ISSN(Online) : 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Special Issue 7, October 2015

In silico prediction of interactionsbetween MAPK and WRKY proteins in coconut

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ABSTRACT: Mitogen-Activated Protein Kinase (MAPK) cascade is one of the most important and crucial conserved signaling mechanisms found in all eukaryotes. It is at the heart of a molecular cell signaling network that necessary for the proliferation, growth, differentiation and survival of all cell types. This unique and essential phosphorelay signaling mechanism, also involved in various developmental and vital processes in plants, consists of three components such as MAPKKK, MAPKK and MAPK. WRKY transcription factors are one of the largest families of transcriptional regulators found throughout the green lineage. This key regulator is involved in many biological processes such as senescence, dormancy development, drought tolerance, response to wounding, metabolism and hormone signaling and cold. Protein-protein interactions between different WRKY-MAPK are involved in generating tolerance against abiotic/biotic stresses.We have modeled two MAPKs and twelve WRKY protein sequences derived from coconut by homology modeling and protein-protein docking were performed to predict the potential interacting partners of MAPK and WRKY. We found that all WRKYs are potentially common interacting partners for MAPK group A. Prediction of protein-protein interaction networks could be used for designing signal transduction pathways and to find targets for responses against biotic/abiotic stresses.

KEYWORDS: Mitogen-Activated Protein Kinase (MAPK), WRKY, homology modeling, docking

I. INTRODUCTION

Protein-protein interactions are essential for virtually all cellular processes comprising of complex mechanisms, some are transient and temporal and specific to certain environmental condition, growth and developmental stages of the cell, whereas others stably persist throughout most cellular conditions [1]. Mitogen Activated Protein Kinase (MAPK) cell cascades are extremely conserved andwell-studied signaling modules in all eukaryotes [2]. MAPKs phosphorylate the interacting proteins with three components of protein kinases such as MAPKs, MAPK Kinases (MAPKKs) and MAPKK Kinases (MAPKKKs). In plants, MAPK pathways are involved in the regulation of various processes such as defense responses against bacterial and fungal pathogens, abscission and programmed cell death in responses to a diversity of environmental signals [2],[3]. Several MAPK cascades are induced by different stresses such as cold, drought, salinity and heavy metals and mediate transduction of signals from cell surface to nucleus [4]. Elucidation of the three dimensional models of all members of MAPK family have revealed that they are structurally similar and functionally conserved in nature. MAPK functions by various protein-protein interactions and post translational modifications [5]. MAPK are grouped into different classes; A, B, C and D. MAPK3,6 and 10 belong to group A, group B include MAPK4,5,11,12,13, group C members are MAPK1, 2, 7, 14 and group D include MAPK 8,9,15,16,17,18,19 and 20 kinases [6].WRKY genes are one of the largest transcription regulators families in plants and shape vital parts of signaling webs that alter many plant processes. A single WRKY transcription factor might be involved in the regulation of several apparently different processes [7]. These regulatory factors contain an N-terminal end of WRKYGQR amino acid sequence and a zinc-finger motif and regulate the expression of target genes having W-box elements (C/T)TGAC(C/T) in the promoter regions by particularly binding to the (C/T)TGAC(C/T) sequence [8]. *WRKY* genes are involved in response to environmental stimuli, abiotic stresses, plant growth and regulating antimicrobial/mechanical injury pathways, which showing its complex and important role in regulation [9].Studies have shown that MAPK phosphorylate WRKY transcription factors, and this phosphorylation-dephosphorylating state of protein may transform the interacting partners and patterns [10]. AtWRKY33, an *Arabidopsis* transcription factor, is important for plant resistance to necrotrophic pathogens [10]. AtWRKY 25 and 33

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interact with MKS1 (MAP KINASE SUBSTRATE 1) and also with MAPK4 [11]. In rice, studies using yeast two hybrid system have identified that upon elicitor treatment such as salicylic acid,jasmonic acid(JA) and hydrogen peroxide, a number of proteins OsWRKY33 interact with OsBWMK1 MAPK involved in SA-dependent defense responses [12]. Information regarding interaction of MAPK and WRKY is still scarce. In order to understand their protein-proteininteraction,molecular modeling and docking approachescan play a vital role. To the best of our knowledge, there has not been any work carried out to comprehensively address the interaction of MAPKs with WRKY proteins in coconut. In this study, we have performed *in silico*analysis for studying MAPK-WRKY protein- protein interactions.

