The Pharma Innovation Journal 2023; 12(10): 591-599

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(10): 591-599

www.thepharmajournal.com
Received: 02-07-2023
Accepted: 06-08-2023

Dhanvarsha M
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Saranya N
Department of Plant Molecular Biology & Bioinformatics, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Infant Richard J
Department of Plant Molecular Biology & Bioinformatics, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Mary Roshni A
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Mohankumar S
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
Mohankumar S
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

SNP analysis and *in silico* docking studies on serotonin pathway gene CYP71A1 for yellow stem borer resistance in rice

Dhanvarsha M, Saranya N, Infant Richard J, Mary Roshni A and Mohankumar S

Abstract
Rice (*Oryza sativa* L.) is a widely grown cereal crop consumed by half of the world's population. The Yellow Stem Borer (YSB), a monophagous pest causes yield loss of up to 25% in rice. It damages the crop in all stages, producing dead hearts in the vegetative stage and white ears in the reproductive stage. In response to stress, the plant's defence mechanism induces the accumulation of serotonin through the serotonin biosynthetic pathway. Serotonin has a major role in pest immunity and rendering the crop susceptible to pests. Therefore, this study aimed to analyse the single nucleotide polymorphism (SNP) of gene *CYP71A1* involved in the serotonin pathway, in both susceptible and resistant varieties of YSB in rice. Two functional SNPs were identified at position 9581596 (G/A) and 9581369 (G/T) in the YSB susceptible and resistant varieties through multiple sequence alignment tool CLUSTAL omega. Further, modelling and docking studies were performed by I-TASSER server and Discovery studio software respectively to explore these mutations in the gene *CYP71A1* that encodes Tryptamine 5-hydroxylase (T5H) enzyme and produces serotonin with its substrate tryptamine. The docking results showed that (T5H) in the susceptible variety (ASD16) showed high binding energy of -16.66 kcal mol^{-1} compared to the resistant variety (TKM 6) -19.02 kcal mol^{-1}respectively. In addition alanine scanning mutagenesis was done using ABS scan promotes insight into the T5H binding site residue LEU (378) on tryptamine binding. The development of YSB resistant rice lines could be made possible through targeted gene editing of this region, alleviates the infestation of YSB and improves rice productivity.

Keywords: Rice, serotonin, yellow stem borer, single nucleotide polymorphism, tryptamine 5-hydroxylase

1. Introduction
Rice, a major cereal crop that provides food for two-thirds of the world's population [1], is facing numerous challenges due to the presence of various biotic and abiotic stresses. The biotic stress includes pests which also act as vectors for indirect transmission of viruses, pathogens causing disease and parasitic plants. Pests and plant pathogens reduces agricultural productivity and quality that ultimately weakens global food security and leads in greater economic losses. According to Savary *et al*. 2019, 30% of rice crop loss caused by 137 diseases and pests globally [2]. Among these, stem borers were mostly damaging the rice field of Asia which includes yellow stem borer (YSB), stripped stem borer (SSB), white stem borer (WSB) and pink stem borer that contributes the maximum percentage of yield loss of 36.26, 24.54, 17.74 and 22.19 respectively [3, 4]. YSB stands out as a prominent monophagous pest specifically affecting lowland rice crops. Infested plant showed symptoms of dead hearts during the vegetative stage and white ears during the reproductive stage, resulted in major yield losses of around 25-30% [5, 6]. Despite multiple attempts, chemical methods were unsuccessful in completely eliminating the YSB [7]. Moreover, the use of pesticides disrupts natural enemies and biodiversity. The development of Monosomic Alien Addition Lines (MAALS) has shown moderate resistance to YSB due to the appearance of extra unpaired chromosomes known as univalent [8]. Additionally, manipulating genes involved in host-herbivore interactions has shown as promising strategy in conferring resistance to YSB, particularly to host plant populations lacking inherent resistance genes [9]. In regard of SSB infestations, an increase in tryptamine metabolites like serotonin has been observed in gramineae family as a defence mechanism [10].
The biosynthetic pathway of serotonin involves two enzymes, Tryptophan decarboxylase (TDC) [EC: 4.1.1.105] and Tryptamine 5-hydroxylase (T5H) [EC: 1.14.-. -].

Gene TDC1 encodes Tryptophan decarboxylase enzyme (TDC), converts tryptophan to tryptamine and the SL gene encoded by Cytochrome P450 monoxygenase (CYP1A1), converts tryptamine to serotonin [11]. The inhibition of TDC enzyme decreases the serotonin production and also increases the susceptibility to fungal disease, *Bipolaris oryzae* in rice [12]. Downstream of serotonin pathway involves two enzymes in melatonin biosynthesis, namely Serotonin N Acetyl Transferase (SNAT) [EC: 2.3.1.87] and Acetyl Serotonin Methyl Transferase (ASMT) [EC: 2.1.1.4] [13] (Fig.1). Abiotic stress activates the genes SNAT1 and ASMT1 leading to melatonin accumulation [14]. Earlier studies have reported that serotonin was important for gaining body weight, fecundity and oviposition in SSB. Furthermore, supplementing the SSB with artificial diet of serotonin improved their growth [15]. Serotonin plays a vital role in pest immunity. In pests, it activates the serotonin receptor which has a unique function in host recognition and behaviour [16].

Single nucleotide polymorphism (SNP) favours the analysis of functional genetic variations in the genes for the development of marker assisted breeding approaches. Finding non-synonymous SNPs, in the coding region of gene remains an important step in understanding the link between variation and trait [17]. The current study focuses on identifying SNP variation of CYP71A1 gene involved in serotonin biosynthetic pathway for YSB resistance trait. Based on the identified SNPs, three-dimensional structure of resistant and susceptible rice T5H were modelled and the interaction mechanism was studied through molecular docking of tryptamine to T5H (CYP71A1). Further the SNPs mutation energy was calculated, to check whether the SNP changes alter the binding energy of tryptamine in resistant and susceptible varieties. Alanine scanning mutagenesis through ABS scan provided insights on the interacting residues of T5H that favours in the binding of tryptamine. Thus, the present study provides information for the development of YSB resistant rice lines using genome-editing technology.

