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In-silico Approaches for Studying Cross-talk of Different Kinases Associated in Diverse Biological Processes with their Interacting Substrates Partners

Gohar Taj*, Payal Agarwal and Anil Kumar

Molecular Biology and Genetic Engineering, College of Basic Science and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar (Uttrakhand)

Abstract

The Signal transduction pathway uses docking interaction with kinases which may also be regulated by phosphorylation. Regulation of these docking interactions by phosphorylation allows an additional level of control over the diverse biological processes including Crosstalk induction of defense. Amino acid sequences surrounding the phosphorylation motif have been extensively studied. However, the present study, attempts have been made to identify the presence of important determinants/motifs for substrate specificity of Mitogen activated protein kinase (MAPK), CaM kinase II, Protein kinase C, Receptor tyrosine kinase in the 54 identified MAPK 3 and MAPK 6 substrates of *Arabidopsis thaliana*. All identified substrates do not possess the known (p) S/ (p) T-P phosphorylation sites. Out of 54 substrates, 47 have S/T-P site and 7 are lacking such sites for interaction. Likewise 8 shows XXRRX(p)S pattern, 28 shows HYDXRXX(p)SXX pattern , 35 shows HYDXXRXX(p)S pattern, 12 shows R/$K_{(1-3)}$X(p) S/T(HYD)R/$K_{(1-3)}$ pattern and 17 shows $Na_{(1-3)}$-X-(p)Y-XX-HYD pattern. In order to ascertain the role of surrounding hydrophobic amino acids in the interaction, the other conserved pattern/Motif were also identified in the MAPK substrates which include XXRRX(p)S, HYDXRXX(p)SXX, HYDXXRXX(p)S, R/$K_{(1-3)}$X(p)S/T(HYD)R/$K_{(1-3)}$, $Na_{(1-3)}$-X-(p)Y-XX-HYD (Na-Hydrophilic; HYD- Hydrophobic; X- Any amino acid) pattern. These conserved patterns show activation sites for other kinases viz. MAPK Activated Protein kinase-1, MAPK Activated Protein kinase -2, CaM Kinase II, Protein kinase C, Tyrosine protein kinase respectively. Identification of interacting partners based on the surrounding amino acids of phosphorylation sites could be useful in the understanding of such complex hierarchical networks involved in controlling the defense signaling pathways.

Keywords: MAPK3 and MAPK6 Substrate; Kinases; Phosphorylation site; Conserved pattern; Homology; Crosstalk

Introduction

Phosphorylation is catalyzed by protein kinases (pks) and activity of these kinases are associated with many biological processes such as development, cell division, cell death, response towards biotic and abiotic stimuli [1]. Considering these reasons, it is important for better understanding how protein kinase select and recognize their interacting partner. Numerous researches have been performed to elucidate the intrinsic mechanisms of phosphorylation in many life phenomena such as cell cycle [2,3]. Protein phosphorylation can occur on serine, threonine and tyrosine residues, as well as histidine and aspartate residues in the case of two-component phosphorelays [4]. Kinases and phosphatase both recognize their substrates through different patterns, or motifs, present near the phosphorylation site in the amino acid sequence of the substrate [5,6].

Phosphorylation usually results in a functional change of the target protein (substrate) by changing enzyme activity, cellular location, or association with other proteins. There is more than 500 protein kinase [7] and ATP binding site in kinases is highly conserved. The three-dimensional structures of several protein kinases, some with bound substrates and nucleotides, have been determined [8]. All protein kinases show a common fold, consisting of two lobes hinged through a short linker region. The extra cellular domain serves as the ligand-binding part of the molecule. The intracellular or cytoplasmic domain is responsible for the (highly conserved) kinase activity, as well as several regulatory functions [9].

The numbers of known protein kinases have increased at an ever-accelerating pace, it has become more challenging to determine which protein kinases interact with which substrates in the cell and also which kinase interact with other kinase. Likewise there are some Kinases which have mixed kinase activities, like MAP Kinase Kinase (MAPKK) dual specific kinase is involved in the MAP kinase cascade, are a mixed serine/threonine and tyrosine kinase. Some of the kinase deal within this study are Mitogen Activated protein kinase(MAPK), Protein kinase C(PKC), Ca/Calomodulin dependent kinase II(CaM Kinase II), Tyrosine protein kinase, MAPK Activated protein kinase-1 & MAPK Activated protein kinase -2.

