 43°C with a gradient of 1°C for 45 s and extension at 72°C for 1 min. The cycle was repeated 40 times before a final extension at 72°C for 5 min. The amplified products were run for 90 min on 1.5% agarose (XcelGen®, India #XGA), containing 0.5 μg/ml ethidium bromide (Himedia®, India #MB071) at 80 V. The optimized annealing temperature was incorporated in hot start PCR to amplify the corresponding gene sequence in teak.

2.3. DNA sequencing, homology search and phylogeny construction

The single band of size 750 bp amplified in teak genotypes by the above primer was eluted from the agarose gel and purified through gel purification kit (Qiagen®, India #28704) and subjected to DNA sequencing using Sanger sequencing chain termination method in ABI applied biosystem. Teak nucleotide sequence recovered a size of 713 bp (Available as Online Supplementary Data S1, GeneBank Acession No. KY781201). The obtained sequence was blasted to find out the nucleotide and amino acid similarity with the available sequences in NCBI database (Available as Online Supplementary Data S1-S3), employing BLASTn (http s://BLAST.ncbi.nlm.nih.gov) and BLASTx (https://BLAST.ncbi.nlm.nih.go v), respectively. Subsequently, the teak nucleotide and the translated protein sequence was aligned with the top BLAST hit of NCBI via CLC Genomics Workbench version (10.1.1) (https://www.qiagenbioinformatics.com) to demonstrate similarities and conservancy of nucleotide sequences and protein motif sequence among the species (Available as Online Supplementary Data S2). The most consensus nucleotide sequences of teak obtained through BLAST hit

i.e. pectin methylesterase inhibitor 51 (PMEI) were aligned and then used to construct phylogenetic tree comparing *PMEI* and *CCR* gene sequence of the same plant species (Available as Online Supplementary Data S2) following maximum likelihood method based on the Tamura-Nei model with 1,000 bootstrap simulations through MEGA7 software (Kumar et al., 2016).

3. Results

3.1. Amplification of PMEI gene and homology search

The annealing temperature range of 51°C – 48°C exhibited amplification of reproducible band size of 750bp. However, 52°C annealing temperature obtained a very intense band (Fig. 1) and was incorporated in the hot start PCR program for amplification of target gene in teak genotypes (Fig. 2). Sanger sequencing method recovered teak nucleotide sequence of a size of 713 bp (GeneBank Acession No.KY781201.1) that showed noteworthy identity with *PMEI* gene of different species in the database: 79% identity and 93% query cover with *Sesamum indicum* (XM_011101847.2), 77% identity and 97% query cover with *Erythranthe guttata* (XM_012998281.1), 66% identity and 42% query cover with *Eucalyptus grandis* (XM_010027268.2), 67% identity and 66% query cover with *Populus euphratica* (XM_011009258.1) and so forth (S3). On the other hand, the pairwise blast result of teak amplified nucleotides revealed 1% (13nt) query cover with *CCR* gene of *Brassica rapa* (XR 628039.2) only. *CCR* gene sequences of other species did not match (Available as Online Supplementary Data S2). Furthermore, the alignment of putative *PMEI* nucleotide and amino acid sequences with *PMEI* nucleotide and amino acid sequences of selected species showed maximum number of nucleotide and amino acid similarities (Figs. 3 and 4). The highest nucleotide and amino acid similarity were found with *Sesamum indicum*.

Phylogeny of teak nucleotide sequence

Phyolgenetic tree exhibited two separate clusters of *PMEI* and *CCR* gene obtained through NCBI database. The DNA sequence amplified in teak by *E. pilularis* CCR primer mingled with *PMEI* gene sequences of *Sesamum indicum* (XM_011101847.2) and *Erythranthe guttata* (XM_012998281.1) with bootstrap value 87%. The high bootstrap values supported clustering of teak sequences with *PMEI* gene sequences (Fig. 5).

For nucleotide sequence similarity of *PMEI* and *CCR* gene and their primer specificity, *PMEI* gene was blasted with CCR gene of selected species to check the nucleotide similarity existed between them and found maximum of 89% identity between *Sesamum indicum* (XM_011101847.2) and *Eucalyptus pilularis* (FJ834299.1) with 11% query cover followed by 89% identity with 9% query cover between both genes of *Nicotiana tabacum* and so forth. Primer blasted against both genes showed similar distribution of their complementarity site with CCR as well as PMEI gene (Available as Online Supplementary Data S4).