2. Materials and Methods

2.1 Choice of varieties used in the study

The varieties reported in literatures as resistant and susceptible based on the IRRI-Standard Evaluation Score in the varietal screening of YSB at both vegetative and reproductive stages were used in this study (Table 1). The accession ID of the rice varieties in 3K RG dataset provided in the International Rice Informatics Consortium (IRIC) (http://iric.irri.org/) was used for the retrieval of the CYP71A1 gene sequences. For ASD16, the gene sequence of CYP71A1 was obtained from the whole genome sequence available at National Centre for Biotechnology Information (NCBI) database (SRX9260514).

2.2 Retrieval of gene sequence

Oryzabase was used for identifying locus ID of the gene (https://shigen.nig.ac.jp/rice/oryzabase/). Using these locus ID, start and end position of the genes were found via SNP seek database (https://snp-seek.irri.org/). The gene sequences of different rice varieties were retrieved from the Rice Functional Genomics and breeding Database (RFGB version 2.0) (https://www.rmbreeding.cn/) by providing the accession ID of the variety, chromosome location, start and end position of the gene sequence (Table 2). The gene sequence of the *CYP71A1* for reference cultivar Nipponbare (*Oryza sativa* japonica group) was obtained from NCBI database (Accession no. AP014964.1).

2.3 Identification of SNPs

The coding sequences of the reference gene *CYP71A1* was retrieved from NCBI (Accession no. XM_015793215.2). Using Expasy translate tool (https://web.expasy.org/translate/), the translated protein sequences of the reference gene and the gene sequence of resistant and susceptible varieties retrieved from the RFGB database were compared using multiple sequence alignment tool - CLUSTAL Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to distinguish non-synonymous SNPs from synonymous SNPs. It confines heuristics, parallel processing and other optimization techniques to achieve fast alignment and making it suitable for analysing extensive sequence datasets [31].

2.4 Protein modelling

FASTA sequence of T5H protein (CYP71A1) corresponding to the resistant variety (TKM6), and susceptible variety (ASD16) was blasted against the Protein Data Bank database (PDB) for homology modelling of the protein. In PDB, the suitable template of protein structure was not found. So we employed the Iterative Threading Assembly Refinement (I-TASSER) server (http://zhanglab.ccmb.med.umich.edu/I-TASSER) to generate a protein model. I-TASSER server is an integrated platform for automated protein structure and function prediction and it uses various threading alignments to construct three dimensional molecular models. Finally, the modelled protein structure was validated using Ramachandran plot (Fig.2).

2.5 Protein-ligand docking

For docking, the ligand tryptamine (2-(1H-Indol-3-yl) ethan-1-amine) was downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. The modelled protein T5H and the ligand tryptamine were imported into the Discovery studio (DS) software. The protein and ligand were prepared for docking by minimizing energy using CHARMm force fields and the protocol involves inclusion of missing atoms, assignment of charges and the insertion of missing loops. The information of ligand binding sites of the modelled proteins was predicted by I-TASSER COFACTOR algorithm. In addition the CASTp tool was used to predict the ligand binding pocket and active sites of the protein T5H (Fig.3). From these ligand binding sites were defined and docking performed using C-DOCKER protocol.

2.6 Mutation Binding energy

The effect of SNP mutations was evaluated by Discovery Studio mutation energy protocol. The resistant protein T5H was mutated in correspondence to the SNP found in susceptible ASD16 protein (SER33LEU and PRO107THR) to check for the stability of mutation.

2.7 Alanine Binding-Site Scan

The ligand binding residues of the protein T5H were mutated to alanine through Alanine Binding-Site Scan server (http://proline.biochem.iisc.ernet.in/abscan/). The resistant T5H protein-ligand complex in pdb format was given as input. Binding site residues were identified through cut-off distance of 4.5Å [32].

3. Results and Discussion

The YSB infestation invasively affects the yield that reduced up to 87.66 percent when the crop left untreated with insecticides [33]. Even with continuous application of insecticides, effective management of YSB is still challenging due to its behaviour and concealed feeding patterns [34]. Extensive molecular research aimed at identifying the genes and quantitative trait loci (QTL) responsible for imparting resistance to YSB [35]. Previous studies have reported the involvement of CYP450 genes in insect resistance [36]. The enzyme Tryptamine 5-hydroxylase encoded by CYP71A1 gene belongs to cytochrome P450 monooxygenase family [37]. In that connection serotonin biosynthetic pathway gene CYP71A1 was analysed for functional SNPs associated with insect resistance traits. In a report, external application of serotonin in *Setaria viridis*, resulted in a decrease of survival rate and body weight of *Rhophalosiphum padi* adults (aphid), thus it indicates serotonin is susceptible to pests [38]. The variety TN1 was most susceptible to brown plant hopper, the relative gene expression studies revealed that, the gene CYP71A1 was up regulated to 2.7 fold compared to resistant varieties [15]. It was evident that susceptibility to pest infestation was higher due to accumulation of serotonin in rice

3.1 Genetic variation assessment of serotonin biosynthesis gene CYP71A1

In the SNP analysis, CYP71A1 gene sequences of resistant and susceptible varieties of rice were compared to reference nipponbare sequence in which the TDC gene remained conserved with no SNPs detected across the studied varieties. However, the downstream gene CYP71A1 exhibited two SNPs in which one transition and one transversion mutation was observed in the coding region (Table 3). These variations led to notable protein changes, specifically serine to leucine (position: 9581596) and proline to threonine (position: 9581369) respectively. The rice mutants generated by knocking out of CYP71A1 gene attributes resistance to stem borers, the sequencing result of the mutant gene CYP71A1 reported that the insertion of "A", adenine at the 71th position in the first exon of the gene through CRISPR/cas9 technology. The intended mutation at 9581617 position in the gene leads to frame shift of the codon CUU that codes for leucine, resulted in reduced serotonin levels [15]. From this it was hypothesised that the CYP71A1 gene exhibited SNPs can change the serotonin levels in plants. The location of the gene CYP71A1 in chromosome 12 was not previously studied for insect resistance traits. Discovery of novel SNPs in genes will lead to the development of genetic markers for the resistant trait [17].

