International Journal of Chemical Research and Development
www.chemicaljournal.in
Online ISSN: 2664-6560; Print ISSN: 2664-6552
Received: 01-11-2019; Accepted: 02-12-2019; Published: 09-01-2020
Volume 2; Issue 1; 2020; Page No. 01-09

MAPK signaling in plant disease resistance

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DOI: https://doi.org/10.33545/26646552.2020.v2.i1a.13

Abstract
During their life time, plants always suffer from invasion of potential pathogenic microorganisms in the environment. To defend themselves against pathogen attack, plants have evolved a sophisticated immune system. Two types of innate immune responses, which are precisely regulated upon infection from different types of pathogens, have been recognized in plants so far. The first innate immune response is the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), which is activated by a number of PAMPs such as flagellin and chitin. The other one is the effector-triggered immunity (ETI), which is modulated by recognition of pathogen-derived avirulence effectors by plant R genes. Much progress has been made in understanding the mechanisms by which plants detect and defend themselves against microbial attack. These include the identification of components involved in the signal transduction pathways coupling pathogen recognition to the activation of defense responses and the demonstration that three endogenous plant signaling molecules, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), are involved in plant defense. In the past few years, it has become apparent that mitogen activated protein kinase (MAPK) cascades play some of the most essential roles in plant signal transduction pathways from cell division to cell death. The first reports of plant MAPKs in 1993 identified extracellular signal regulating kinase (MsERK1) in alfalfa and D5 kinase in pea. Sequential phosphorylations ensue as MAP3Ks activate downstream MAP kinase kinases (MAP2Ks; also called MKKs or MEKs) that in turn activate MAPKs. MAPKs then target various effector proteins in the cytoplasm or nucleus, which include other kinases, enzymes, or transcription factors. Li *et al*., 2014 identified a total of five SlMKK genes with one new member, SlMKK5 in tomato. qRT-PCR analyses revealed that expression of SlMKK2 and SlMKK4 was strongly induced by *B. cinerea* and by jasmonic acid and ethylene precursor 1-amino cyclopropane-1-carboxylic acid. Virus-induced gene silencing (VIGS)-based knockdown of individual SlMKKs and disease assays identified that SlMKK2 and SlMKK4 but not other three SlMKKs (SlMKK1, SlMKK3 and SlMKK5) are involved in resistance against *B. cinerea*.

Keywords: MAPK cascade, MPK kinase, SlMKK2/SlMKK4, botrytis cinerea, defense response, tomato (*Solanum lycopersicum*)

Introduction
Eukaryotic mitogen-activated protein kinase (MAPK; also called MPKs) cascades transduce environmental and developmental cues into intracellular responses. In a general model, stimulated plasma membrane receptors activate MAP kinase kinase kinases (MAP3Ks; also called MAPKKKs or MEKKs) or MAP kinase kinase kinase kinases (MAP4Ks). Sequential phosphorylations ensue as MAP3Ks activate downstream MAP kinase kinases (MAP2Ks; also called MKKs or MEKs) that in turn activate MAPKs. MAPKs then target various effector proteins in the cytoplasm or nucleus, which include other kinases, enzymes, or transcription factors (Khokhlatchev *et al*., 1998) [14, 15]. More specifically, MAP3Ks are serine or threonine kinases that phosphorylate MAP2Ks at a conserved S/T-X3−5-S/T motif, and MAP2Ks phosphorylate MAPKs on threonine and tyrosine residues at a conserved T-X-Y motif (Chang and Karin, 2001) [5]. The deactivation and regulation of MAPK activity are mediated by tyrosine and serine/threonine-specific phosphatases (Luan, 2003) [20]. The formation and integrity of a specific MAPK cascade can be mediated by scaffold proteins, shared docking domains, and adaptor or anchoring proteins (Takekawa *et al*., 2005) [30]. The first reports of plant MAPKs in 1993 identified MsERK1 in alfalfa (Duerr, 1993) [10]. and D5 kinase in pea (Stafstorm *et al*., 1993). During that year, MAPKs were cloned from Arabidopsis thaliana (thale cress) and tobacco as well. A seminal work that assigned a function in ethylene signaling to a specific Raf-like kinase, CTR1, was also published (Kieber *et al*., 1993) [16]. This study was an early example of the power of genetic screens in A. thaliana. These discoveries were guided by studies of MAPK cascades in yeast and metazoans. At that time, four MAPK pathways were charted in mammalian cells: two extracellular-signal regulated kinase (ERK) modules and two other pathways that involve the MAPKs c-Jun Nterminal kinase/stress-activated protein kinase (JNK /SAPK) and p38/Hog (Chang and Karin, 2001) [5].

Cross talk among pathogen defense signaling pathways
There is a growing body of literature that reports that the JA, SA and ET defense signaling pathways do not function independently. Rather, they are involved in a complex signaling network in which the different pathways influence each other through positive and negative regulatory interactions. In Figure 1, the results of studies are summarized, carried out primarily in A. thaliana, that provide evidence for cross talk among the SA, JA and ET signaling pathways. Incorporating the results from

these studies into a single model is difficult as several different plant signaling mutants, pathogen systems and defense reporter genes have been used. Thus, it is often hard to compare the results from different studies directly.