3.2. PMEI protein characterization

The amplified teak nucleotide of size 713 bp showed 57.36% GC and 42.64% AT. The in-silico translation of teak nucleotide comprised 237 amino acid sequence in length. The Pfam search showed that the teak nucleotide belonged to the PF04043 protein family representing the pectin methylesterase inhibitor (PMEI) protein. The PMEI region spanned from 24 to 171 amino acid and comprised 147 amino acid in length (Fig. 6). The molecular weight of teak PMEI protein was 15.94 kDa, a theoretical pI 8.97 and a protein formula $C_{697}H_{1117}N_{199}O_{211}S_9$ collectively. 25.9% protein residues were hydrophilic, 42.7% hydrophobic and 31.2% neutral. The 3D configuration of putative teak PMEI protein (Fig. 7) showed four long and three distorted helical bundle stabilized with two disulfide bridges (Fig. 7f). On alignment, the 3D configuration of putative teak PMEI protein aligned well with the known configuration of PMEI protein of *Arabidopsis thaliana* (PDB code 1X8Z) (Fig. 7d). Similarly, the Ramachandran plot (Fig. 8) validated the 3D configuration of putative teak PMEI protein that contained 96% amino acid residues from the favored region and 4% from the allowed region.

4. Discussion

Pectin comprises primary structure (the outermost middle lamella) and lignin secondary and tertiary structure (the innermost layers) of cell wall that provides rigidity/strength, on the one hand, and barriers against various stresses, on the other, in timber species. In the present investigation, CCR gene specific primer from *E. pilularis* that is responsible for lignin biosynthesis amplifies pectin methylesterase inhibitor (*PMEI*) gene in phylogenetically highly divergent teak belonging to a different Clade-Asterids (Zeng et al., 2017). Apparently, there exists no relationship between biosynthesis pathways of pectin and lignin. From evolutionary point of view, pectin incorporation in cell wall precedes lignin vis-à-vis emergence of terrestrial plant life from fresh water charophycean green algae during mid Paleozoic Era (Sørensen et al., 2011). Presumably, the suites of enzymes modifying and restructuring cell wall components may have co-evolved to maintain dynamic structure of cell walls that can be altered in response to both biotic and abiotic stimuli (Sørensen et al., 2011). This explains to some extent cross amplification *PMEI* gene in teak in the present investigation. Weikard et al. (2006) have obtained amplification of X- and Y-specific amelogenin (*AMELX* and *AMELY*) genes in a group of animals of Bovidae family, employing a pair of cattle specific primer of the same gene. However, an amplification of different gene across phylogenetically diverse taxonomic Clade as in our investi-

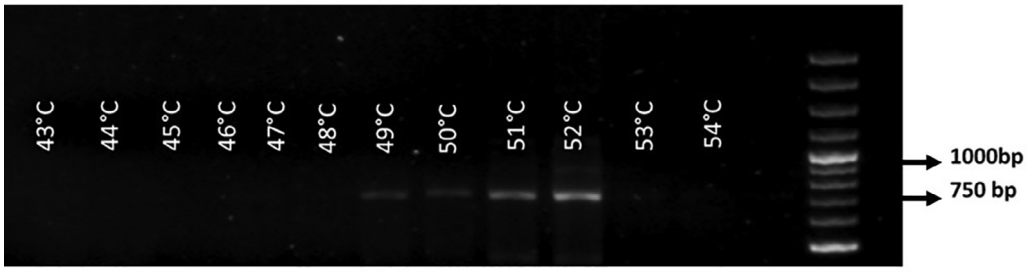

Fig. 1. Annealing temperature standardization for teak (*Tectona grandis* L.f.) genomic DNA using *E. pilularis* CCR gene specific primer through gradient PCR. 5 μl O'GeneRuler Ready-to-use 100 bp Plus DNA Ladder loaded on extreme right.

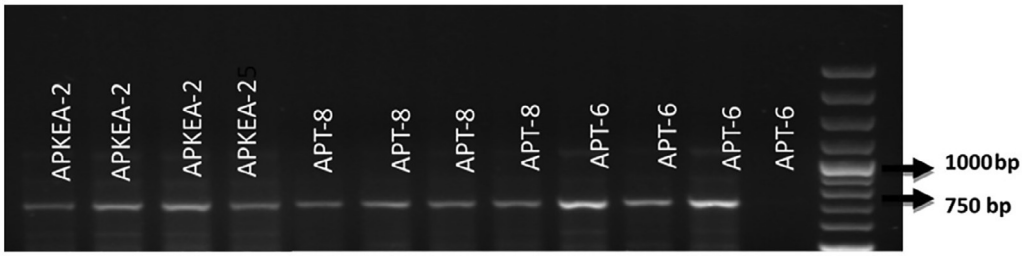

Fig. 2. Amplification of a single band (*PMEI* gene) across four ramets each of three teak (*Tectona grandis* L.f.) genotypes. 5 μl O'GeneRuler Ready- to-use 100 bp Plus DNA Ladder loaded on extreme right.