3.2 Molecular docking

In-silico analysis was carried out to study the role of serotonin in pest resistance. Molecular docking explores the variations in binding affinity between resistant and susceptible varieties concerning the compound tryptamine and the protein T5H encoded by CYP71A1. The resistant variety TKM6 was selected based on literatures confirming its resistance to stem borer. Alternatively the susceptible variety ASD16 was chosen due to the existence of two functional SNPs in the CYP71A1 gene. To assess the ligand interaction affinity of two mutations reported in the present SNP analysis, molecular docking was performed using DS software to analyze the interaction between the compound tryptamine and the protein T5H in both resistant (TKM6) and susceptible (ASD16) variety (Fig.4,5). The primary objective of this analysis was to identify the optimal binding residue and assess the strength of interaction with the receptor (T5H). The determination of these factors relied on the CDOCKER ENERGY scores, which was found to be 16.66 kcal mol^{-1} for resistant variety and -19.02 kcal mol^{-1} for the susceptible variety. Additionally, the CDOCKER interaction energy scores were observed to be -21.65 kcal mol^{-1} for the resistant and -22.27 kcalmol^{-1} for the susceptible variety, respectively. From the docking scores and interaction energy scores, interaction between tryptamine and the protein T5H in the susceptible variety is higher, compared to the resistant variety, which indicates the binding affinity of enzyme-substrate complex led to higher production of the product compound (serotonin). In the resistant variety, the compound exhibited significant interactions with LEU (379) through a conventional hydrogen bond, VAL (379), ALA (310) through pi-alkyl bond and CYS (453) through pi-sulfur bond. Compared with the susceptible variety the compound shows conventional hydrogen bond interaction with ARG (451) and ARG (106) residues and pi-alkyl interactions with LEU (378), ALA (310) and CYS (453) residues, respectively. The proteins are naturally flexible and have different ligand binding conformations on docking when the binding site residues are mutated [39]. Docked complex was predicted to have a hydrogen bonding formed as a result of electrostatic attraction between hydrogen atom and electronegative atom N or O, with the hydrogen bond distance 2.5 to 3.2 Å between donor and acceptor [40]. The conventional hydrogen bonds of susceptible variety protein having the bond length of 1.83 Å between atoms HH21-N2 and 2.24Å between atoms of O-H24 respectively, the shorter distances of 2.3Å in hydrogen bonds depicts the covalent bond character and larger binding energy [41]. Thus the two conventional hydrogen bond in the protein of susceptible variety attributes a stronger interaction. π-π interactions between the aromatic residues of side chain and ligands contribute to the ligand-binding affinity [42]. The cation-π systems in aqueous solution presenting 10 kJ mol^{-1} binding affinity compared to the strength of salt bridges [43], here pi-alkyl bonds formed between the uncharged amino acids. The residual mobility, made the ligands to move freely in the binding pocket of the protein cytochrome P450 $_{cam,}$ thus it influences the interaction positively [44]. This study further supports, the susceptibility of the variety ASD16 was attributed by higher serotonin accumulation on infestation of the pest.

3.3 Mutation binding energy & Mutagenesis of ligand binding residues

The mutation energy (ΔΔGmut) was calculated (Table 4) to study the stability of mutations SER33LEU and PRO107THR in the docked complex structures. In the mutation energy graph, the binding energy was neutral to decreasing upon mutation of these residues with the binding energy difference of -0.07 kcal mol^{-1} (Fig 6)**.** The altering binding energy due to mutations can be calculated by the difference in binding free energy of the mutated protein to the wild type protein (ΔΔGmut = ΔΔGfold (mutant) - ΔΔGfold (wild type)). Van der Waals (EvdW) interactions, electrostatic interactions (ΔGelec), an entropy contribution (-TSsc) due to changes in side-chain mobility, and a non-polar, surface-dependent, contribution to solvation energy (ΔGnp) are all added together

to get the total energy [45]. Moreover the SNPs mutational energy was computed to evaluate its influence on binding in comparison with the TKM6 (resistant variety). The two SNPs of ASD16 showing mutation energy between -0.5 to 0.5 kcal mol^{-1} which represents the mutated structure was neutral to decreasing stability. Studying the ligand binding residues was a key in understanding the cellular and metabolic processes, in spite the role of individual amino acids involved in interaction remains unclear. To found the importance of each residue at a specific site, the technique site directed mutagenesis was mostly used to generate single base mutations [46] so that ABS scan analysis was performed. In ABS scan analysis, nine ligand binding residues of T5H were mutated to alanine (Table 5). The energy of each mutated structure was predicted using Autodock 4.1 and the details were provided in Table 5. The lowest binding energy was observed for the residue VAL (379) was -1.86 kcal mol^{-1} and the highest binding energy was observed for the residue LEU (378) was -2.13 kcal mol^{-1} when it was mutated to alanine. Therefore compared to the other ligand binding residues LEU (378) has more impact in interaction. In future, exploring the mutation of LEU (378) in protein T5H via genome editing might reduce the binding affinity to its substrate tryptamine, thereby it reduces the serotonin production which facilitates resistance to YSB in rice