The Protein kinase C also known as PKC is a family of enzymes which is involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PKC enzymes play an important role in several signal transduction cascades. Beside this, Ca^{2+}/calmodulin-dependent protein kinases II or CaM kinases II are serine/threonine-specific protein kinases which are primarily regulated by the Ca^{2+}/calmodulin complex and reveals a memory effect on activation, likewise, Tyrosine kinase protein play role in disease resistance signaling. The Receptor Tyrosine Kinase family is present more abundantly in animal then in plant [10]. By searching for Tyr-kinase-specific sequence motifs, several dual specific kinases (DSKs) are identified but no true tryrosine-specific kinases are found in plant [11]. MAPK activated proteins kinase is well known in animal system rather in plant, get

***Corresponding author:** Dr. Gohar Taj, Molecular Biology and Genetic Engineering, College of Basic Science and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar -263145, Dist. Udham Singh Nagar (Uttrakhand), Tel: +91-5944-233898; Fax: +91-5944-233473; E-mail: gohartajkhan@rediffmail.com

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activated by MAPK, and regulates cell growth and survival in response to hormonal signals [12].

The Mitogen activated protein Kinases (MAPKs) a class of protein kinase shown to play pivotal roles in eukaryotic systems establishes a cognation between sensors and the intrinsic responses, and leads to changes in cellular organization or altered gene expression [13]. A surprisingly large number of genes encoding MAPK pathway components have been uncovered by analyzing model plant genomes. Among them MAPK3 and MAPK 6 are best characterized and are closely related proteins which shows high level of functional redundancy. They play an important role in the development of induced resistance to biotic and abiotic stress in plants [13] and also act as a positive intercessor of defense responses [14]. Their key role in plant growth and development has already been explained.

Until recently it was viewed that signal transduction work as cascade (Simple chain of consecutive states). However, recent research has focused on the divergence, crosstalk, and redundancy between signaling pathways [15,16]. The known MAPK substrate

S.No	Accession number	Gene Code	Function	Substrate of MAPK3/ MAPK6
1	AY091243	AT5G23200	Unknown protein	MAPK3
2	BT001177	AT2G18020	60s ribosomal protein l2	BOTH
3	BT002083	AT5G58620	Putative protein	BOTH
4	BT026422	AT2G40510	40s ribosomal protein s26	BOTH
5	BT025612	AT5G02560	Unknown protein	MAPK3
6	NM_102437	AT1G26740	Structural constituent of ribosome	MAPK3
7	BT008538	AT1G77450	Grab1-like protein	BOTH
8	BT006316	AT1G02840	Ribonucleoprotein sf-2 like protein	BOTH
9	BT025609	AT3G11510	Putative 40s ribosomal protein s14	BOTH
10	BT002367	AT5G19290	Phospholipase-like protein	BOTH
11	NP_001031374	AT2G19730	Putative ribosomal protein l28	MAPK3
12	AAP37774	AT4G03260	Putative protein phosphatase regulatory subunit	MAPK3
13	AAP21361	AT4G39880	Ribosomal protein l23 family protein	BOTH
14	AY096731	AT4G15000	Ribosomal protein	MAPK3
15	AY122957	AT5G48990	Kelch repeat-containing f-box family protein	BOTH
16	NP_196341.1	AT5G62070	Calmodulin binding protein	MAPK3
17	NP_190462.1	AT3G48930	Cytosolic ribosomal protein s11	MAPK3
18	AY096689.1	AT1G64370	Unkown protein	MAPK3
19	NP_568358	AT5G17870	Plastid-specific ribosomal protein 6	MAPK3
20	NP_001154565	AT2G39460	60s ribosomal protein l23a	BOTH
21	NM_104152	AT1G52740	Putative histone h2a	MAPK3
22	NP_190826	AT3G52580	Putative ribosomal protein s14	BOTH
23	NP_001078737	AT5G48760	60s ribosomal protein l13a	BOTH
24	AY063100.1	AT3G06730	Thioredoxin putative	BOTH
25	NP_195631	AT4G39200	Ribosomal protein s25	MAPK3
26	NP_176726	AT1G65480	Flowering time locus t	BOTH
27	NP_201339	AT5G65360	Histone h3	MAPK3
28	NP_568649	AT5G45775	Ribosomal protein l11-like	BOTH
29	NP_194898	AT5G10360	40s ribosomal protein s6	MAPK3
30	BT021097	AT5G44100	Casein kinase i	BOTH
31	NP_187090	AT3G04400	60s ribosomal protein l17	MAPK3
32	NP_566303	AT3G07350	Unknown protein	BOTH
33	NM_001123870	AT1G23860	9GB-like splicing factor	BOTH
34	NP_563787	AT1G07350	Transformer sr ribonucleoprotein,putative	BOTH
35	BT028892	AT1G02070	Unknown protein	MAPK3
36	AY122965	AT4G17390	60s ribosomal protein l15 homolog	BOTH
37	NP_568299	AT5G14320	30s ribosomal protein s13	BOTH
38	NP_849585	AT1G03680	Thioredoxin m1	BOTH
39	BT026343	AT2G02820	ATmyb 88	MAPK3
40	BT029523	AT5G66940	DNA-binding protein-like	MAPK3
41	AY081631	AT1G16700	NADH-ubiquinone oxidoreductase	BOTH
42	NP_568684	AT5G47570	Unknown protein	MAPK6
43	BT006528	AT1G56220	Unknown protein	MAPK6
44	AY114615	AT1G22160	Senescence-associated protein-related	MAPK6
45	NP_973695	AT2G46020	Putative snf2 subfamily transcriptional activator	MAPK6
46	AF361097	AT4G11280	ACC synthase	MAPK6
47	NP_188924	AT3G22845	Emp24/gp25l/p24 protein-related	MAPK6
48	NP_191429	AT3G58700	60s ribosomal protein l11	BOTH
49	BT003407	AT5G21100	Ascorbate oxidase like protein	MAPK6
50	BT008713	AT3G60390	Homebox-leucine zipper protein hat3	MAPK6
51	NP_174808	AT1G35680	Chloroplast ribosomal large su protein l21	MAPK6
52	NP_199178	AT5G43650	Putative bhlh transcription factor	MAPK6
53	NP_200274	AT5G54630	Zinc finger protein-related	MAPK6
54	NP_196232	AT5G06140	Sorting nexin-like protein	MAPK6