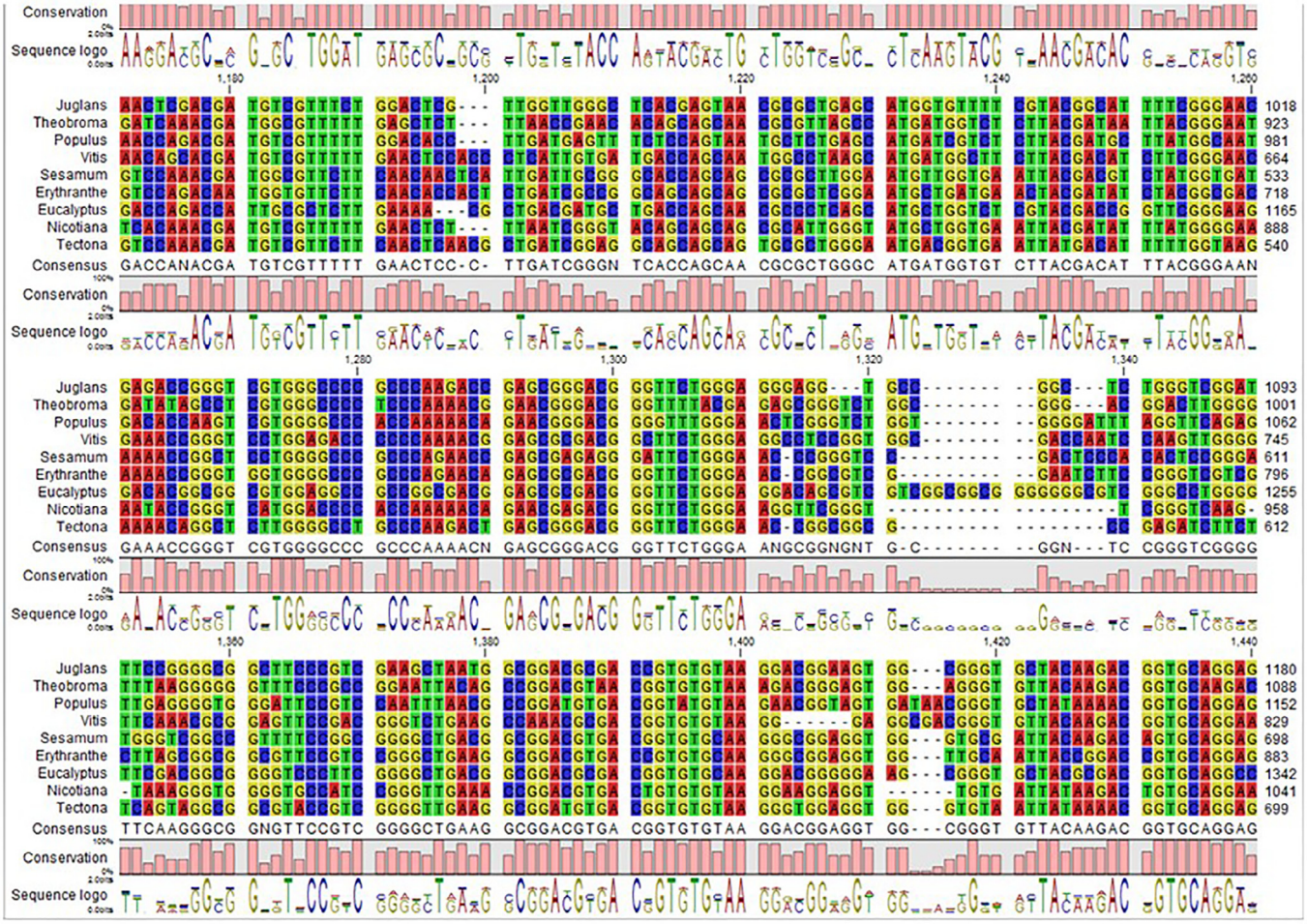

Fig. 3. Alignment of putative *PMEI* nucleotide sequence of teak with nucleotide sequences of *PMEI* of *Erythranthe guttata* (XM_012998281.1), *Sesamum indicum* (XM_011101847.2), *Nicotiana tabacum* (XM_016595759.1), Vitis vinifera (XM_002275156.3), *Juglans regia* (XM_019003548.1), *Theobroma cacao* (XM_007048266.2), *Eucalyptus grandis* (XM_010027268.2) and *Populus euphratica* (XM_011009258.1) through CLC Genomics Workbench version (10.1.1) software.

gation has not been reported in the literature. Therefore, the present investigation attempts to characterize and compare partially amplified teak *PMEI* gene and its translated protein with those of other species available in the database.