Cross talk between the JA and ET signaling pathways

Several studies provide evidence for positive interactions between the JA and ET signaling pathways. Both JA and ET signaling are required for the expression of the defense-related gene PDF1.2 in response to infection by A. brassicicola (Penninckx *et al*., 1996) [26], and for the expression of PDF1.2, HEL, and CHIB in response to treatment with E. carotovora culture filtrates (Norman *et al*., 2000) [22]. Further, when exogenously applied together to plant tissue, JA and ET appear to function synergistically to induce PDF1.2, HEL and CHIB in A. thaliana (Penninckx *et al*., 1998) [25], and osmotin and PR1b in tobacco (Xu *et al*., 1994) [35]. Evidence that JA and ET coordinately regulate many other defense-related genes was obtained in an A. thaliana microarray experiment that monitored gene expression in response to various defense-related stimuli. In this study, nearly half of the genes that were induced by ET were also induced by JA treatment (Schenk *et al*., 2000) [28]. Not surprisingly, the study revealed that JA and *Et al*so independently regulate separate sets of genes. Little evidence exists suggesting antagonistic interactions between the JA and ET defense pathways.

Cross talk between the SA and ET signaling pathways

Limited data suggest both positive and negative regulatory interactions between the ET and SA signaling pathways. In tomato, the development of disease symptoms following infection by X. campestris pv. vesicatoria requires both SA and ET, and the accumulation of SA in infected plants is dependent on ET synthesis (O'Donnell *et al*., 2001) [23]. Results from the microarray experiment mentioned previously suggest that, in A. thaliana, SA and ET may function together to coordinately induce several defense-related genes (Schenk *et al*., 2000) [28]. Although the induction of SA-dependent expression of PR genes does not require an intact ET signaling pathway in A. thaliana, exposure to ET has been reported to potentiate the SA-mediated induction of PR-1 in this species. However, genetic data from the same study suggest that the ET signaling pathway also negatively affects SA-dependent responses: the basal level of PR-1 mRNA appears to be significantly elevated in ein2 plants (Lawton *et al*., 1994). These data, which appear contradictory at first, may reflect the complexity of regulatory cross talk between the SA and ET signaling pathways.

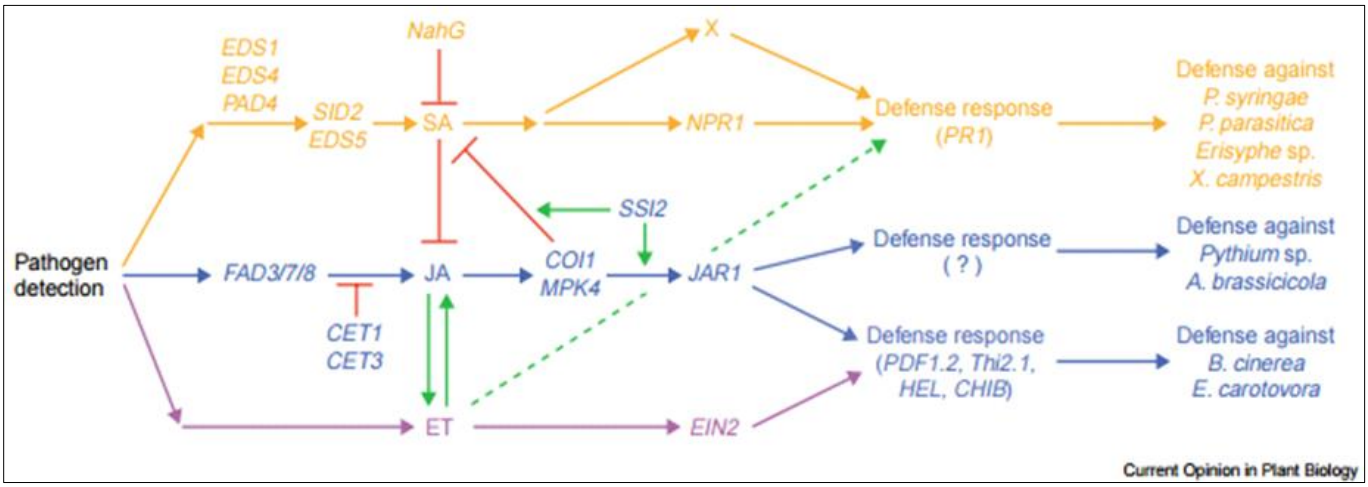

Fig 1

Cross talk between the SA and JA signaling pathways

The interactions between SA and JA signaling appear to be complex, and there is evidence for both positive and negative interactions between these pathways. However, the primary mode of interaction between these pathways appears to be mutual antagonism. The inhibitory effect of SA on JA signaling in tomato is well substantiated (Doares *et al*., 1995) [8]. Several recent genetic studies also provide evidence for an antagonistic effect of SA on JA signaling in A. thaliana. The eds4 and pad4 mutants, which are impaired in SA accumulation, exhibit enhanced responses to inducers of JA-dependent gene expression (Gupta *et al*., 2000) [11]. In the cpr6 mutant, which accumulates elevated levels of SA and constitutively expresses both SA- and JA-dependent defenses, reducing the level of SA by crossing in an eds5 mutation results in a further increase in PDF1.2 expression (Clarke *et al*., 2000) [6]. There is growing evidence that JA also antagonizes SA signaling. Studies in tobacco reveal that JA inhibits the expression of SA-dependent genes (Niki *et al*., 1998) [21]. Treatment of tobacco plants with elicitors produced by E. carotovora, which we now know activates JA signaling in A. thaliana, resulted in inhibited expression of SA-dependent genes (Vidal *et al*., 1997) [31]. The characterization of three JA-signaling mutants, mitogen-activated protein kinase4 (mpk4), suppressor of SA insensitivity2 (ssi2) and coi1, has provided genetic evidence that JA signaling also negatively regulates the expression of SA-mediated defenses in A. thaliana (Petersen *et al*., 2000) [27]. In addition to exhibiting impaired JA signaling, mpk4 and ssi2 plants constitutively express SA-mediated defenses and exhibit enhanced resistance to P. syringae and P. parasitica (Kachroo *et al*., 2001). Importantly, the impairment of JA signaling in these mutants is not due to an inhibitory effect of elevated levels of SA; JA-dependent gene expression was also impaired in mpk4 nah G and ssi2 nah G plants that do not accumulate high levels of SA. Thus, constitutive SA signaling in