Table 1: Explaining all Known MAPK 3 and MAPK 6 substrate with accession number, gene code & their functions.

has also been studied to canvas the crosstalk of different kinases. Conventional experimental methods that measure kinase-substrate interaction require some hypothesis as to the kinase involved in the phosphorylation. Computational methods to predict kinase-substrate interactions from structural information [9] or from other known kinase substrates [17] have also been introduced, but such additional information is not completely available.

Gene code	Pattern	Gene code	Pattern
AT5G48990	NQKSSPNP NQKSSPNPS SILTSPELY DIKDSPCSN	**AT5G10360**	QGVLTPGRV LHRGTPCFR
AT5G02560	KTPKSPSKA KATKSPKKS STTKTPKSP	**AT5G62070**	NQKSSPNPS SILTSPELY DIKDSPCSN
AT3G48930	LGFKTPREA	**AT5G21100**	LIVRSPKER
AT4G11280	KLNVSPGGS FSPHSPVPP	**AT3G60390**	AGVSSPNST GPMSPWAA
AT5G19290	LAMASPRRT RSRQTPSDL	**AT5G43650**	RMMSPKISS ANFSPQEF
AT4G03260	DRITSPNSL SEKSSPFK	**AT2G40510**	RVRTPPPR
AT2G02820	IVTWSPEED RGGWSPEED AESESPLTK DLHDSPASS LGVESPSPY VESPSPYPS DGISTPLKA	**AT1G23860**	VRRRSPSPR RRSPSPRRR RRRRSPDYG RRSISPRGR RGRRSPPRR SRRDSPRRR RRRDSPYGR YGRRSPYAN RRSVTPPRR
AT1G52740	VKRISPRHL	**AT3G11510**	RDESSPYAA
AT3G52580	RDESSPYAA	**AT5G06140**	GSMQSPRSP QSPRSPSSH
AT3G06730	NTPLSPILS NLPFSPLTR LFFISPDPS	**AT2G39460**	YVRLTPDYD
AT1G65480	PDVPSPSNP YENPSPTA	**AT5G44100**	TTTSSPRDR KKVSTPIEV
AT5G48760	EGVPTPYDK	**AT5G54630**	PPISSPRSI LRPGTPMHY
AT1G02840	KDSRSPSRG SSRRSPAKS STSRSPGPR STSRSPGPR SKSRSPSPR SRSPSPRRS SRSRSPLPS EGSKSPSKP PSKPSPAKS SPAKSPIHT	**AT5G58620**	EVEVSPPRG TPPLSPNGV GATTTPPLS
AT1G56220	SAPASPAGS TPPLSPFSP LSPFSPPLS	**AT1G77450**	WYFFSPRDR EQAVSPEFT
AT1G22160	LAMVSPRGT	**AT1G16700**	KGPLSPRFR
AT4G39880	ERANSPTRG	**AT1G03680**	RIASSPTGS
AT5G23200	SQSTSPRPP	**AT3G07350**	EDDDSPCLS
AT5G47570	KVPFTPRVY	**AT5G67360**	KESRSPRDD
AT1G07350	LENRSPMSY RSRYSPSLS SPSLSPYDK SPSYSPRRS DRSYSPYYR ARDRSPYYM SRSYSPRYR DRSYSPHYQ RRGRTPTPG	**AT2G46020**	SSAASPSSS NFASSPGSM QRQISPAIG SSGSSPESH KEMASPVSS WDGTSPISS TEPSSPQRS HTDESPILA GGSSSPVSP SSPVSPPPA RGLRSPVSG VSTPTPRGA
AT5G48760	EGVPTPYDK	**AT3G22845**	NPYSTPETV
AT5G45775	LSGQTPVFS	**AT3G07110**	EGVPTPYDK

