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In Silico Identification of Potential Mimicking Molecules as Defense Inducers Triggering Melanin Biosynthetic Pathway in Magnaporthe Grisea

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Abstract—*Magnaporthe grisea is a major phytopathogenic fungi which cause Rice blast, a recalcitrant disease of Oryza sativa (rice) throughout the world, which is highly destructive and responsible for significant yield losses. Since no resistant source is available against Rice blast, therefore, efforts have been made in the present study to identify defense inducer molecules which can induce melanin pathway. This fungus produces a specialized structure called appressorium, infecting the plant. In this study. Trihydroxynaphthalene reductase (THNR) of M. grisea is used as drug targets against rice blast to inhibit the melanin pathway which is responsible for appressorium formation. In this study, high quality structural model of THNR was downloaded from Protein Data Bank (PDB). This was followed by 1000 analogs were virtually screened from ZINC database for interaction with THNR. After docking analysis, it was found that two analogs viz. ZINC66511493 showed more binding affinity with THNR as compared to ZINC47622465. ZINC66511493 possesses efficient, stable and good cell permeability properties. Based on the obtained results and its physiochemical properties, it is capable of mimicking melanin pathway and may be used as defense inducers for triggering melanin resistance against Rice blast, only after further validation through field trials.*

Keywords*: Magnaporthe grisea, Trihydroxynaphthelene Reductase, Scytalone Dehydratase, Virtual Screening.*

1. INTRODUCTION

Rice (*Oryza sativa*) is the most important staple food crop of grass (Poaceae) family that originated in India. It has been cultivated for more than 7000 years as a major crop, and it currently sustains more than half the world's population. It covers the world's largest area (28%) covering 42.3 million hectares with a total production of 80 million tonnes annually. But globally, it stands next to wheat in harvested area [1-2]. Rice is the basic food crop and being a tropical plant, it flourishes comfort in hot and humid climate. Rice is mainly grown in rain fed areas that receive heavy annual rainfall. That is why it is fundamentally a kharif crop in India [3]. It demands temperature of around 25 degree Celsius and above and rainfall of more than 100 cm. Rice is also grown through irrigation in those areas that receives comparatively less rainfall. Rice is the staple food of eastern and southern parts of India [4].

The natural environment for plants is composed of a complex set of abiotic stresses and biotic stresses [5]. Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels. The abiotic stresses like temperature (heat, cold chilling/frost), water (drought, flooding/hypoxia), radiation (UV, ionizing radiation), chemicals (mineral deficiency/excess, pollutants heavy metals/pesticides, gaseous toxins), mechanical (wind, soil movement, submergence) are responsible for over 50% reduction in agricultural production [6-7]. Abiotic stress conditions cause extensive losses to agricultural production worldwide, while biotic stress caused by living- organisms, both macro and micro organisms such as viruses, fungi, bacteria, weeds, insects and other pests and pathogens are a major constraint to agricultural productivity from fields to markets in the developing world [8-9].

M. grisea is the fundamental agent of Rice blast. The asexual stage of *M. oryzae* is described by the name *Pyricularia oryzae*) [10]. It is an extremely effective plant pathogen as it can reproduce both sexually and asexually to produce specialized infectious structures known as appressoria that infect aerial tissues and hyphae that can infect root tissues [11].

The present study aims to search for potent anti-fungal agents that could be developed as successful fungicides against the rice blast. The fungal agent targeted in this study is *M. grisea* [12]. The melanin biosynthetic pathway is a potential target for antifungal agent discovery. Fungal melanin can influence the immune response of the host. Melanin is

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critical to host invasion in plant pathogens as well. Fungi produce appressoria, structures that penetrate plant tissue, allowing the organisms to invade the host [13]. Melanin in the cell wall of these structures provides mechanical strength to the appressoria that aids in tissue penetration. Two pathways of melanin synthesis are found in fungi [14]. The first step, formation of 1, 3, 6, 8-tetrahydroxynaphthalene (1, 3, 6, 8-THN), is catalyzed by a polyketide synthase (PKS). After 1, 3, 6, 8-THN production, a series of reduction and dehydration reactions produce the intermediates scytalone, 1, 3, 8-trihydroxynaphthalene, vermelone, and finally 1,8-dihydroxynaphthalene (DHN). Polymerization of DHN leads to formation of melanin [7].