In the present investigation, a single reproducible band of size 750 bp in teak genome has been amplified. On the contrary, the same set of primer amplifies a 1628 bp amplicon in *E. pilularis* (Sexton et al., 2010). Shiferaw (2013) has expressed that the primer is considered transferable if the size of the amplicon is comparable. The present investigation, however, exhibits both disparity in amplicon size and amplification of a different gene, i.e. *PMEI*, suggesting a low transferability performance outside the family/order. The transferability of EST- based primers has been reported in the literature in Rosaceae, i.e. Malus to Prunus, 69% and Malus to Fragaria, 61% (Sargent et al., 2009) as well as Prunus to Fragaria, 66% (Dantec et al., 2010). Similarly, the transferability of SSR to the Sapindaceae family, Litchi to Rambutanake (Sim et al., 2005) and 58 % from Litchi to Blighia (Ekué et al., 2009). However, a very

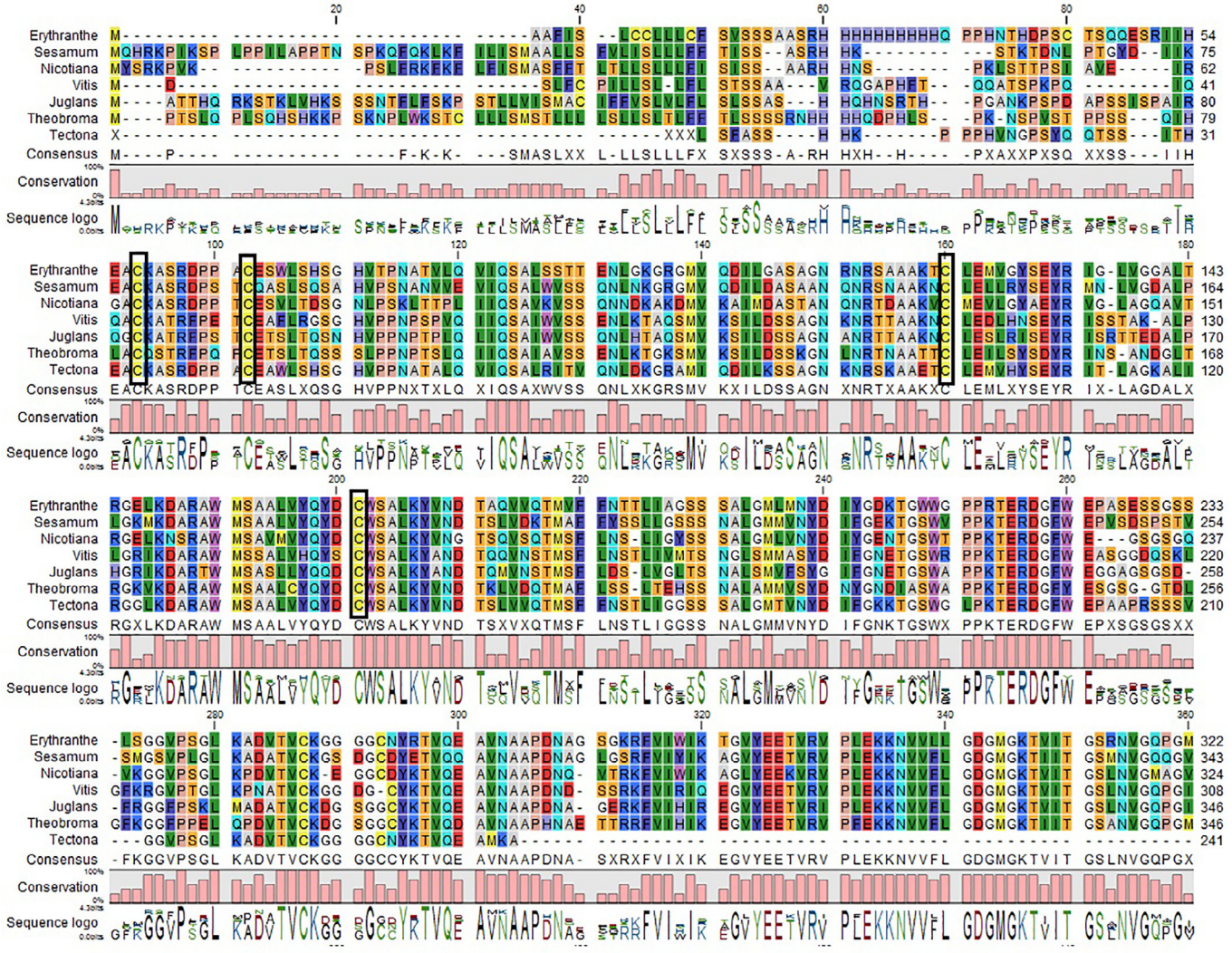

Fig. 4. Alignment of in-silico translated PMEI amino acid sequences of teak with PMEI protein sequences of *Erythranthe guttata* (XP_012853735.1), *Sesamum indicum* (XP_011097189.1), *Nicotiana sylvestris* (XP_009759332.1), Vitis vinifera (XP_002275192.1), *Juglans regia* (XP_018859093.1) and *Theobroma cacao* (XP_007048328.2) through CLC Genomics Workbench version (10.1.1) software. The marked yellow line showing the conserved cysteine amino acid residues among the species (Di Matteo et al., 2005).

low intergeneric transferability, i.e. 2.9%, has been recorded in the Myrtaceae family between the *Eucalyptus* and *Eugenia* genera (Zucchi et al., 2002). Low transferability suggests a rapid change in the genome due to evolutionary influences. Further, the sequencing and BLAST analysis of the newly amplified teak nucleotide displays a maximum identity with *PMEI* gene rather than the CCR gene. In consonance, the translated teak protein BLAST result also possesses the highest homology with PMEI proteins of various species. The above findings may imply that the reshuffling of nucleotide between genes due to transposable elements can lead to amplification of different target genes (Du et al., 2010). Factors that participate in the biological evolution of a species can also correspond to the different target gene amplification (Yurchenko et al., 2011).