Table 1: Varieties chosen for SNP analysis

S. No	Accession ID	Variety	Susceptibility to YSB	Reference
1	CX120	TKM6	R	[18]
2	CX160	W1263	R	[19]
3	CX157	PTB33	R	[20]
4	IRIS_313-10523	PTB18	R	[21]
5	IRIS_313-10985	Balam	R	[22]
6	IRIS_313-7688	IR22	R	[23]
7	IRIS_313-8657	Co19	R	[24]
8	IRIS_313-8755	Norin 6	R	[25]
9	IRIS_313-11208	Parijat	R	[26]
10	CX236	Vandana	MR	[27]
11	B032	Rohini	MR	[27]
12	PRJNA666312	ASD16	S	[27]
13	CX162	TN1	S	[28]
14	CX94	Swarna	S	[27]
15	CX227	Kasalath	S	[29]
16	CX46	Pusa Basmati1	S	[30]
17	CX146	Co43	S	[30]

* R-Resistant * MR- Moderately resistant * S-Susceptible

Table 2: Physical positions of the gene CYP71A1

S. No	Gene	Locus ID	Chromosome	Start position	End position
1	CYP71A1	Loc_os12g16720	12	9579747	9582014

Table 3: SNPs for CYP71A1 gene

SNP Position	9581596	9581369	R/S
Varieties			
NIPPONBARE	G	G	S
TKM6	G	G	R
PTB33	G	G	R
W1263	G	G	R
PTB18	G	G	R
IR22	A	G	R
BALAM	G	G	R
PARIJAT	G	G	R
NORIN 6	G	G	R
CO19	G	G	R
VANDANA	G	G	MR
ROHINI	A	G	MR
ASD16	A	T	S
TN1	G	G	S
KASALATH	G	G	S
SWARNA	G	G	S
PUSA BASMATI 1	G	G	S

Table 4: Mutation binding energy

Mutation type	Mutatation site	Mutation energy (kcal mol^{-1})	Effect
Single mutation	SER33>LEU	-0.07	Neutral to decreasing
Single mutation	PRO107>THR	-0.07	Neutral to decreasing

Table 5: ABS scan analysis report

Ligand-binding residues	Binding energy Kcal mol^{-1}	Electrostatics	Van der waals	Desolvation energy	Torsional energy
THR_123_ALA	-1.95	-0.13	-3.16	0.75	0.59
PHE_218_ALA	-1.98	-0.14	-3.24	0.79	0.59
ASP_306_ALA	-2.02	-0.06	-3.29	0.74	0.59
VAL_309_ALA	-2.04	-0.14	-3.34	0.83	0.59
ALA_310_ALA	-2.11	-0.14	-3.38	0.81	0.59
VAL_375_ALA	-1.90	-0.14	-3.18	0.81	0.59
LEU_378_ALA	-2.13	-0.12	-3.42	0.81	0.59
VAL_379_ALA	-1.86	-0.14	-3.15	0.82	0.59
LEU_495_ALA	-1.97	-0.14	-3.27	0.84	0.59