THNR is a key enzyme of melanin pathway of *M. grisea* and by inhibiting the pathway we can reduce the rate of reaction and checks the melanin production. So, the aim of this study is to identify the potential agrochemicals as a fungicides by inhibiting melanin biosynthesis pathway [10].

2. MATERIALS AND METHODOLOGY

Retrieval of target structures

Three dimensional structure of THNR protein is publicly available on Protein Data Bank (www.rcsb.org/pdb), a repository for the three-dimensional data of large biological molecules. THNR [PDBID: IYBV] was downloaded which are experimentally determined by X-Ray diffraction method. It structure consists of two chains i.e. A and B.

Retrieval of ligand molecules

Literature studies were followed to retrieve the 3D structures of ligand compounds from ZINC database with cut off of 50% structural identity [15]. We have found 1000 analog structures and these compounds were downloaded in sdf file format. Further, sdf file was converted into pdb using local python script and subsequently there was prepared pdbqt file of each ligand for virtual screening. Virtual screening was performed against THNR using AutoDock vina.

Molecular docking approach

Molegro Virtual Docker (MVD) was used for docking studies. MVD requires a three 3D-structure of both protein and ligand molecules. It performs flexible ligand docking, so the optimal geometry of the ligand is determined during the docking. The candidates with the best conformation and energetic results were selected for further analysis. MVD was used to calculate the interaction energies between three dimensional macromolecular structures of ligands and proteins.

3. RESULTS AND DISCUSSION

Among various diseases, Rice blight is the most destructive with no known source of resistance available so far. The chemical control of disease is biohazardous and costly. It is believed that the disease can be controlled successfully by developing some novel fungicides in the form of defense inducers which can mimick the action of various plant hormones in triggering the plant's own immune response. Traditionally, necrotrophic fungal pathogens have been shown to be the primary activators of melanin synthesis pathway through activation of the receptor, THNR.

In the present study, computational approaches have led to identification of defense inducer molecules for triggering melanin biosynthetic pathway through interaction with THNR against the infection of Blast species on Oryza crops. Therefore, a 3D structural model of THNR was downloaded and analyzed through a number of computational tools to accesses the stereo chemical quality.

Virtual screening can provide precious support in discovery of novel lead molecules. In several drug discovery projects, the virtual screening technology has been the key contributor to find out new ligand molecules on the basis of biological macromolecular structures and their binding site residues. The predicted binding site area of THNR was targeted in present study to evaluate the binding affinity of ligand molecule and its structural analogs through virtual screening. Total of 1000 molecules was screened to investigate the new molecules.

The top identified molecule ZINC66511493 (3-carbamoylphenyl) formed eight hydrogen bonds with amino acid residues ARG85, ARG121, ARG349, ARG410, and ARG500 whereas the amino acid residues such as ARG85, ALA86, MET88, PHE89, LEU91, ARG349, TYR387, ARG410, VAL412, ALA444, TYR446, LEU471, GLU498, ARG500, and TRP523 were also interacted through hydrophobic bonding (Fig. 1). The second top identified molecule ZINC47622465 (1-[[3-(piperidine-1-carbonyl) phenyl] also form eight hydrogen bond with amino acid residues ARG85, ARG121, ARG349, TYR387, and ARG410 whereas the amino acid residues ARG85, MET88, PHE89, ARG349, ALA385, TYR387, ARG410, VAL412, ALA444, TYR446, LEU471, and ARG500 were also interacted through hydrophobic bonding (Fig. 2).