In addition, the blasted *PMEI* and *CCR* genes exhibit 89% identity with 11% query cover between *Sesamum indicum* and *E. pilularis*. The blasted primer pair also exhibits equal distribution of their complementarity site in both genes. The above result indicates, though the function of the two enzymes formed by their genes is distinct, but they share several nucleotide similarities suggesting that the duplicating gene of a common ancestor has evolved due to speciation (Fitch, 1970). That is why the phylogenetic tree makes two separate clusters for *CCR* and *PMEI* genes where teak nucleotide lies in the *PMEI* cluster with proximity to sister clad of *Sesamum indicum* and *Erythranthe guttata*. In addition, the Pfam protein database also classified the teak nucleotide as a pectin methylesterase inhibitor allocated to a family PF04043.

The translated teak PMEI protein comprises 147 amino acids in length. A comparable size of 151 amino acid residue of PMEI protein has earlier been obtained in plants (Di Matteo et al., 2005; Hong et al., 2010; Pinzon-Latorre and Deyholos, 2013; Husna et al., 2021). The molecular weight of teak translated protein is 15.95 kDa, which is comparable to the 18 kDa of kiwi fruit PMEI proteins (Giovane et al., 1995; Di Matteo et al., 2005). The alignment of PMEI amino acid sequence reveals four conserved cysteine residues involved in the formation of disulfide bridges. A similar result has been obtained in case of *Actinidia deliciosa*, which contains four conserved cysteine residues in PMEI chain (Di Matteo