Table 2 explains all those sequences which contain (p)S/T-P-site with threshold value above 0.800 when analyzed by using NetPhos 2.0 server.

Table 2: Predicting the (p)S/TP-site in all studied sequences.

Gene code	Pattern	Gene Code	Pattern
AT2G40510	RRVRTPPPR RKEDTPKPG	**AT2G39460**	KISATPRNK YVRLTPDYD
AT3G48930	LGFKTPREA	**AT5G48760**	EGVPTPYDK
AT5G45775	LSGQTPVFS	**AT5G10360**	QGVLTPGRV LHRGTPCFR QRLVTPLTL
AT3G58700	LSGQTPVFS	**AT5G14320**	PASNTPNKQ
AT5G47570	KVPFTPRVY	**AT3G07110**	EGVPTPYD
-	-	**AT3G22845**	NPYSTPETV

Table 3 explains the presence of (p)TP site in all those sequences where (p)SP-sites are absent, having threshold value above 0.500 when analyzed by NetPhos 2.0 server.

Table 3: Predicting sequences having (p)TP site, but lacking (p)SP site.

Materials and Methods

Phosphorylation site prediction of Known substrate of MAPK3 and MAPK6, *Arabidopsis* [18] (Table 1) is done by using NetPhos 2.0 server [17] and then nearby sequence of Phosphorylation site was studied to find out the consensus sequences or motif which is phosphorylated by other kinases.

The sequences/motif which is searched for this study is SP/TP for MAPK, HYDXXRXXS for CaM Kinase II,R/$K_{(1-3)}$X(p)S/T(HYD)R/$K_{(1-3)}$ for PKC and $(Na)_{1-3}$-X-Y-XX-HYD for Receptor tyrosine kinase, HYDXRXXSXX for MAPKAP Kinase-2 and XXRRXSXX for MAPKAP Kinase-1 (X- Any amino acid, Na-hydrophilic; HYD-Hydrophobic).

Results

After the study of *Arabidopsis thaliana* genome it can be concluded that the plants have acquired signal transduction pathway component different from animals as Tyrosine phosphorylation is less common in plants because plant genome do not encode for such receptor tyrosine kinase, where as animal contain a large family of receptor tyrosine kinases. Due to this reason the study emphasized more on Serine-Threonine phosphorylation site then on tyrosine site.

Phosphorylation site prediction of Known substrate of MAPK3 and MAPK6, *Arabidopsis* is done by using NetPhos 2.0 server (http://www.cbs.dtu.dk/services/NetPhos/). NetPhos results shows that a single substrate have so many phosphorylation sites. And interestingly not all phosphorylation sites in a single substrates posses SP/TP(MAPK) motif. So it was hypothesized that these phosphorylation sites might be recognized by other kinases

Analysis for Proline residues at + 1 phosphorylation site

The Proline residue was analyzed at +1 site of phosphorylation, as MAP Kinase are proline directed serine/threonine kinase, which phosphorylated the serine/threonine in the dipeptide motif S/T-P [19]. The Serine residue which shows threshold value above 0.800 was taken into consideration in our study and out of 54 protein sequence 42 showed the presence of (p)SP-site, as revealed in Table 2.

There are some substrate which have (p)TP(Threshold value 0.5) site but that do not contain any (p)SP site, when analyzes it was found that out of 54, 11 contained such pattern as shown in Table 3.

Some of the sequences lack (p)S/T-P site as shown in Table 4. Out of 54, 7 sequences lack (p)S/T-p site which are MAPK3 Substrate and

in that 2 sequences i.e. AT2G18020, AT4G17390 are the substrate for both kinases i.e MAPK 3 and MAPK 6.