the mpk4 and ssi2 mutants is likely due to loss of an antagonistic effect of JA signaling on the SA pathway. MPK4 is predicted to encode a mitogen activated (MAP) kinase that is required for JA-dependent gene expression. The SSI2 gene encodes a steroyl-ACP fatty-acid desaturase, which is hypothesized to catalyze the synthesis of a fatty-acid-derived signal that is involved in mediating both JA signaling and negative cross talk between the JA and SA pathways (Kachroo *et al*., 2001). The coi1 mutant also exhibits enhanced expression of SA-dependent defenses and enhanced resistance to P. syringae (Kloek *et al*., 2001) [17]. However, unlike mpk4 and ssi2 mutants, coi1 plants do not exhibit constitutive expression of SA-dependent defenses. Rather, the SA-mediated defense pathway is sensitized in coi1 plants, such that SA-dependent defenses are hyperactivated in response to attack by P. syringae (Kloek *et al*., 2001) [17]. These findings are consistent with the hypothesis that the JA signaling pathway negatively regulates the expression of SA-dependent defenses. COI1 encodes an F-box protein that is hypothesized to regulate JA-signaling by inactivating negative regulators of JA-mediated responses (Xie *et al*., 1998) [34]. The observation that a JA-insensitive tomato mutant (i.e. jai1) exhibits enhanced resistance to P. syringae suggests that JA antagonizes SA-dependent pathogen defenses in tomato as well as in A. thaliana (G Howe, personal communication). There is limited evidence for positive interactions between the JA and SA pathways. Results from early experiments with tobacco indicate that SA and JA act synergistically to induce PR1b expression (Xu *et al*., 1994) [35]. In A. thaliana, microarray analysis of plants that had been exposed to a variety of defense-inducing treatments has revealed that more than 50 defense-related genes are co-induced by SA and JA (Schenk *et al*., 2000) [28], suggesting that the two signals coordinately regulate these genes.

A typical MAPK cascade

Sequence and functional analyses of the Arabidopsis genome have revealed that there are 20 MAPKs, 10 MAPKKs and 80 MAPKKKs, with a similar repertoire of genes observed in other plant genomes (Colcombet and Hirt, 2008) [7]. Based on structural motifs and sequence similarities, Arabidopsis MAPKs can be divided into four groups (A–D). Except for members of the most distant D group, which carry a T-D-Y phosphorylation motif, all other MAPKs (A, B and C groups) are activated in the T-E-Y motif (Ichimura *et al*., 2002) [13]. The best studied MAPKs, MPK3, MPK4 and MPK6 have been implicated in plant innate immunity (Asai *et al*., 2002), cytokinesis and microtubule organization (Zeng *et al*., 2011), epidermal patterning (Wang *et al*., 2007), ovule development (Wang *et al*., 2008), and also activation by abiotic stresses and ABA (Ichimura *et al*., 2000) [12]. MPK7 and MPK11 also appear to play a role in innate immunity (Bethke *et al*., 2012) [4].