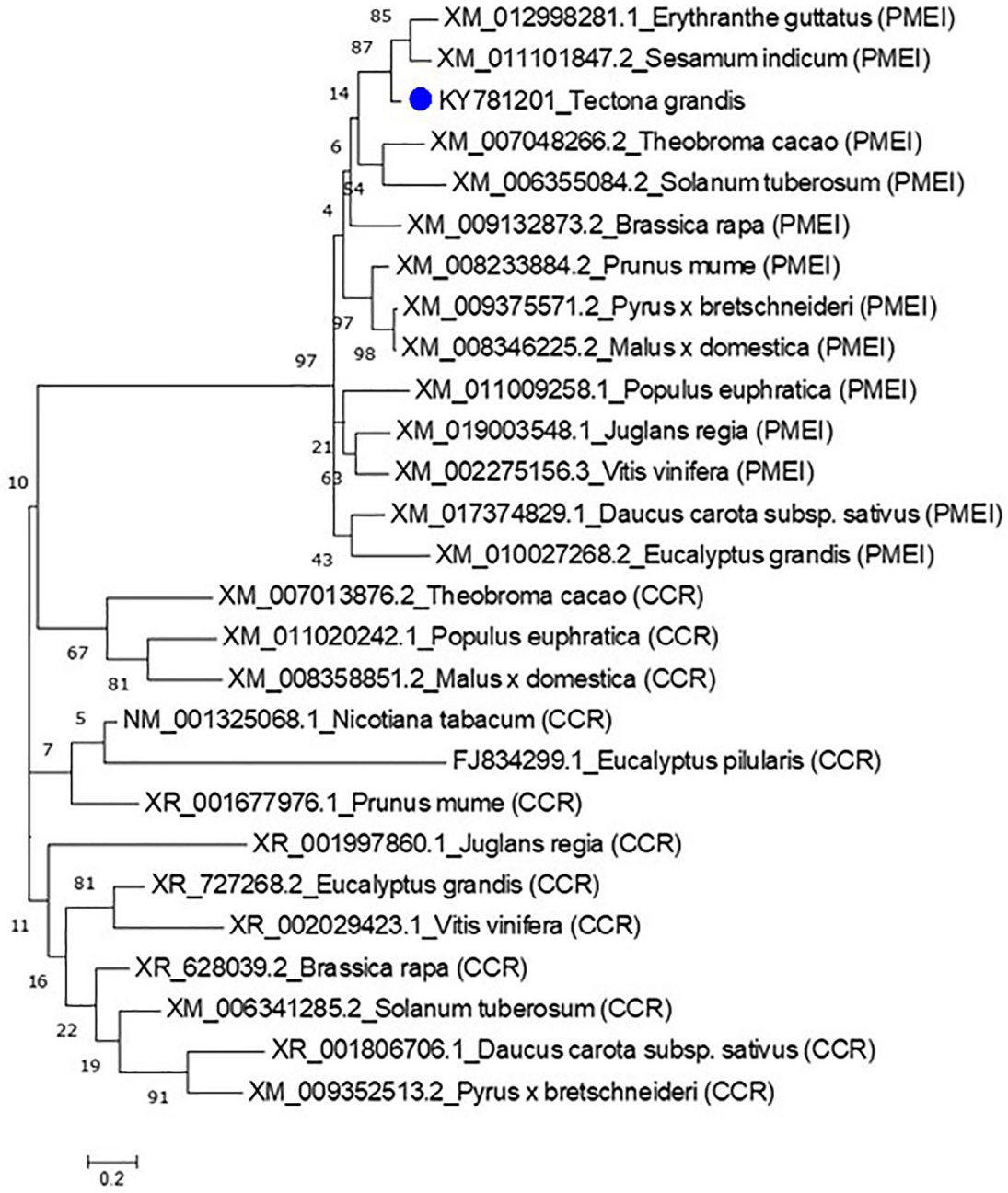

Fig. 5. A phylogenetic tree generated using top BLAST hit of newly amplified teak nucleotide. A Maximum Likelihood Method based on the Tamura - Nei model was used with 1,000 bootstrap value for inference of significantly related clusters. The *CCR* nucleotide sequences of the same species were also used to find the most closely related cluster of teak nucleotides. The teak nucleotide is showing in blue circle and bootstrap value (%) on each node.

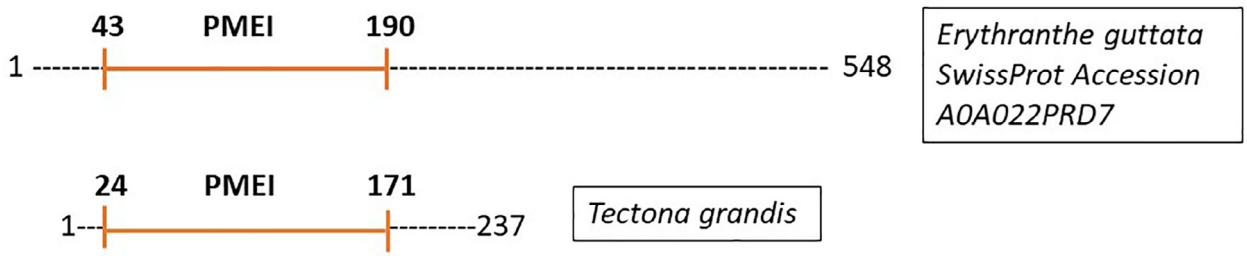

Fig. 6. Linear structure of in-silico translated amino acid sequence of putative PMEI protein of teak (*Tectona grandis* L.f.) with Pfam alignment of *Erythranthe guttata* amino acid sequence (SwissProt accession number: A0A022PRD7). The PMEI region (yellow) spanned from 43 to 190 (147 amino acid) in *Erythranthe guttata* and 24 to 171 (147 amino acids) in *Tectona grandis* L.f.

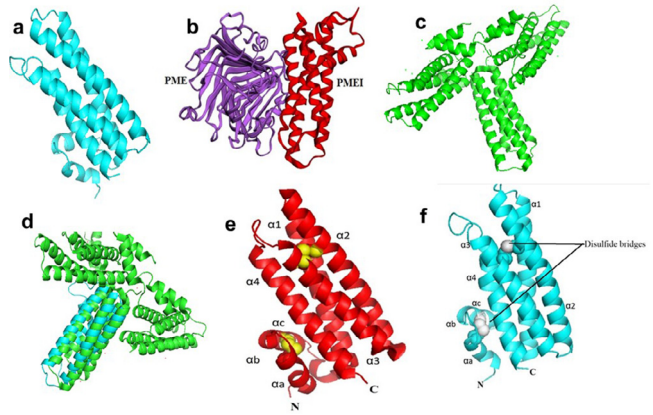

Fig. 7. Structure of putative pectin methylesterase inhibitor of teak and its comparison with the known structure of PMEI of *Arabidopsis thaliana* and *Actinidia deliciosa*: (a) 3D protein structure of putative partial PMEI of teak (*Tectona grandis* L. f.) obtained through Swiss model, (b) Crystal structure of pectin methylesterase and pectin methylesterase inhibitor complex from *Solanum lycopersicum* and *Actinidia deliciosa* (PDB code 1XG2) (Di Matteo et al., 2005), (c) Crystal structure of pectin methylesterase inhibitor from *Arabidopsis thaliana* (PDB code 1X8Z) (Hothorn et al, 2004), (d) Alignment result of teak putative PMEI and *Arabidopsis* PMEI protein through PyMOL, (e) Crystal structure of PMEI from *Actinidia deliciosa* with four helices and disulfide bridges (PDB code 1XG2) (Di Matteo et al., 2005) and (f) Teak 3D PMEI structure with four helices and disulfide bridges.