Analysis for CaM-Dependent Protein Kinase motif (HYDXRXXS; HYD-Hydrophobic, X-any amino acid)

Calcium signaling is one of the best documented pathway in plants and the best known Ca^{2+} sensor is calmodulin (CaM). The active Ca^{2+}/CaM complex interacts with target proteins and regulates their activity [20]. Ca/calmodulin (CaM)-dependent protein kinase (CaMK II) is a ubiquitous mediator of Ca -linked signaling that phosphorylates a wide range of substrates to co-ordinate and regulates Ca- mediated alterations in cellular function. The core consensus sequence for CaMK II HYDXRXX(p)S where X is any amino acid; HYD- Hydrophobic amino acid [21]. CaM kinase II, *Homo sapiens* (Accession number-Q13554), sequence has been retrieved, and from NCBI BLASTp it reveals 42% homology with CPK 14, Calmodulin dependent protein kinase in *Arabidopsis thaliana* (Accession number NP_973661.1) which play a pivotal role in amplifying and diversifying the action of Ca^{2+}-mediated signals [22]. When CaM Kinase II conserved pattern was analyzed in our sequences it was found that out of 54 sequences 35 shows the pattern as shown in Table 5.

Table 6 explains the presence of Conserved pattern for protein kinase C, which shows that MAPK substrate also contain recognition motif for Protein Kinase C.

Analysis for Tyrosine Kinase Protein -$Na_{(1-3)}$-X-(p)Y-XX-HYD(Na-Hydrophilic; HYD-Hydrophobic)

The Intracellular signaling is mediated by association of multi-protein complex where tyrosine phosphorylation initiates downstream signaling by creating sequence-specific recognition sites and phosphortyrosine-binding domains which facilitate the assembly of multi-protein complex [23,24]. Although Tyrosine phosphorylation is less common in plants, a recent study indicates that Tyr phosphorylation has an important role in plant signaling [25]. Several other reports also implicated the role of Tyr phosphorylation in disease-resistance signaling [26]. Tyrosine protein kinase 6, *Homo sapiens* (Accession number Q13882), sequence has been retrieved and from NCBI BLASTp it reveals 32% homology with protein kinase family protein of *Arabidopsis thaliana* (Accession number NP_179361.1), as these kinases phosphorylated protein which has been implicated in responses to many signals, including light, pathogen invasion, hormones, temperature stress, and nutrient deprivation. When its conserved patterns i.e. $Na_{(1-3)}$-X-(p)Y-XX-HYD (Na-hydrophilic; HYD- hydrophobic) was observed in all MAPK substrate that are used in our study, it shows that out of 54, 17 shows tyrosine kinase protein recognition pattern as shown in Table 7.

Gene code	Pattern
AT2G19730	VXFSKX XVXSYK VAXSGA
AT4G15000	AXXSXV
AT5G65360	AXXSTGG AXXSAXA
AT2G18020	AGXSVFX IVXSGCX
AT3G04400	FXMSLG XLXSAC
AT4G39200	XAXSGG
AT4G17390	XXXSVAX XXXSRRA

Table 4 shows that 7 sequences out of 54 lack (p)S/T-P- site

Table 4: Predicting sequences lacking (p)S/TP site.

Gene Code	Pattern	Gene Code	Pattern
AT3G52580	AXRXXS	**AT5G66940**	VXRXXS
AT2G40510	VXRXXS	**AT5G48760**	FXRXXS
AT1G26740	FXRXXS AXRXXS	**AT5G23200**	VXRXXS
AT4G15000	VXRXXS	**AT5G58620**	YXRXXS GXRXXS
AT3G48930	LXRXXS	**AT1G02840**	HXRXXS GXRXXS
AT1G64370	GXRXXS	**AT5G19290**	HXRXXS
AT5G48990	LXRXXS FXRXXS	**AT4G03260**	AXRXXS MXRXXS
AT5G10360	FXRXXS GXRXXS	**AT4G39880**	GXRXXS
AT1G22160	GXRXXS	**AT5G02560**	AXRXXS
AT2G19730	LXRXXS	**AT3G58700**	IXRXXS
AT5G62070	FXRXXS	**AT5G45775**	FXRXXS
AT5G54630	MXRXXS IXRXXS	**AT5G06140**	IXRXXS
AT5G44100	YXRXXS	**AT4G11280**	FXRXXS
AT3G07350	LXRXXS AXRXXS	**AT1G23860**	YXRXXS
AT5G21100	MXRXXS	**AT5G65360**	AXRXXS
AT5G47570	AXRXXS	**AT2G46020**	WXRXXS
AT3G04400	LXRXXS	**AT2G18020**	VXRXXS
AT3G60390	IXRXXS		

Table 5 explains the presence of Conserved pattern for CAM kinase II, which shows that MAPK substrate also contain recognition motif for CaM Kinase.