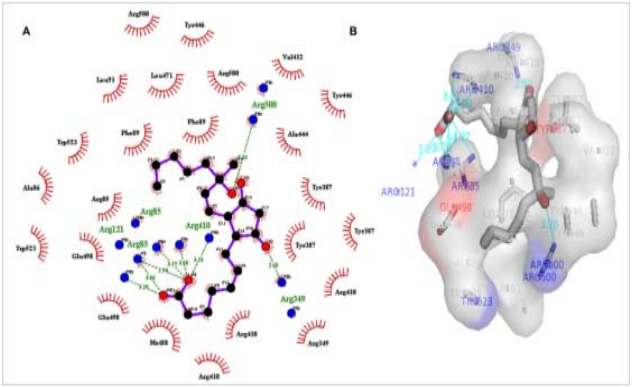

Fig. 1: (A) 2D and (B) 3D representation of docked structure of THNR, depicted H-bond interaction with ZINC66511493 generated by Ligplot and pyMOL software.

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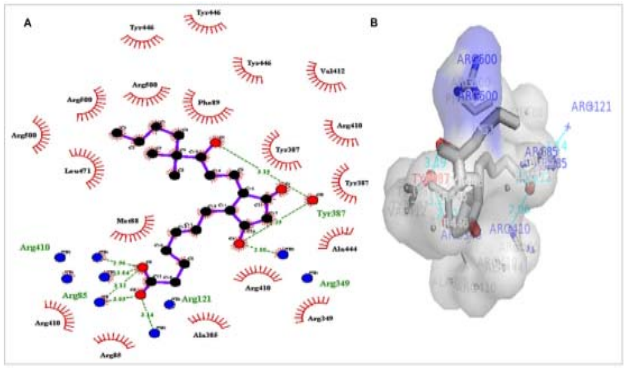

Fig. 2: (A) 2D and (B) 3D representation of docked structure of THNR, depicted H-bond interaction with ZINC47622465 generated by Ligplot and pyMOL software.

Hydrogen bond calculation analysis showed that the amino acid residues TYR446, LYS496, and ARG85 plays key role during melanin biosynthesis stabilization, whereas, Ala86 provides the stability during COI1-ZINC66511493 binding and ALA87, TYR387, GLU351 plays key role during the stabilization of THNR-ZINC47622465 complex. The results of PCA suggest that the binding of ZINC66511493 and ZINC47622465 is novel as compare to other molecule. As per Lipinski's rule of five a drug will illustrate good ADME (absorption, distribution, metabolism, and excretion) properties if it's logP value is less than 5, Hydrogen bond donor should be less than 5, Hydrogen bond acceptor should be less than 10 and Molecular weight should be less than 500 (Lipinski et al., 2001). A molecule has less than 140 Å of PSA showed good cell membrane permeability.

In 1960s more than 1 kg of agrochemical was generally applied per ha due to lack of knowledge about the potential molecular target, today the use rates can be considerably reduced as 10 g/ha, it is only 1% of that previously required because of advances in structural biology and use of bioinformatics tools for identification of novel, efficient and potent molecules. The results of present study clearly revealed that the ZINC66511493, could act as a lead molecule as defense inducer for the prevention and management of Rice Blast disease of Oryza. ZINC66511493 is showed greatest binding affinity along with hydrogen bond interaction as compare to other compounds selected in this study, it could cross cell membranes due to ideal logP value and low molecular weight as well as its hydrophobic nature, and are able to triggering melanin biosynthetic pathway in Oryza by interaction with THNR for production of antimicrobial compounds to develop a resistant systems that controlling crop systems and maintaining its integrity during Oryza–Blast Interaction to destroy effect of Blast toxins. It might be also useful for protection of other crops against the infection of plant pathogens.

4. CONCLUSION

The present computational study provides an insight about the interactions between defense molecule and its analogs with THNR of *M. grisea*. ZINC66511493 showed greater binding affinity as compare with ZINC47622465. Our finding suggests that the ZINC66511493 is able to work as mimicking molecule to triggering melanin biosynthetic pathway during *Magnaporthe* infection to prevent and manage Rice blast for securing food and nutritional security of the rapidly growing world population. However, field trial is required to validate its efficacy and potency to provide new molecule for farmers that will directly replace the use of hazardous fungicide.

5. ACKNOWLEDGEMENT

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