et al., 2005). A 3D structure through Swiss-model of the translated teak protein reveals four helical chains (α1, α2, α3 and α4) arranged in an antiparallel direction up and down. The N-terminal protruding region has three small, distorted helices (αa, αb and αc). The complete protein bundle is stabilized by hydrophobic interaction and disulfide bridges. The disulfide bond is formed between amino acid residue C74 and C114 located in α2 and α3 helical chain and C9 and C18 residue located in distorted chain αa and αb (Di Matteo et al., 2005). The 3D image of teak PMEI is in an excellent agreement with the 3D image of *Actinidia deliciosa* (PDB code 1XG2), where four antiparallel helices with three distorted N-terminal helices are stabilized by two disulfide bridges (Di Matteo et al., 2005). The four helical bundles bearing 3D image of teak is very nicely and significantly aligned with the PMEI protein of *Arabidopsis thaliana* (PDB code 1X8Z). A Ramachandran plot validates the helical rotation of protein by estimating the angle-rotation of C-C (psi) and N-C (phi) bonds of amino acid, along with 96% of the residues belonging to the favored region and 4% to the allowed region. The forecast structure analysis gives a strong indication of the good quality of the projected 3D structure of PMEI protein in teak. Hothorn et al. (2004) have demonstrated a complete model of interaction of PMEI protein and PME enzyme where the four helical bundles of PMEI protein bind directly with the cleft of the active site of PME enzyme and prevent their binding affinity with pectin and regulate the activity of PMEs.

As for biological function, the overexpression of the *PMEI* genes in *A. thaliana* decreases PME activity and increases degree of methyl esterification in pectin vis-à-vis alleviation *of B. cinerea* symptoms (Lionetti et al., 2007). Lionetti et al. (2017) have further evaluated the infection rate of *B. cinerea* in the mutant *A. thaliana* containing T-DNA/transposon insertion region located in the 5′ UTR of *PMEI* gene, and in the start codon and exon. The transformed mutant plants have exhibited higher PME activity and become substantially more vulnerable to *B. Cinerea* than wild-type plants. In addition, the level of PME activity has been recorded to be higher in mutant plants than in wild species after infection, suggesting that PMEI protein plays an inhibitory regulatory role for PME activity during the disease containment. In wheat (Volpi et al., 2011) and cotton (Liu et al., 2018), the degree of methyl esterification of pectin also tremendously increases with overexpression of the *PMEI* gene. The highly methyl esterified pectin inhibits hydrolyzing function of pathogen-secreted polygalacturonase (pectin degrading enzyme). Therefore, the infection caused by *Fusarium gramineum* and *Bipolaris sorokiniana* in the transgenic wheat and infection caused by *V. dahlia* in transgenic cotton is restricted. However, *PMEI* gene-silenced plant has shown increased susceptibility to a fungal attack (Liu et al., 2018).

PMEI protein also plays an essential role in the protection against the virus. It has been found that PME is necessary for the systemic transmission of the tobacco mosaic virus (TMV) in the hosts (Dorokhov et al., 1999; Chen et al., 2000; Chen and Citovsky, 2003). A study using western blotting, affinity chromatography, and microsequencing confirms that the tobacco mosaic virus movement protein (TMV MP) binds to the host PME enzyme that facilitates the virus movement from one cell to the other. The mutation in binding site of PME triggers the inactivation of TMV movement in the host (Chen et al. 2000). The overexpression of *PMEI* gene in tobacco increases the degree of methyl esterification up to 70%. The inoculation of TMV virus in transgenic tobacco leaves shows no symptoms as compared to the typical symptoms observed in the wild type after 8-days of infection (Lionetti et al., 2014). As for bacteria, *Capsicum annuum* upregulates expression of the *PMEI* gene and circumvents infection caused by *Xanthomonas campestris* pv. Vesicatoria (Xcv). However, silencing of *PMEI* gene in the species increases susceptibility toward the Xcv. Similarly, the overexpression of the *PMEI* gene in *Arabidopsis* shows decrease in susceptibility toward *Pseudomonas syringe* (An et al., 2008). *PMEI* gene has also been linked with abiotic stress tolerance; however, their molecular mechanism is poorly understood to date. Through transcriptional profiling, it has been found that the *PMEI* genes are overexpressed during cold, drought, H_2O_2 stimulus, salinity, water stress, abscisic acid treatment, and anaerobic condition (An et al., 2008; Nguyen et al., 2016; Hong et al., 2010; Liu et al., 2018).