Table 5: Predicted the CaM Kinase II conserved pattern i.e HYDXXRXX(p)S.

Gene code	Pattern
AT4G15000	XKXSAKK XKXTAKK
AT5G10360	RRXSVRX
AT5G44100	XKXTLKX
AT3G07350	XRXSLRX XRXSLRX
AT1G23860	XRXSVRR
AT4G17390	XRXTWKK
AT3G58700	XRXSVRX
AT2G18020	XRXSFRX XRXSGRX
AT5G65360	XKXSARX
AT3G58700	XRXSVRX
AT5G54630	XRXTVKX
AT5G45775	XRXTRRX

Table 6 explains the presence of Conserved pattern for protein kinase C, which shows that MAPK substrate also contain recognition motif for Protein Kinase C.

Table 6: Predicted the protein kinase C, conserved pattern i.e R/$K_{(1-3)}$X(p) S/T(HYD)R/$K_{(1-3)}$.

Analysis for MAPK AP kinase-1 and MAPK AP kinase-2 (XXRRXSXX & HYDXRXXSXX)

Many cellular responses of MAPK cascades have been shown to be mediated by MAP kinase-activated protein kinases (MAPKAPK). The MAPKAP-1 family is activated by the ERK and JNK in mammalian system whereas MAPKAP-2 is capable of directly phosphorylating transcription factors [27].

MAPK AP-1, *Homo sapiens* (Accession number- Q9BPZ7), sequence has been retrieved and from NCBI BLASTp it reveals 38% homology with pentatricopeptide (PPR) repeat-containing protein in *Arabidopsis thaliana* (Accession number- NP_194007.1) which help in restoring fertility to cytoplasmic male-sterile plants [28]. Similarly MAPK AP-2 *Homo sapiens* (Accession number- P49137) sequence has been retrieved and from NCBI BLASTp it reveals 37% homology with CDPK9 (Calmodulin-like domain protein kinase 9) in *Arabidopsis thaliana* (Accession number NP_197748) which has been implicated in the regulation of cell cycle and transcription [29].

The conserved pattern for MAPK AP-1 and MAPK AP-2 is XXRRX(p)SXX [30], HYDXRXX(p)SXX, respectively [31]. When this pattern was analyzed in all MAPK substrates sequences which are examined in this study, it depict that 8 protein sequences out of all shows conserved pattern for MAPK AP-1 and 28 shows MAPK AP-2

Gene code	Pattern
AT5G23200	QXYXXI
AT1G02840	PXYXXV TXYXXM SXYXXV
AT3G11510	SXYXXM
AT5G19290	TXYXXI
AT4G03260	NXYXXG
AT5G48990	SXYXXL
AT5G62070	SXYXXL
AT3G52580	SXYXXM
AT5G44100	QXYXXI PXYXXL
AT3G07350	SXYXXV
AT1G23860	PXYXXL PXYXXA SXYXXG
AT1G07350	PXYXXL SXYXXG
AT4G17390	SXYXXY
AT2G02820	SXYXXG SXYXXA
AT5G66940	SXYXXV
AT2G39460	PXYXXA
AT5G21100	NXYXXY

Table 7 explains the presence of Conserved pattern for tyrosine kinase protein, shows that MAPK substrate also contain recognition motif for tyrosine kinases protein.

Table 7: Predicted the Tyrosine kinase protein conserved pattern i.e. $(Na)_{1-3}$-X-(p) Y-XX-HYD(Na-hydrophilic;HYD-Hydrophobic).

Gene Code	Pattern
AT5G23200	XXRRWS-----
AT1G02840	XXRRXSX
AT1G64370	XXRRXSX
AT5G10360	XXRRXSX
AT1G23860	XXRRXSX XXRRXSX XXRRXSX XXRRXSX
AT1G22160	XXRRXSX
AT1G35680	XXRRXSX
AT5G54630	XXRRXSX

Table 8 explains the presence of Conserved pattern for MAPK AP kinase-1 present in MAPK substrate.

Table 8: Predicted the conserved pattern RRX(p)SXX for MAPKAP kinase-1.