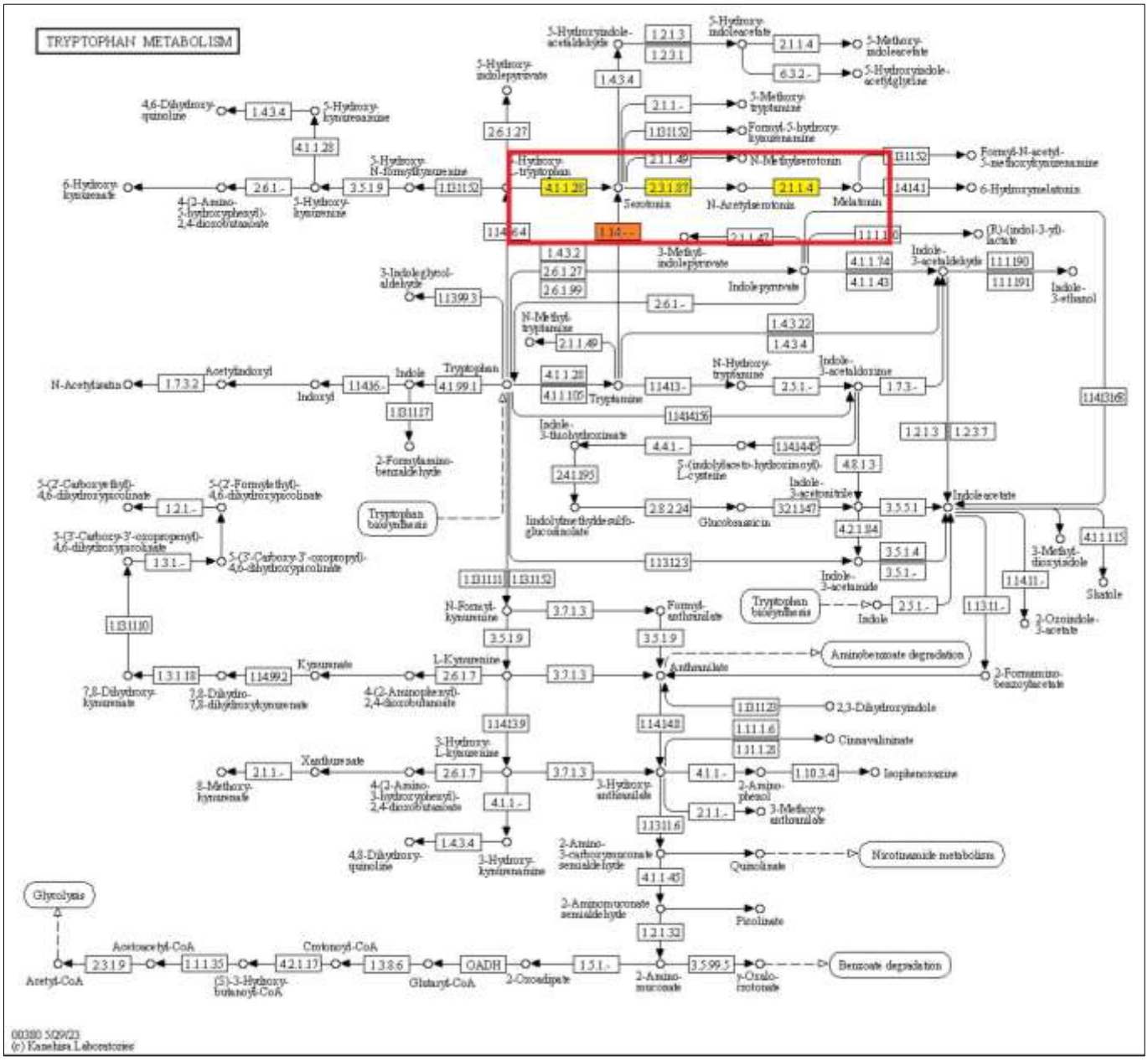

Fig 1: Biosynthetic pathway of serotonin and melatonin in rice

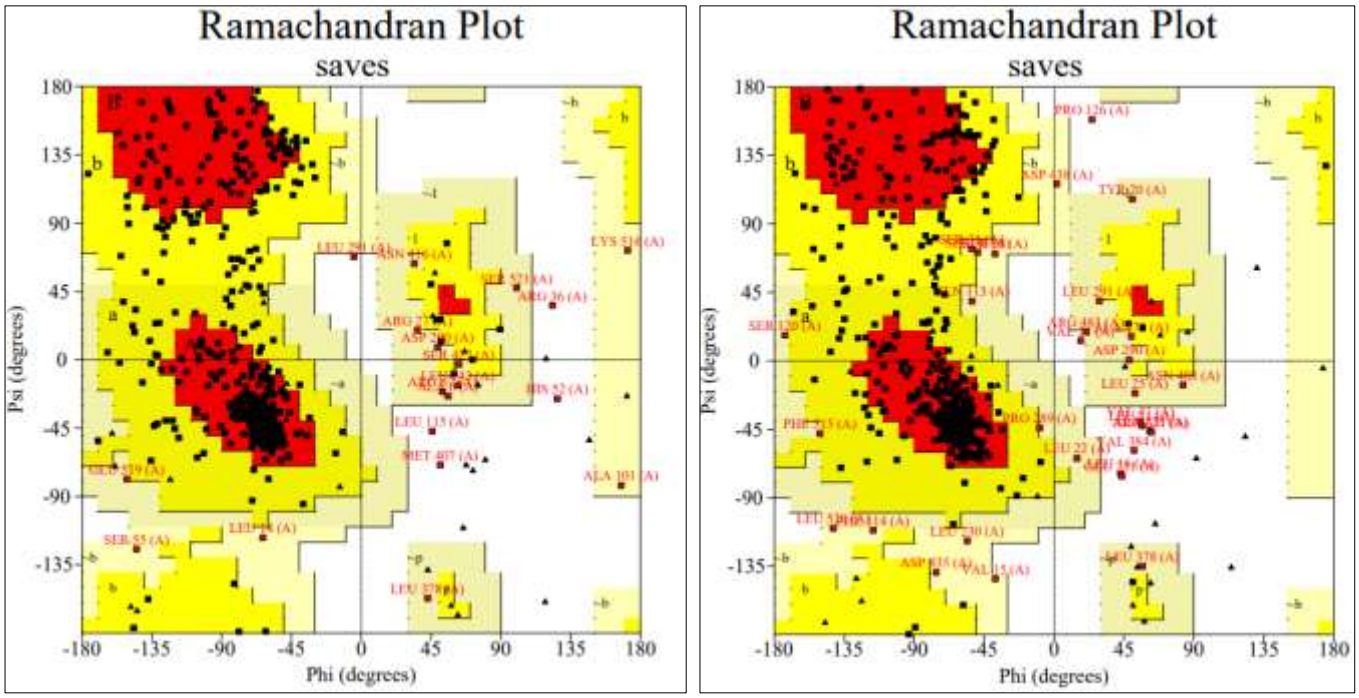

Fig 2: Validation using Ramachandran plot, Residues in favored regions- 331 (73.6%) and 334 (74.1%), Residues in additionally allowed regions-100 (22.2%) and 88 (19.5%), Residues in generously allowed regions-14 (3.1%) and 21 (4.7%) and Residues in disallowed regions- 5 (1.1%) and 8 (1.8%) for resistant and susceptible variety protein T5H respectively