II. MATERIALS AND METHODS

Sequence retrieval: The MAPK and transcription factor WRKY protein sequences of coconut were retrieved from publically available NCBI database (http://www.ncbi.nlm.nih.gov/) and the Accession No.are provided inTable 1.

Table 1: Coconut MAPK and WRKY protein sequences with their accession numbers

MAPK/WRKY	Accession No
MAPK1	580402
MAPK2	FJ200378
WRKY1	gi|83596311
WRKY2	gi|83596313
WRKY5	gi|83596317
WRKY6	gi|83596319
WRKY7	gi|262088594
WRKY10	gi|83596321
WRKY12	gi|262262684
WRKY13	gi|83596323
WRKY21	gi|83596329

In silico homology modeling: The three dimensional structures of MAPK and WRKY transcription factor were derived by using MODELLER (http://www.salilab.org/modeller/) 9v7 program. For selection of templates for homology modeling, PSI BLAST was performed against the PDB database (http://www.pdb.org/pdb/home/home.do). Hits with > 30% sequence identity were selected.The generated 3D models were refined with the help of loop refinement (MODELLER and looper algorithm based) and side chain refinement protocols. Structural refinement through energy minimization was performed using energy minimization tool keeping parameter value constant for all structures. Elucidation of model quality was performed by drawing Ramachandran plot. 'Prepare protein protocol' was finally run on protein models. The protocol performs some of the following restructuring: 1) optimizes side-chain conformation for residues with inserted atoms 2) removal of water molecule etc. Kinase specific phosphorylation sites were assessed using KinasePhos (http://KinasePhos.mbc.nctu.edu.tw/), which identifies serine, threonine and tyrosine phosphorylation sites.

Protein-protein docking: It plays a crucial role in interaction between two proteins and prediction of global binding energy. Fully modeled, refined structure of coconut MAPK1 and MAPK2 were taken as receptors and the WRKYs considered as ligands for the docking interaction studies on the online server FireDock (http://bioinfo3d.cs.tau.ac.il/FireDock/) and PathDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/).

III. RESULTS AND DISCUSSION

Based on SMART domain analysis programme, two predicted domains were clearly spanned in coconut MAPK1 protein. One wasEDR1 (PF14381), ethylene responsive protein kinase domain (1-207aa) and the other one S_TKc (SM00220), serine-threonine protein kinase catalytic domain (412-666 a. a.). Similarly MAPK2 protein was comprised of two domains such as PKinase_Tyr (10-136aa, PF07714). Kinase phosphorylation sites were predicted in two of

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these MAPK proteins and isprovided in Fig.1. The structures of MAPK1 were phosphorylated at Serine (S) at 10 sites of different positions, one Threonine(T) and Tyrosine (Y)of occupied domain (Fig.1a). MAPK2 phosphorylation site analysis revealed the presence of single phosphorylated aminoacid Serine (S), Threonine (T)and Tyrosine (Y) (Fig.1b).