Gene code	Pattern	Gene code	Pattern
AT1G02840	VXRXXSX GXRXXSX	**AT5G66940**	VXRXXSX
AT5G19290	HXRXXSX	**AT1G16700**	AXRXXSX
AT2G19730	FXRXXSX	**AT5G21100**	TXRXXSX
AT4G03260	MXRXXSX	**AT5G67360**	SXRXXSX YXRXXSX
AT4G39880	GXRXXSX	**AT2G40510**	HXRXXSX
AT4G15000	VXRXXSX	**AT1G77450**	VXRXXSX
AT3G52580	AXRXXSX	**AT3G11510**	AXRXXSX
AT5G48760	HXRXXSX	**AT1G56220**	SXRXXSX
AT3G07350	IXRXXSX	**AT1G22160**	IXRXXSX TXRXXSX
AT5G44100	TXRXXSX	**AT1G23860**	GXRXXSX
AT3G04400	LXRXXSX	**AT4G17390**	VXRXXSX
AT5G45775	LXRXXSX	**AT3G58700**	LXRXXSX
AT5G54630	TXRXXSX	**AT5G43650**	GXRXXSX
AT1G07350	VXRXXSX CXRXXSX YXRXXSX GXRXXSX MXRXXSX HXRXXSX IXRXXSX	**AT5G58620**	GXRXXSX CXRXXSX GXRXXSX

Table 9 explains the presence of Conserved pattern for MAPK AP kinase -2 present in MAPK substrate.

Table 9: Predicted the conserved pattern, HYDXRXX (p)SXX for MAPKAP kinase-2.

conserved pattern which implies that homologue of MAPK activated protein kinase 2 might also be present in plants so this opens a pathway for further analysis. Table 8 and Table 9 shows sequences which show above conserved pattern.

Discussion

This study is based on the study of consensus sequences for short stretches of primary sequences which is required for Phosphorylation by different kinases in the known substrate of MAPK-3 and MAPK-6 and postulated that these substrate might be the substrate/ partner of other kinases. It has been already stated that all known MAPK substrate carrying minimal consensus sequence i.e. (p) S/T [32]. The present study demonstrates that the most of the MAPK substrate that has been studied in our analysis also contain some non S/T (p)-site which clearly indicates that their might also be some other recognition motifs, which are also responsible for MAPK Substrate phosphorylation. In contrast to this, these substrate might also contain recognition motif for some other kinases, like, HYDXXRXX(p)S motif is present in CaM Kinase II (Ca2+/Calmodulin dependent Kinase protein) that mediate signal transduction pathway where calcium plays an important role as it play role in Ca signal transduction pathway. It has been already stated that in some plants like tobacco, CaM Kinase II plays an important role in its growth and development [33]. Its homologue also play important role in animals too [34]. CaM kinase II shows 42% homology with CPK 14, Calmodulin dependent protein kinase in *Arabidopsis thaliana*. Another kinase whose conserved pattern was analyzed is Protein Kinase C, which is found to be involved in desensitization in modulating membrane in structural event, and it has also been partially purified in *Brassica compestries* [35]. Protein kinase C shows 39% homology with S6K2- Serine/Threonine protein kinase 2 in *Arabidosis thaliana*. Presence of the consensus sequences in these substrate indicate that their must be a crosstalk between MAP Kinase and Protein kinase C homologue of plant.

Activity of Tyrosine Kinase protein was also studied as it was

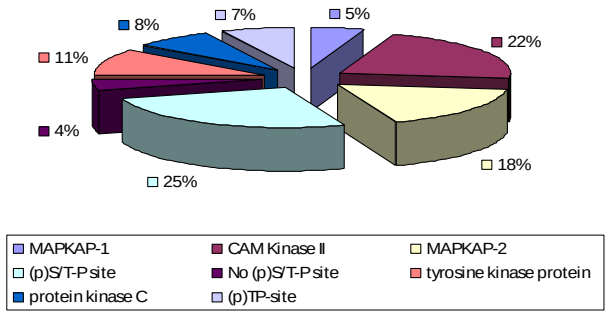

Figure 1: Percentage of conserved pattern seen in all MAPK substrate.

known that is essential for signal transmission as phosphorylation of tyrosine residue modulates enzymatic activity creates binding sites for downstream signaling in molecules. Although Arabidopsis genome does not encode receptor tyrosine kinase unlike animal which indicate that tyrosine phosphorylation occurs less frequently in plants [36]. The presence of the consensus sequences which is recognized by Tyrosine protein kinase in these substrate indicate that their homologue must be present in plants which might show the functional homology as it plays a role in disease-resistance signaling when its homology was seen against *Arabidopsis thaliana* it shows 32% homology with protein kinase family protein.