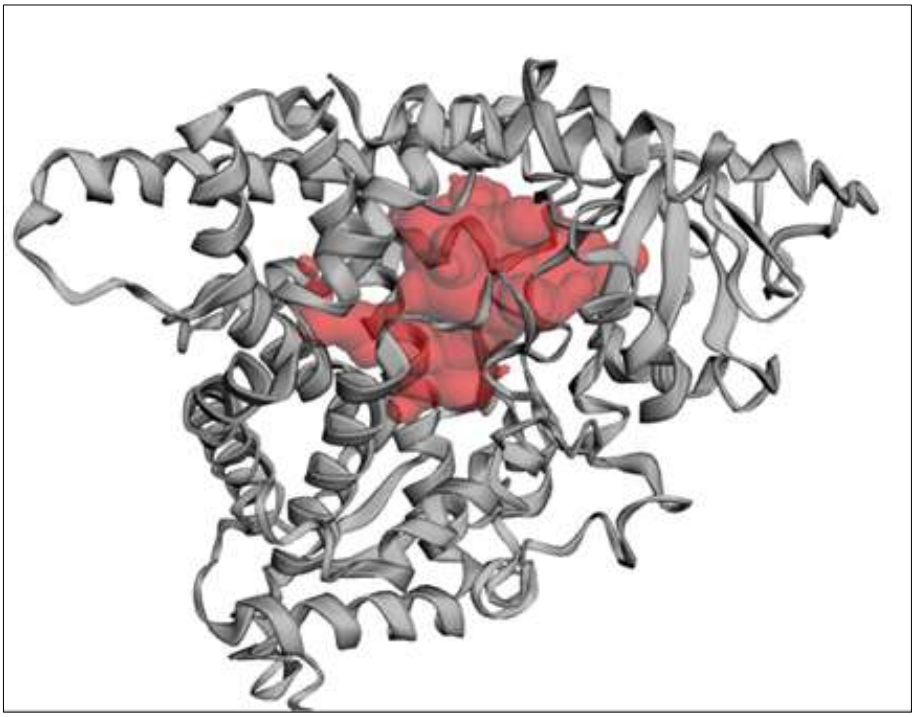

Fig 3: Binding pockets of resistant and susceptible protein T5H using CASTp

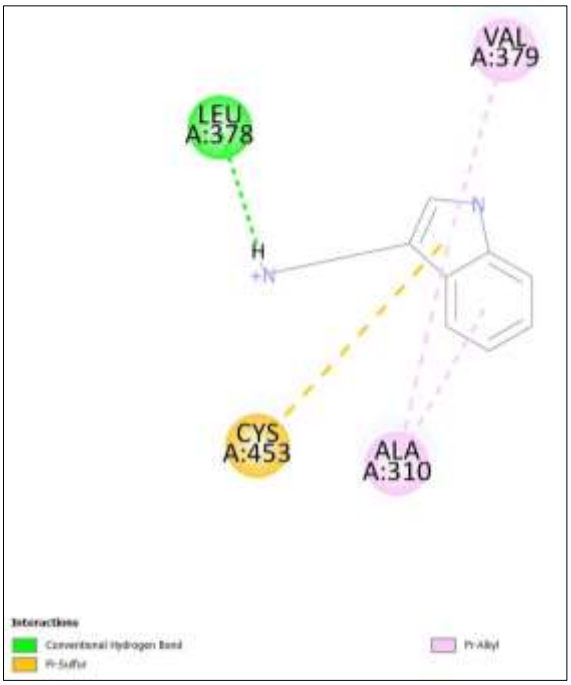

Fig 4: Interaction between tryptamine and T5H in resistant variety (TKM6)

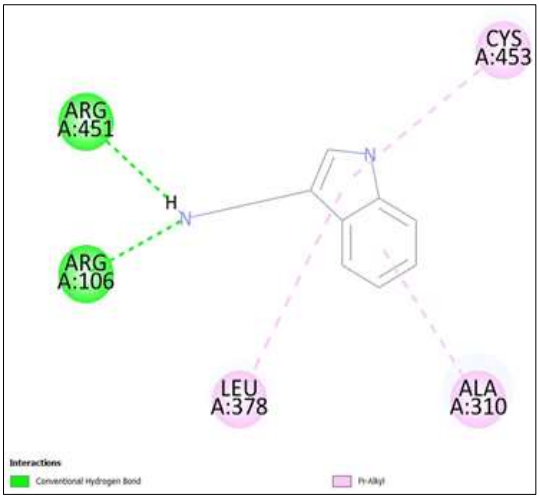

Fig 5: Interaction between tryptamine and T5H in susceptible variety (ASD16)

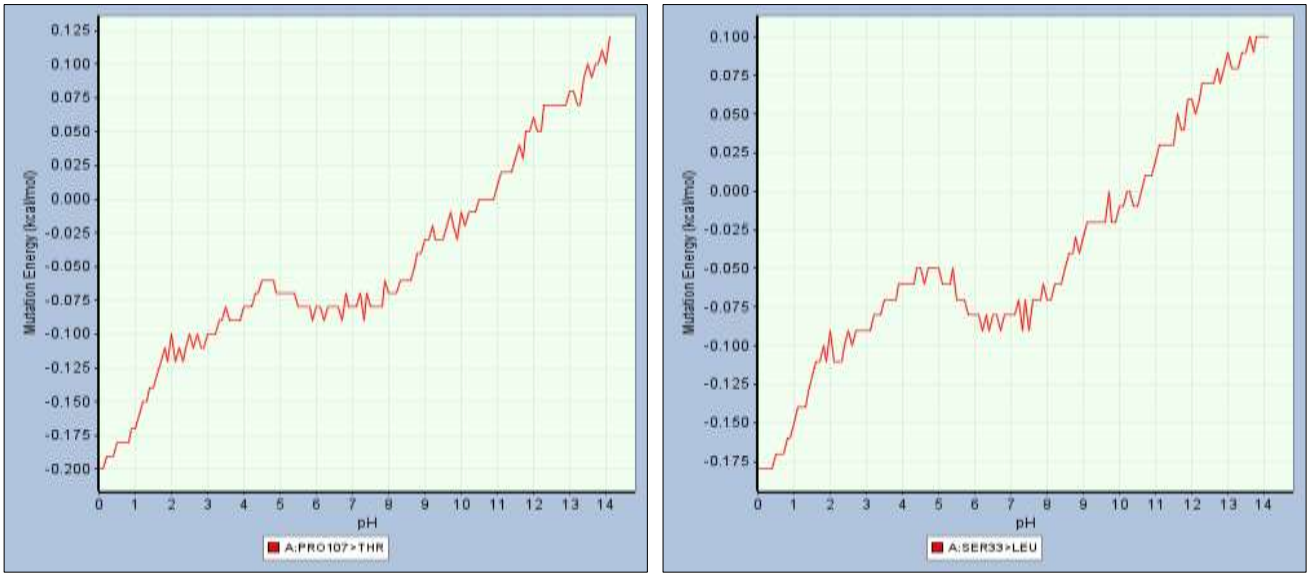

Fig 6: Stability of mutation binding energy for the mutations PRO107THR and SER33LEU are shown

4. Conclusion

The symptoms caused by the YSB in rice cultivation poses a significant threat to crop yield. Conventional management practices for YSB control have proven to be challenging. Therefore, a molecular approach involving gene manipulation emerges as a promising strategy to combat YSB infestation. In this study, we examined the functional SNPs of the gene CYP71A1 in the serotonin biosynthetic pathway in resistant and susceptible genotypes of YSB. The protein T5H encoded by CYP71A1 of susceptible variety (ASD16) possessing two functional SNPs were identified and modelled along with the resistant variety (TKM6) and comparative docking studies of the protein T5H with the tryptamine compound revealed a stronger interaction in susceptible variety (ASD16). These findings provide valuable insights into the functional mechanism of serotonin in pest resistance and highlight the significance of gene CYP71A1 in conferring resistance to YSB. Consequently, targeted genome editing of this region holds potential for developing YSB resistance in rice.