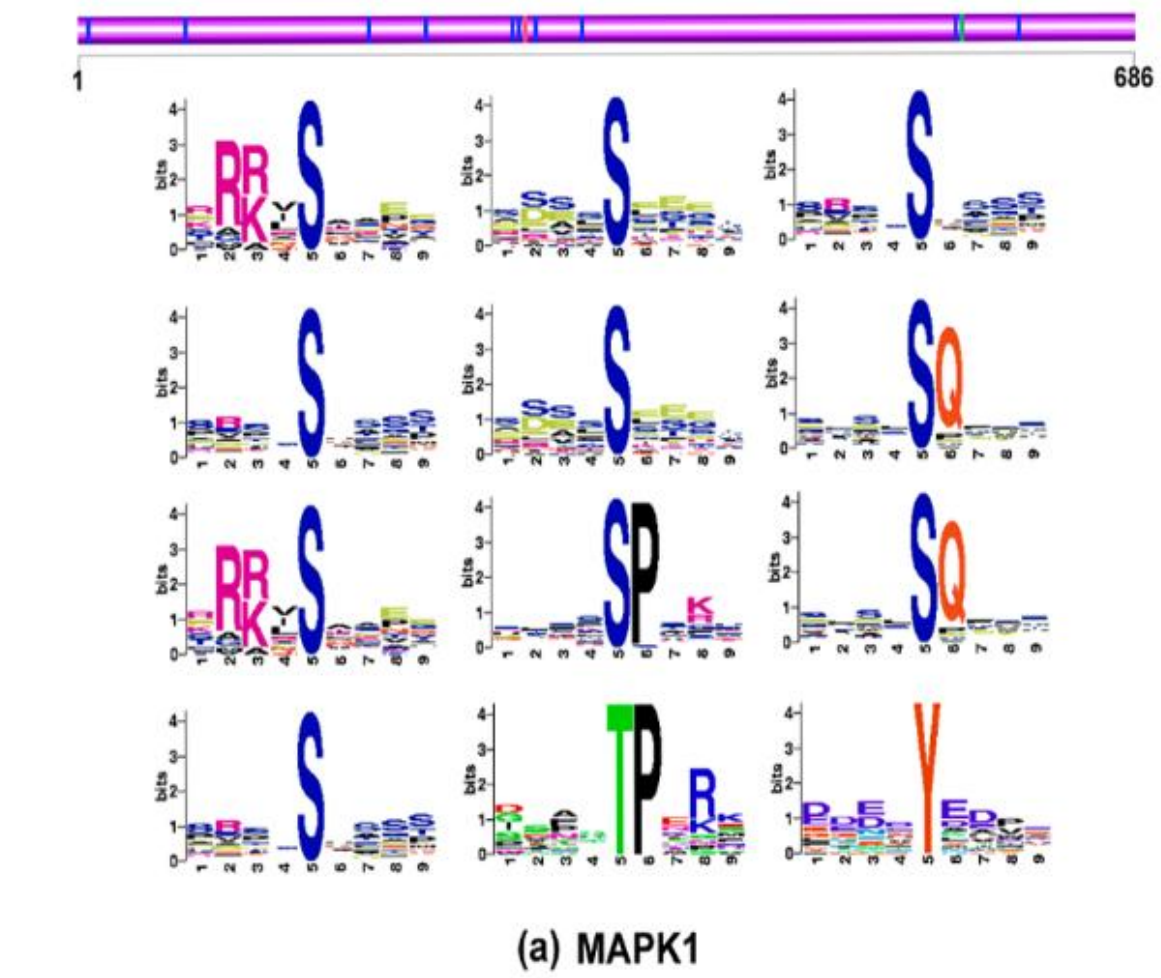

Fig. 1.Kinase phosphorylation site analysis of coconut MAPKs; (a)MAPK1 protein having ten predictedsites of Serine (S), only one site of Threonine (T) and Tyrosine (Y) (b) MAPK2, contained single site of each S,T and Y amino acid residues.

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Due to the lack of an available X-ray crystal structure of coconut MAPKs, we constructed MAPK1 and MAPK2 homology models based on the structures of its close homologues. Selected templates, their PDB IDs and their identity with target proteins isshown in Table 2. The secondary structure prediction of MAPK1, MAPK2 and WRKY2 are presented in Fig.2 (a-c). Like mammalian MK5 [13], predicted models coconut MAPKs have an N-terminal end consisting of a conserved α-helix, a bundle of β-sheets, and a conserved P-loop. The C-terminal end is largely helical and contains the activation segment, including Thr164 (S_TKc domain of MAPK1) and Thr103 (PKinase domain of MAPK2) which becomes phosphorylated when MAPK1 is activated. ATP binding site were seen in the hinge region, whichis the space where two lobes are connected. The most prominent differences between the models are seen in the C-terminal activation segment and shapes of the projected ATP binding pockets (Fig. 3).In turn, the resolved 3D structure of coconut WRKY protein consisted of β-sheet strands, forming a zinc-binding pocket with the zinc ion bound by two cysteine and two histidine residues, where the WRKYGQK residues exist. This might be the interactive domain of WRKYs corresponding to most N-terminal- β-strand(Fig. 3).