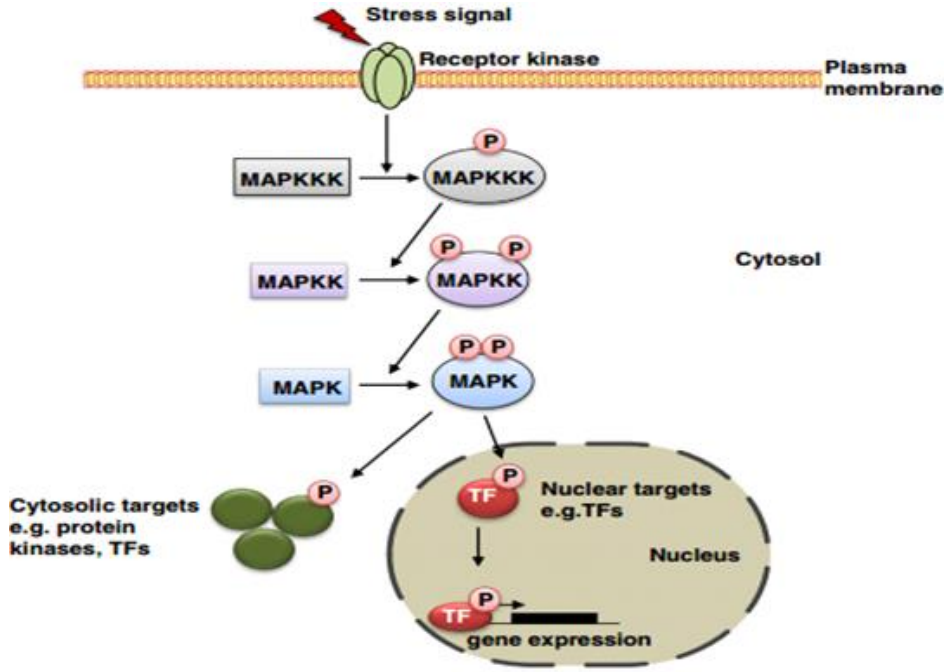

Fig 2

MAPK cascades in diverse plant signal transduction pathways

Activation of the plant MAPK cascade confers resistance to a broad spectrum of bacterial and fungal pathogens. It suggests that signaling events initiated by diverse microbes converge into conserved MAPK cascades. MAPK cascades in diverse plant signal transduction pathways. A general schematic presentation of signal transduction pathways is shown on the left. FLS2 is the putative receptor for the flagellin peptide elicitor flg22. AtHK1 is the putative histidine kinase osmosensor. The functionally defined MAPK-cascade components are shown in bold. MAPK, MAPKK and MAPKKK homologs in three plant species, tobacco (Nt), alfalfa (Ms) and *Arabidopsis* (At) are shown. Alfalfa MsSAMK is MsMMK4, MsSIMK is MsMMK1. Tobacco NtMEK1 is the same as NtNQK1, and tobacco NtNTF6 is NtNRK1. The negative regulators shown here are limited to known MAPK-specific phosphatases: dualspecificity MAPK phosphatase (AtMKP1), phosphotyrosine phosphatase (AtPTP1),

and protein phosphatase 2C (MsMP2C). ER7, auxin-inducible enhancer; GH3 auxin-inducible promoter; GST, glutathione-*S*-transferase; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; HSP, heat shock protein; PAL, phenylalanine ammonia-lyase; (+) positive regulation; (–) negative regulation.

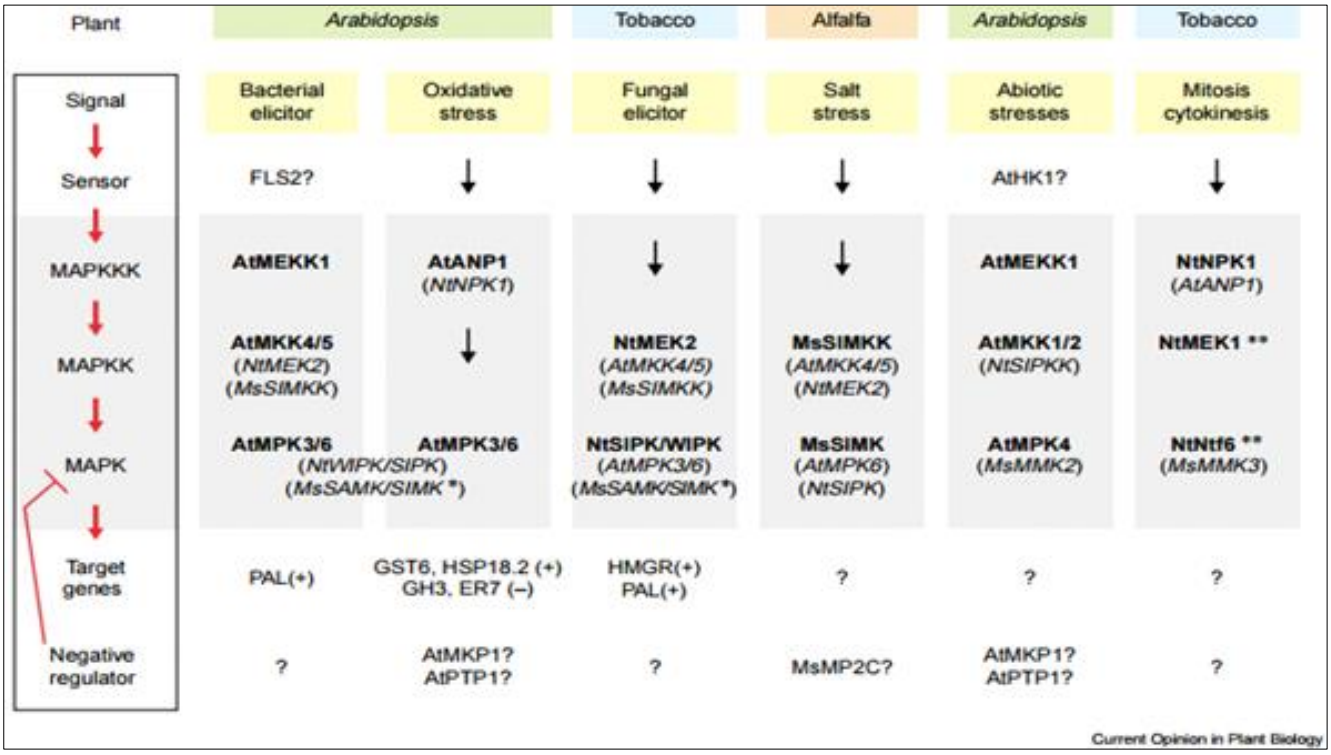

Fig 3

Case study

Despite of extensive studies on the MAPK cascades in immune response in tomato, little is known about the functions of these MAPK cascades in defense response against necrotrophic fungal pathogens such as *B. cinerea*. In the study, they performed functional analysis using virus-induced gene silencing (VIGS) approach of SlMKKs in regulation of defense response against this necrotrophic fungal pathogen.