5. Acknowledgement

The fund granted as fellowship for Dhanvarsha. M by Department of Biotechnology, Ministry of science and technology, Government of India, New Delhi.

6. Conflict of interest

The authors declare no competing interests.

7. Reference

1. Kahani F, Hittalmani S. Genetic analysis and traits association in F_2 intervarietal populations in rice under aerobic condition. J Rice Res. 2015;3(152):2. Doi: http://dx.doi.org/10.4172/2375-4338.1000152
2. Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 2019;3(3):430-439. DOI: https://doi.org/10.1038/s41559-018-0793-y
3. Khan ZR. World bibliography of rice stem borers: 1794-1990. Int. Rice Res. Inst.; Philippines; c1999
4. Rahaman MM, Stout MJ. Comparative efficacies of next-generation insecticides against yellow stem borer and their effects on natural enemies in rice ecosystem. Rice Sci. 2019;26(3):157-166. DOI: https://doi.org/10.1016/j.rsci.2019.04.002
5. Sarwar MU, Ali AK, Ahmad NA, Tofique M. Expediency of different botanical products intended for managing the population of rice stem borers. Proc. 25th Pakistan Cong. of Zoology. 2005;1:1-3.
6. Zainab S, Ram B, Singh RN. Environmental effect on yellow stem borer, *Scirpophaga incertulas* (Walker) and rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) on rice crop. J Environ. Biol. 2017;38(2):291.
7. Padmakumari AP, Katti G, Sailaja V, Padmavathi CH, Lakshmi VJ, Prabhakar M, *et al*. Delineation of larval instars in field populations of rice yellow stem borer, *Scirpophaga incertulas* (Walk.). ORYZA-An International Journal on Rice. 2013;50(3):259-267.
8. Narain A, Kar MK, Kaliaperumal V, Sen P. Development of monosomic alien addition lines from the wild rice (*Oryza brachyantha* A. Chev. et Roehr.) for introgression of yellow stem borer (*Scirpophaga incertulas* Walker.) resistance into cultivated rice (*Oryza sativa* L.). Euphytica. 2016;209:603-613. Doi:10.1007/s10681-016-1633-2
9. Makkar GS, Bentur JS. Breeding for stem borer and gall midge resistance in rice. Breeding insect resistant crops for sustainable agriculture. c2017. p. 323-352. https://doi.org/10.1007/978-981-10-6056-4_11
10. Ishihara A, Hashimoto Y, Miyagawa H, Wakasa K. Induction of serotonin accumulation by feeding of rice striped stem borer in rice leaves. Plant Signal. Behav. 2008;3(9):714-716. https://doi.org/10.4161/psb.3.9.6456
11. Fujiwara T, Maisonneuve S, Isshiki M, Mizutani M, Chen L, Wong HL, *et al*. Sekiguchi lesion gene encodes a cytochrome P450 monooxygenase that catalyzes conversion of tryptamine to serotonin in rice. J Biol Chem. 2010;285(15):11308-11313. DOI: https://doi.org/10.1074/jbc.M109.091371
12. Ishihara A, Nakao T, Mashimo Y, Murai M, Ichimaru N, Tanaka C, *et al*. Probing the role of tryptophan-derived secondary metabolism in defense responses against *Bipolaris oryzae* infection in rice leaves by a suicide substrate of tryptophan decarboxylase. Phytochem. 2011;1;72(1):7-13. DOI: https://doi.org/10.1016/j.phytochem.2010.11.001
13. Byeon Y, Lee HY, Hwang OJ, Lee HJ, Lee K, Back K. Coordinated regulation of melatonin synthesis and degradation genes in rice leaves in response to cadmium treatment. J Pineal Res. 2015;58(4):470-478. https://doi.org/10.1111/jpi.12232
14. Yu Y, Lv Y, Shi Y, Li T, Chen Y, Zhao D, Zhao Z. The role of phyto-melatonin and related metabolites in response to stress. Molecules. 2018;23(8):1887. DOI: https://doi.org/10.3390/molecules23081887
15. Lu HP, Luo T, Fu HW, Wang L, Tan YY, Huang JZ, *et al*. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat. Plants. 2018;4(6):338-344. Doi:10.1038/s41477-018-0152-7
16. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annu. Rev. Med. 2009;60:355-366. DOI: https://doi.org/10.1146/annurev.med.60.042307.110802
17. Yadav CB, Bhareti P, Muthamilarasan M, Mukherjee M, Khan Y, Rathi P, *et al*. Genome-wide SNP identification and characterization in two soybean cultivars with contrasting mungbean yellow mosaic India virus disease resistance traits. PLoS One. 2015;10(4):e0123897. DOI: https://doi.org/10.1371/journal.pone.0123897
18. Joshi S, Tiwari SN. Screening of different rice germplasms against yellow stem borer (*Scirpophaga incertulas*); c2022.
19. Mohankumar S, Thiruvengadam V, Samiayyan K, Shanmugasundaram P. Generation and screening of recombinant inbred lines of rice for yellow stem borer resistance. NISCAIR-CSIR; c2008. p. 346-351.
20. Alam SN, Cohen MB. Durability of brown planthopper, *Nilaparvata lugens*, resistance in rice variety IR64 in greenhouse selection studies. Entomol. Exp. Appl. 1998;89(1):71-78. DOI: https://doi.org/10.1046/j.1570-7458.1998.00383.x
21. Padhi G. Evaluation of selected lowland and medium land rice cultivars against *Scirpophaga incertulas* (Wlk.). Oryza: An International Journal on Rice. 2009;46(2):174-176.
22. Catling HD, Islam Z. The problem of yellow rice borer in Asian deepwater rice. In Proceedings of the 1981