According to our knowledge MAPK Activated protein kinase (MAPKAP-1 and MAPKAP-2), is not present in plant system, and they shows 38% homology with pentatricopeptide (PPR) repeat-containing protein, *Arabidopsis thaliana* and 37% homology with Calmodulin like domain protein kinase 9, *Arabidopsis thaliana,* respectively. The presence of the consensus sequences which is recognized by MAPK-AP-2 in these substrate indicate that their homologue must be present in plants which might show the functional homology as MAPKAP-2 plays role in diseases in animals.

On the bases of these studies it can be hypothesized that these substrates might also act as a substrate for other kinases, this must be a question for further wet lab analysis. Follow-up experiments such as in-vitro verification of phosphorylation site in these substrate for different kinases are essential to evaluate any physiological relevance

Based on this analysis a model is proposed (Figure 2) showing the cross talk between different kinases. There are also some substrate which are unique to MAPK3 i.e. At5g02560, At5g17870, At4g39200, At1g02070 and MAPK 6 i.e At2g46020, At4g11280, At3g22845, At5g06140.

Figure1 Number of conserved pattern studied in all MAPK substrate i.e presence and absence of (p)S/TP-site, (p)TP-site, presence of HYDXXRXXS motif for CAM Kinase II, presence of XXXRRXS motif for MAPKAP-1 conserved pattern, presence of HYDXRXXSX motif for MAPKAP-2 Conserved pattern, presence of $(R/K)_{1-3}XS/T(HYD)R/K$ conserved pattern for Protein Kinase C, presence Na_{1-3}-X-Y-XX-HYD conserved pattern for Receptor Tyrosine Protein studied in our analyses.

A crosstalk between different kinases activating common substrate have been shown in Figure 2.

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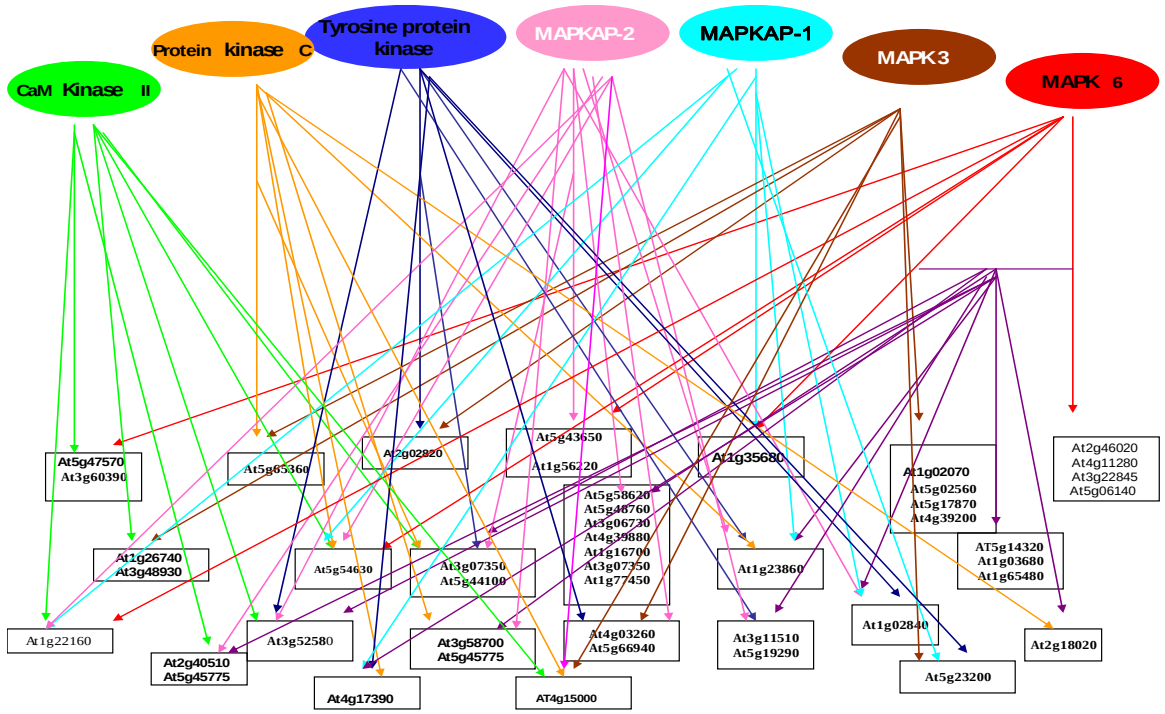

Figure 2: Crosstalk between different kinases, activating common substrate is seen. In a figure Green color reflects CaM Kinase, Orange color reflects Protein kinase C, Blue color reflects Tyrosine protein kinase, Grey color reflects MAPK AP-2, Light blue color reflect MAPK AP-1, Brown color reflects MAPK 3 and red color reflects MAPK 6.

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