Fig 4

Results

- **Identification of tomato SlMKKs**

Five SlMKKs, SlMKK1-5, have been identified from tomato through searching expressed sequence tags. Therefore, it is likely that there are five SlMKKs in tomato genome and each of tomato SlMKKs falls into one group of plant MKKs identified so far. Phylogenetic tree of Slmkks with other plant MKKs

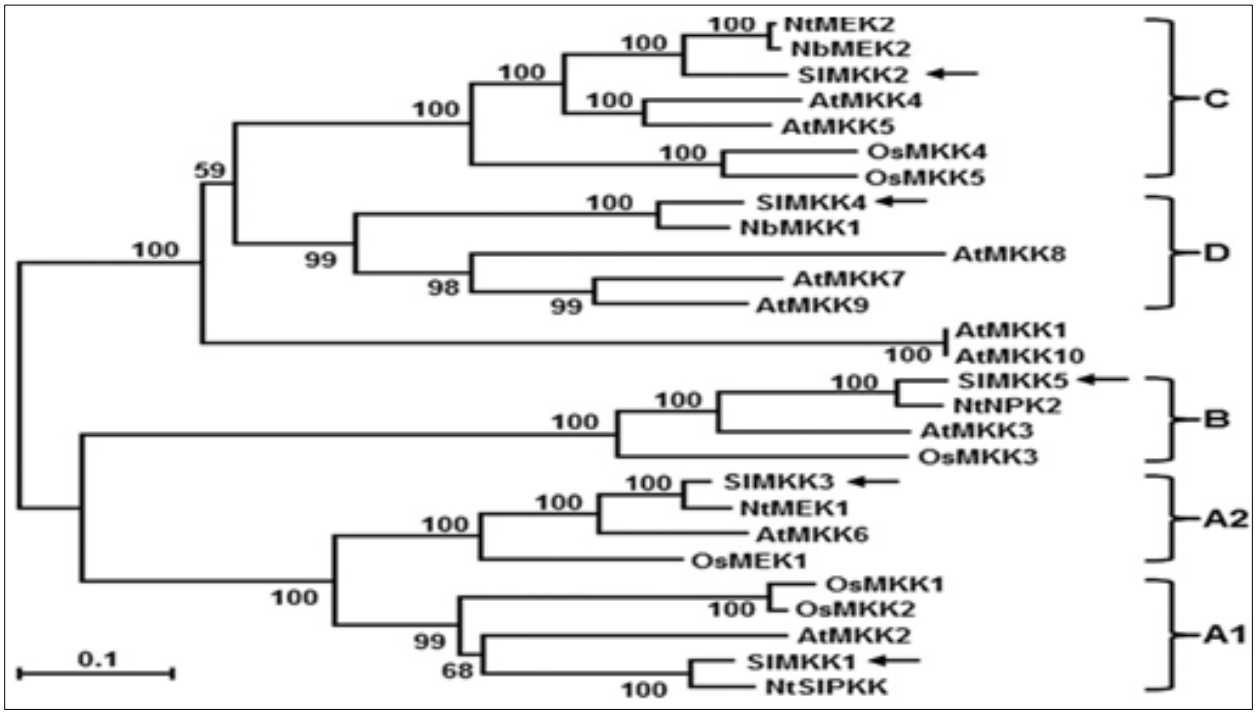

Fig 5

2. Expression of SlMKKs induced by *Botrytis cinerea* infection

To explore the possible involvement of SlMKKs in defense response against *B. cinerea* they first analyzed the expression changes of SlMKKs after infection with *B. cinerea*.

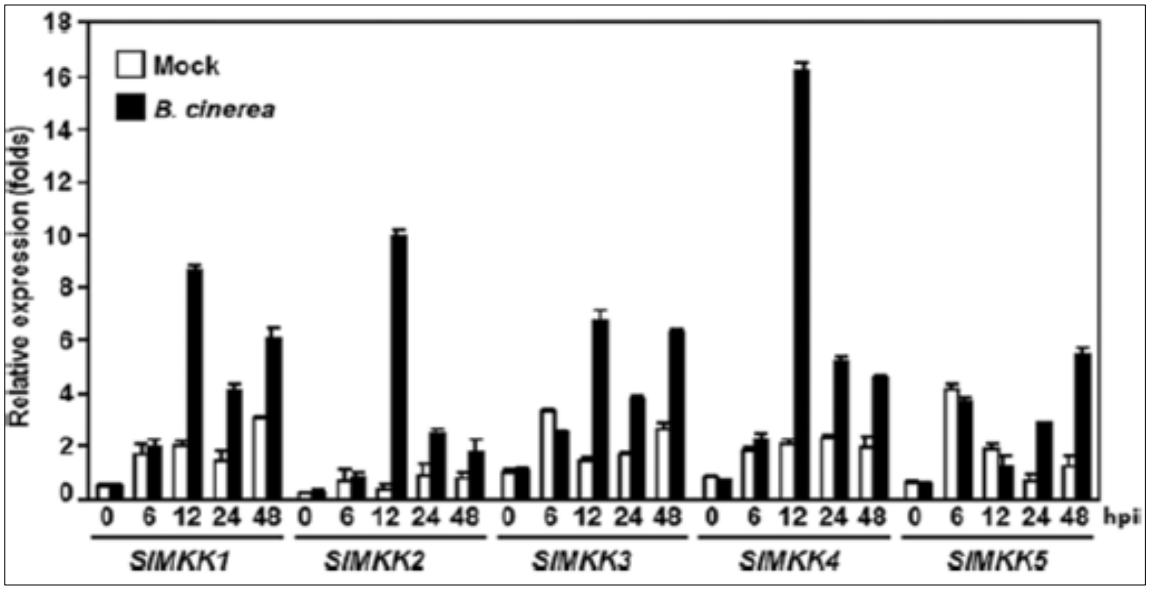

Fig 6

These results indicate that the tomato SlMKKs respond to infection of *B. cinerea* with different dynamics and magnitude of expression and that SlMMK2 and SlMKK4 have stronger induction of expression upon *B. cinerea* infection.