The Ramachandran plot from PROCHECK showed that the modeled MAPK and WRKY proteins possessed more than 90% of the aminoacids in favoured/allowed regions and none of them were in disallowed regions. The stereochemical qualities of the proposed models were assured by Errat together with PROCHECK results indicating that all 3D models were highly acceptable (Fig.3).

Table 2: Selected template proteins, PDB ID and sequence identity towards target coconut MAPKs and WRKYs

Protein	Template	PDB ID	Identity (%)
MAPK1	Serine-threonine protein kinase CTR1	3p86_A	77
MAPK2	MAPK1,structure of a complex[ERK2-PEA15]	4iz5_A	63
WRKY1	Crystal structure of AtWRKY1 protein	2ayd_A	65
WRKY2	Crystal structure of AtWRKY4 protein	1wj2_A	75
WRKY5	Crystal structure of AtWRKY1 protein	2ayd_A	55
WRKY6	Crystal structure of AtWRKY4 protein	1wj2_A	81
WRKY7	Crystal structure of AtWRKY4 protein	1wj2_A	72
WRKY10	Crystal structure of AtWRKY4 protein	1wj2_A	78
WRKY12	Crystal structure of AtWRKY4 protein	1wj2_A	77
WRKY13	Crystal structure of AtWRKY1 protein	2ayd_A	52
WRKY21	Crystal structure of AtWRKY1 protein	2ayd_A	55

The MAPKcascade plays a central role in regulating plant growth and development through protein-protein interactions [14]. Here, we predicted MAPK protein partners through docking studies by taking the global binding energies of interacting partners. Different MAPK interact with downstream WRKY TF interacted with global binding energies ranges from -10 to -25 Kcal/mol(Table 3).Protein-protein interactions comprise the signaling pathways that coordinate diverse cellular functions including senescence, in response to biotic/abiotic stresses, dormancy and germination of seeds [15]. The paper focuses onidentifying different WRKY interacting partners oftwo of MAPKs of coconut.Five closely related coconut WRKY TFs, 2, 6,7,10 and 12 homologous to AtWRKY4 play a positive role in plant resistance toward necrotrophic pathogens, and showed increased susceptibility toward the fungus *Botrytis cinerea*[16]. WRKY1 homologous to AtWRKY1 and VvWRKY1 resulted in resistance to the necrotrophic fungi *Alternariatenuis, Botrytiscinerea* etc. [16]. WRKY6 plays a major role in abiotic/biotic stresses [17]. Coconut WRKY TFs, showing protein-protein interaction network with MAPKs, are shown in Fig.5.

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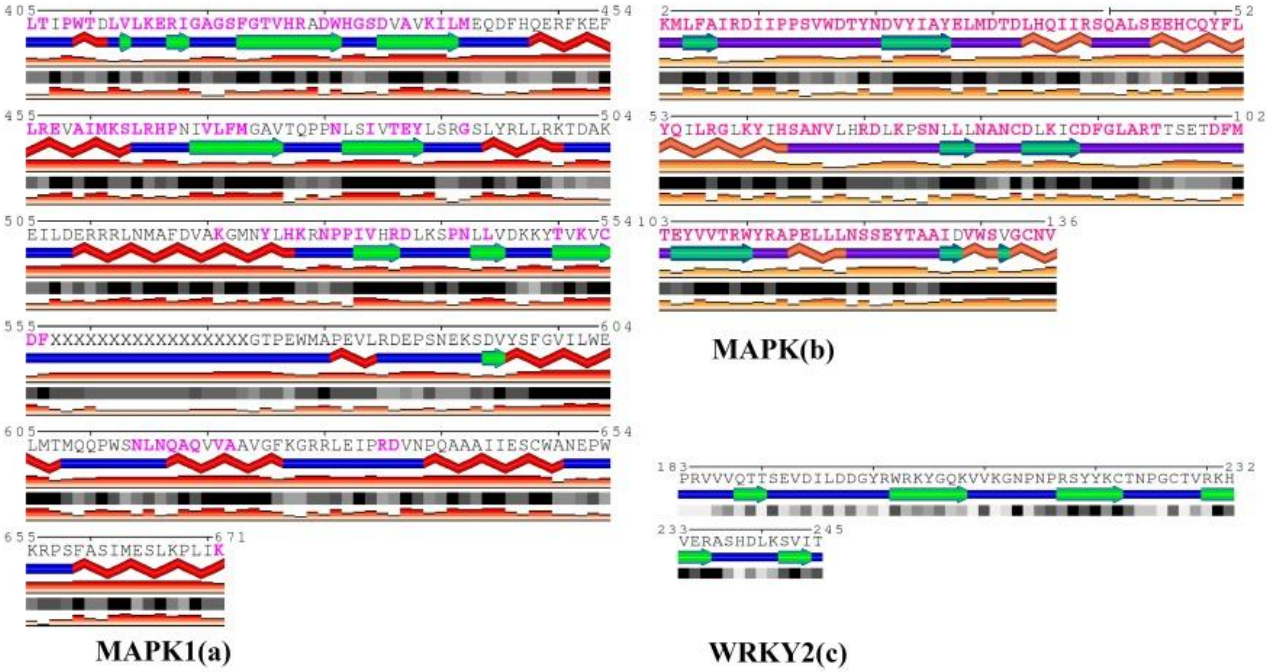