5. Conclusion

E. pilularis CCR primer amplifies a putative *PMEI* gene in teak that constitutes the first report in the literature. The gene can be useful for the study related to its up and down regulation in various biotic and abiotic stress conditions for their modification and creation of genetically engineered tree to incorporate novel trait. It is, therefore, essential to amplify and characterize the full length gene with enormous impact on biotic and abiotic stress tolerance in the teak and other forest trees that often survive in scarce nutrients and water deficit conditions and face natural vagaries, including pathogens, insects, etc. *PMEI* gene may result in wide adaptability as well as luxuriant timber productivity and quality in adverse/ fluctuating climatic conditions, scarce nutrients, water and predisposing insect pests of tropical forests.

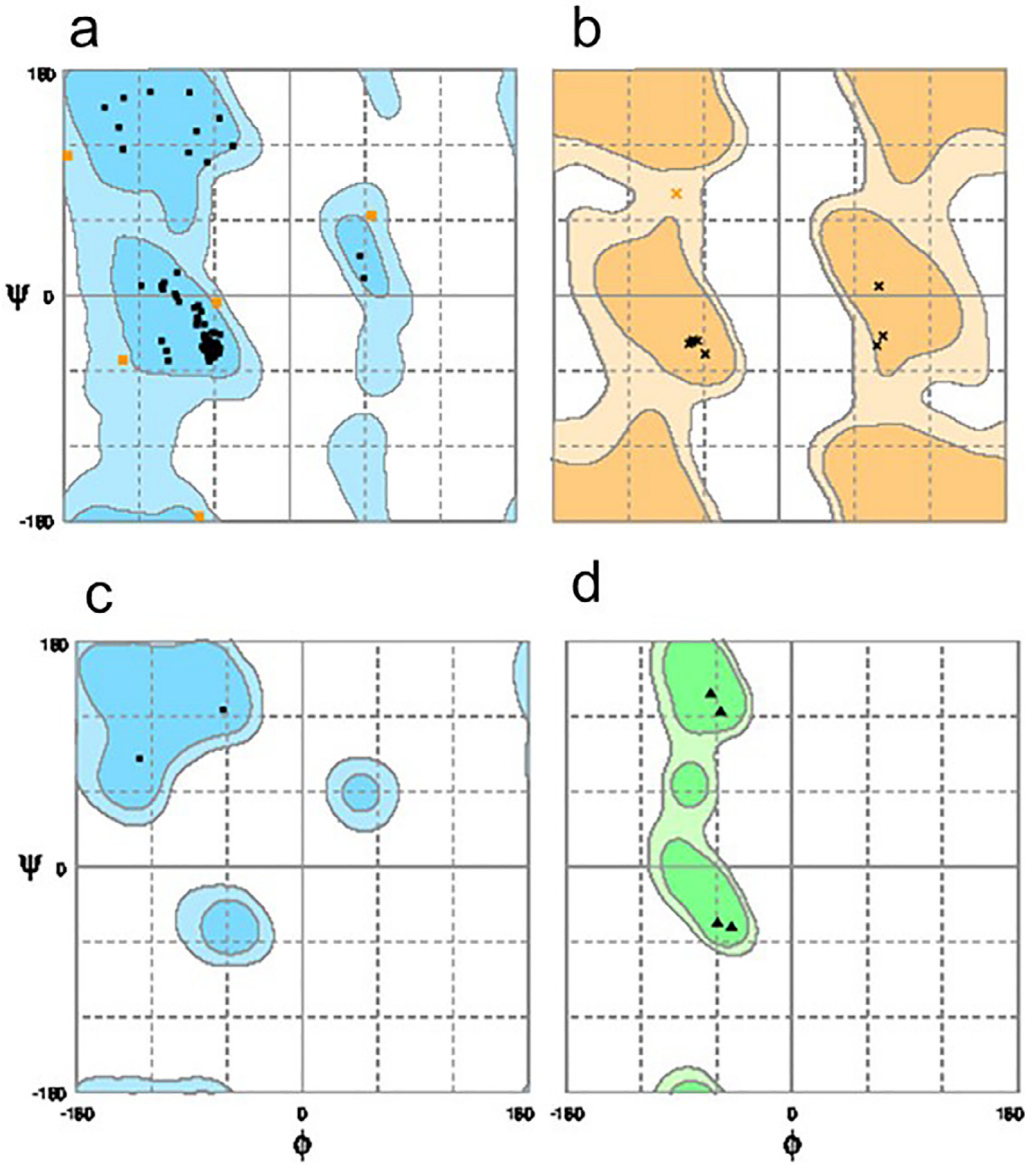

Fig. 8. Ramachandran plot generated using pdb file of 3D image of putative PMEI of teak (*Tectona grandis* L.f.): a. General amino acids, b. Glycine, c. Pre-proline and d. Proline. The graph depicts the angle rotation of C-C bond (psi) and N-C (phi) bond of an amino acid based on the geometric rotation. Allocation of amino acids corresponded to favoured region (dark), allowed regions (light) and not allowed region (white).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval/declarations

Not applicable.

Consent for publication (include appropriate statements)

All authors agree to publish the work.

Availability of data and material/ Data availability

GeneBank Acession No. KY781201.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.07.015.

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