- **Expression of SlMKKs induced by phytohormone treatment**

They further examined the dynamics of SlMKKs expressions in tomato plants after treatment with phytohormone.

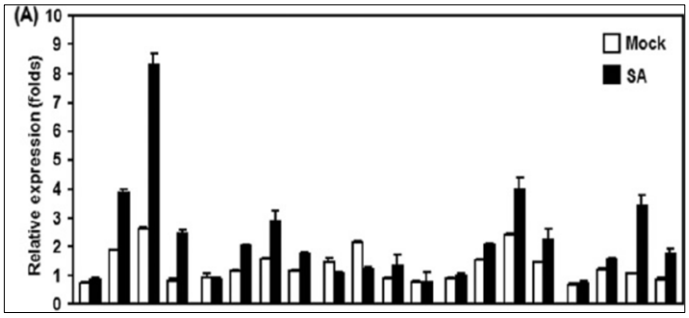

Fig 7(A): Treatment with SA

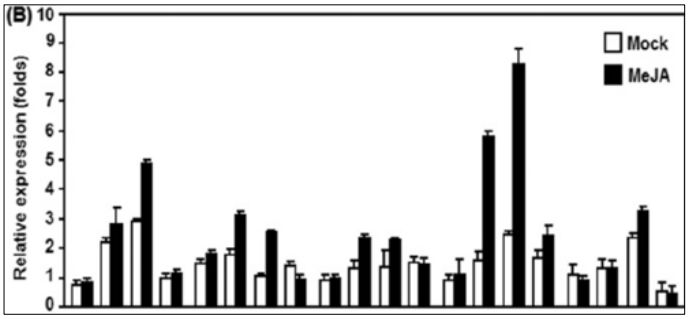

Fig 7(B): Treatment with methyl jasmonate (MeJA)

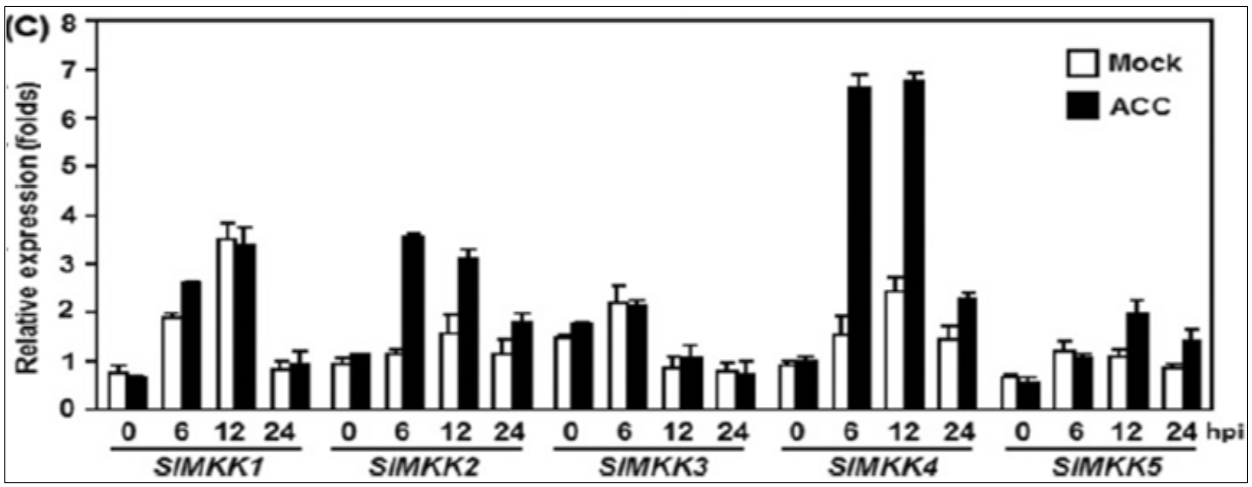

Fig 7(C): Treatment with 1-amino cyclopropane-1-carboxylic acid (ACC) [a precursor of ethylene (ET)].

Therefore, it is clear that the tomato SlMKKs also respond with different expression patterns to SA, JA and ET, three well-known defense signaling hormones.