International Deep water Rice Workshop, Bangkok, Thailand; Manila: International Rice Research Institute; c1982. p. 451-458.
23. Patel KG. Screening of rice varieties against yellow stem borer, *Scirpophaga incertulas* (walker). Biosci. Trends. 2015;8(22):6260-6266.
24. Pawar MS, Reddy R, Murthu S. Scope for breeding rice varieties to major pests. InProc. Rice Res. Workers Conf. Cuttack; c1959. p. 304-305.
25. Okamoto D, Abe Y. Varietal differences in rice stem borer infestation of rice. Nagoya Engei (Agric. Hort.). 1958;33:58-59.
26. Panigrahi D. Reaction of late upland rice varieties against yellow stem borer, *Scirpophaga incertulas* under irrigated condition. J. Insect Sci. Ludhiana. 2012;25(4):350-354.
27. Subudhi HN, Padhi G. Field evaluation of rice cultivars against the yellow stem borer *Scirpophaga incertulas* (wlk.). Oryza: An International Journal on Rice. 2008;45(3):222-225.
28. Chatterjee S, Ghose M, Gangopadhyay C. Field screening of different rice entries against different insect-pests of rice during kharif season. IJAEB. 2016;9(4):667-671.
29. Du B, Zhang W, Liu B, Hu J, Wei Z, Shi Z, *et al*. Identification and characterization of Bph14, a gene conferring resistance to brown plant hopper in rice. PNAS. 2009;106(52):22163-22168.
DOI: https://doi.org/10.1073/pnas.0912139106
30. Devasena N, Soundararajan RP, Reuolin SJ, Jeyaprakash P, Robin S. Evaluation of rice genotypes for resistance to yellow stem borer, *Scirpophaga incertulas* (Walker) through artificial screening methods. J Entomol. Zool. 2018; 6(1):874-878.
31. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, *et al*. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 2011;7(1):539.
Doi: https://doi.org/10.1038/msb.2011.75
32. Anand P, Nagarajan D, Mukherjee S, Chandra N. ABS–Scan: Alanine scanning mutagenesis for binding *in silico* site residues in protein–ligand complex. F1000Research. 2014;3:1-18.
DOI: https://doi.org/10.12688/f1000research.5165.2
33. Pallavi D, Sharanabasappa MP. Relative abundance of yellow stem borer and pink stem borer on paddy. J Entomol. Zool. 2018;6(4):668-667.
34. Li H, Guan R, Guo H, Miao X. New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. Plant Cell Environ. 2015;38(11):2277-2285.
DOI: https://doi.org/10.1111/pce.12546
35. Behura NP, Sen P, Kar MK. Introgression of yellow stem borer (*Scirpophaga incertulus*) resistance genes into cultivated rice (Oryza sp.) from wild species. Indian J Agric. Sci. 2011;81(4):359-362.
36. Smigocki AC, Wilson D. Pest and disease resistance enhanced by heterologous suppression of a *Nicotiana plumbaginifolia* cytochrome P450 gene. Biotechnol. Lett. 2004;26:1809-1814.
DOI: https://doi.org/10.1007/s10529-004-4615-8
37. Kang S, Kang K, Lee K, Back K. Characterization of tryptamine 5-hydroxylase and serotonin synthesis in rice plants. Plant Cell Rep. 2007;26:2009-2015. DOI: https://doi.org/10.1007/s00299-007-0405-9
38. Dangol A, Shavit R, Yaakov B, Strickler SR, Jander G, Tzin V. Characterizing serotonin biosynthesis in *Setaria viridis* leaves and its effect on aphids. Plant Mol. Biol. 2022;109(4-5):533-549.
39. Ota N, Agard DA. Binding mode prediction for a flexible ligand in a flexible pocket using multi-conformation simulated annealing pseudo crystallographic refinement. J Mol. Biol. 2001;314(3):607-617.
DOI: https://doi.org/10.1006/jmbi.2001.5147
40. Jeffrey GA, Jeffrey GA. An introduction to hydrogen bonding. Oxford university press, New York; c1997.
41. Steiner T, Saenger W. Lengthening of the covalent O–H bond in O–H··· O hydrogen bonds re-examined from low-temperature neutron diffraction data of organic compounds. Acta. Crystallogr. B. Struct. 1994;50(3):348-357. DOI: https://doi.org/10.1107/S0108768193011966
42. Hunter CA, Sanders JK. The nature of. pi.-. pi. interactions. J Am. Chem. Soc. 1990;112(14):5525-5534.
DOI: https://doi.org/10.1021/ja00170a016
43. Gallivan JP, Dougherty DA. A computational study of cation− π interactions vs salt bridges in aqueous media: implications for protein engineering. J Am. Chem. Soc. 2000;122(5):870-874.
DOI: https://doi.org/10.1021/ja991755c
44. Raag R, Poulos TL. Crystal structures of cytochrome P-450 CAM complexed with camphane, thiocamphor, and adamantane: factors controlling P-450 substrate hydroxylation. Biochem. 1991;30(10):2674-2684.
DOI: https://doi.org/10.1021/bi00224a016
45. Guo X, Gao Y, Liu F, Tao Y, Jin H, Wang J, *et al*. A short-chain carbonyl reductase mutant is an efficient catalyst in the production of (R)-1, 3-butanediol. Microb. Biotechnol. 2023;16(6):1333-1343.
Doi: https://doi.org/10.1111/1751-7915.14249
46. Morrison KL, Weiss GA. Combinatorial alanine-scanning. Curr Opin Chem Biol. 2001;5(3):302-307.
DOI: https://doi.org/10.1016/s1367-5931(00)00206-4