Fig. 2. Secondary structure analysis of MAPK1, MAPK2 and WRKY2

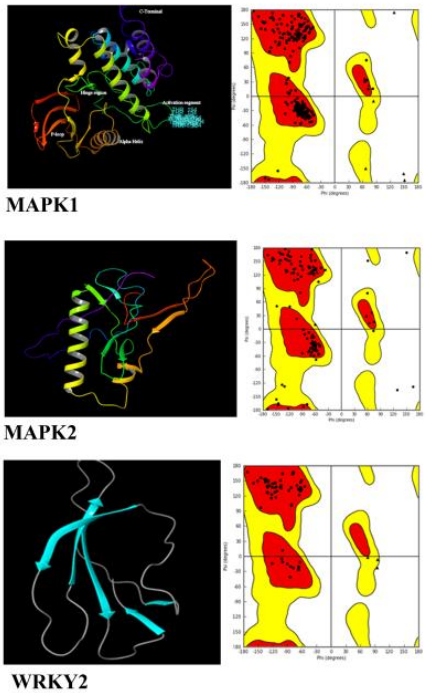

Fig. 3. Final back bone of MAPK1 homology model based on the template (PDB ID: 3p86_A). MAPK1 clearly depicts the hinge region and form ATP binding site. WRKY protein (with template PDB ID: 1wj2_A) shown in figure consist of array of β-strands.

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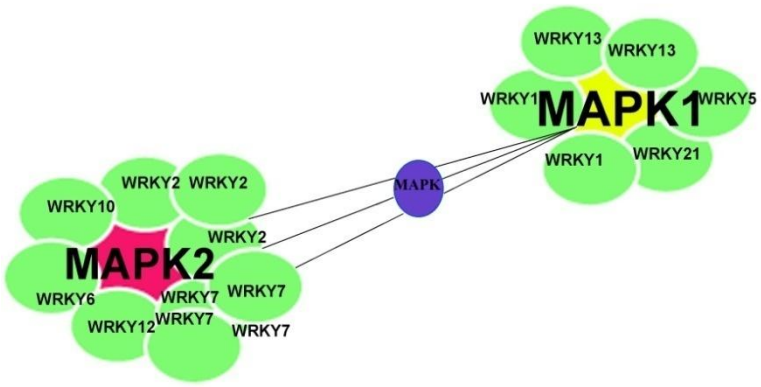

Fig. 5.Protein-protein interaction network of the MAPK (Group A-yellow, Group C-pink colour), with downstream WRKY transcription factors family (light green colour) in coconutwas created by STRING protein network interaction server (http://string-db.org/newstring_cgi/).

Table 3: The range of global binding energy obtained by molecular docking of MAPKs with WRKYs

Serial No.	MAPKs	Global binding energy in (Kcal/Mol) of MAPK with WRKY TF Family
1	MAPK1	-10.01 to -59.83
2	MAPK2	-25.06 to-49.23

IV. CONCLUSION

In this present work, we have predicted the protein-protein interactions between MAPK and downstream WRKY transcription factor by modelling and docking approach. The present study provides protein network of interaction with binding energies in coconut, which can form the basis for future studies in finding out targets in response to various biotic and abiotic stresses.

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