- **Silencing of SlMKK2/SlMKK4 resulted in reduced resistance**

To investigate the roles of SlMKKs in disease resistance against B. cinerea, they used two different strategies, detached leaf disease assays for fast evaluation and whole plant disease assays for confirmation, to compare the disease phenotype and in planta fungal growth in the TRV-target SlMKK-infiltrated plants with those in the TRV-GUS-infiltrated plants. In detached leaf disease assays, typical disease lesions were observed 2 days post inoculation (dpi) (Figure 2A). The lesions on leaves from the TRV-SlMKK2- or TRV-SlMKK4-infiltrated plants were larger than that in the TRV-GUS-infiltrated plants at 2 dpi and began to merge into large necrotic areas at 3 dpi (Figure 2A), showing an approximately 40% of increase in lesion size over those on leaves from the TRVGUS- infiltrated control plants (Figure 2B). The lesions on leaves from the TRV-SlMKK1-, TRV-SlMKK3- and TRV-SlMKK5-infiltrated plants were similar to that in the TRV-GUS-infiltrated plants (Figure 2A and B). Further whole plant disease assays were carried out to confirm the disease phenotype observed in the TRVSlMKK2- and TRV-SlMKK4-infiltrated plants. In the whole plant disease assays, the TRV-SlMKK2- and TRVSlMKK4- infiltrated plants along with the TRV-GU Sinfiltrated plants were inoculated by spraying with spore suspension of B. cinerea and disease phenotype and in planta fungal growth were observed and analyzed, respectively. As shown in Figure 3A, the TRV-GU Sinfiltrated control plants displayed slight disease, whereas the TRV-SlMKK2- or TRV-SlMKK4-infiltrated plants showed severe diseases, showing large necrotic areas and maceration or wilting of full leaves at 5 dpi. Analysis of the transcript for the B. cinerea actin gene Bc Actin A as an indicator of the rate of fungal growth in planta further confirmed that the TRV-SlMKK2- and TRV-SlMKK4-infiltrated plants showed reduced resistance to Botrytis infection than the TRV-GUS-infiltrated control plants (Figure 3B). Growth of B. cinerea in leaf tissues of the TRV-SlMKK2- or TRV-SlMKK4-infiltrated plants had three times higher than those in the TRV-GUS-infiltrated control plants at 24 and 48 hr after inoculation (Figure 3B), indicating much fungal growth in the SlMKK2- or SlMKK4-silenced plants. These data demonstrate that knockdown of the SlMKK2 or SlMKK4 resulted in reduced resistance to B. cinerea and thus both SlMKK2 and SlMKK4 are required for resistance against B. cinerea

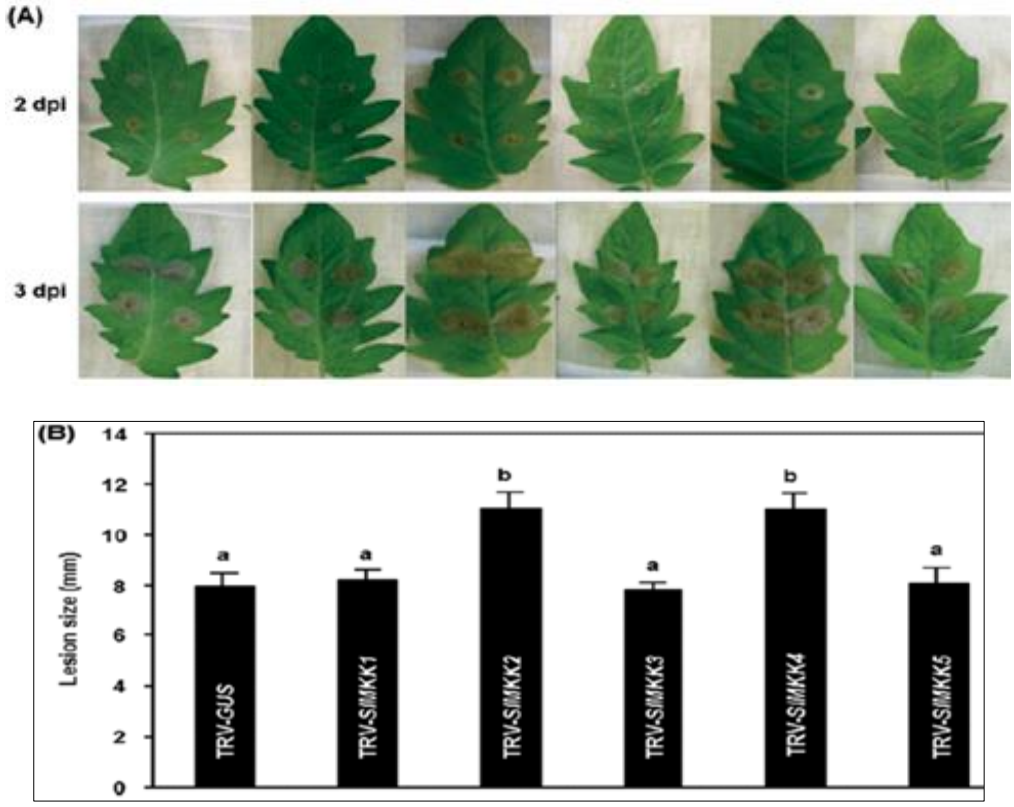

Fig 8: Detached leaf assay

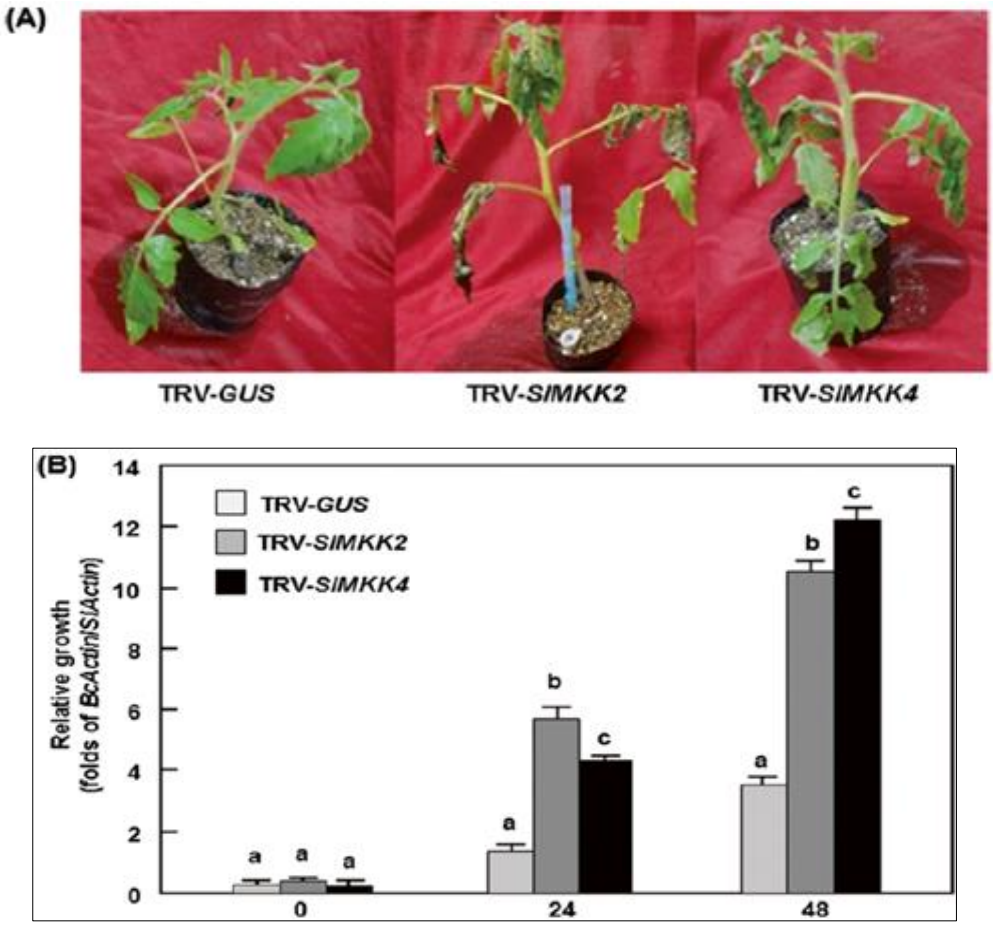

Fig 3: Whole plant disease assay

Knockdown of the SlMKK2 or SlMKK4 resulted in reduced resistance to *B. cinerea* and thus both SlMKK2 and SlMKK4 are required for resistance against *B. cinerea*

Transient expression of SlMKK2/SlMKK4 in *Nicotiana benthamiana* activated defense responses against *B. cinerea*

They examined whether over expression SlMKK2 or SlMKK4 can confer an increased resistance to *B. cinerea*. They were unable to observe typical HR when transiently expressed the wild types of SlMKK2 and SlMKK4 genes in *Nicotiana* leaves. Considering that SlMKK2 and SlMKK4 are components of the MAPK cascades that require protein phosphorylation for their biochemical functions, they thus generated constitutively active phosphor mimicking forms of SlMKK2 and SlMKK4, $SlMKK2^{DD}$ and $SlMKK4^{DD}$ respectively by replacing the conserved Ser/Thr residues in the activation loop ((S/T)XXXXX(S/T)) with Asp.

Conclusion

Plant MAPK cascades are complex networks, which are necessary for defence mechanism in plant. Families of plant MAPK components have expanded during evolution, and different combinations of components accomplish particular functions. By amplifying and transducing pathogen-derived signals perceived at membrane receptors and transducing these signals into altered gene expression, plant MAPK modules play a key role in the induction of defense mechanism. A combination of biochemical and genetic studies will be required to understand the complex roles of MAPKs in plant defense responses. The identification and *in planta* verification of additional MAPKKs modules, will be a future challenge to unravel the sophisticated network of plant MAPK signaling pathways. It is also essential to determine the upstream receptors or sensors that monitor the stimuli as well as the downstream effectors that regulate the responses.

References

1. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL. MAP kinase signaling cascade in Arabidopsis innate immunity. Nature. 2002; 415:977-83.
2. Barbara NK, David MB. Cross talk between signaling pathways in pathogen defense. Current Opinion in Plant Biology. 2002; 5:325-331.
3. Bernoux M, Ellis JG, Dodds PN. New insights in plant immunity signaling activation. Current Opinion in Plant Biology. 2011; 14:512-518.
4. Bethke G, Pecher P, Tsuda K, Katagiri F, Glazebrook J. Activation of the Arabidopsis thaliana mitogen-activated protein kinase MPK11 by the flagellinderived elicitor peptide, flg22. Molecular Plant Microbe Interaction. 2012; 25:471-80.
5. Chang L, Karin M. Mammalian MAP kinase signaling cascades. Nature. 2001; 410:37-40.
6. Clarke JD, Volko SM, Ledford H, Ausubel FM, Dong X. Roles of salicylic acid, jasmonic acid, and ethylene in cpr induced resistance in Arabidopsis. Plant Cell. 2000; 12:2175-2190.
7. Colcombet J, Hirt H. Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes. Biochemistry Journal. 2008; 413:217-26.
8. Doares SH, Narvaez VJ, Conconin A, Ryan CA. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiology. 1995; 108:1741-1746.
9. Dodds PN, Rathjen JP. Plant immunity: towards an integrated view of plant–pathogen interactions. Natural Review of Genetics. 2010; 11:539-548
10. Duerr B, Gawienowski M, Ropp T, Jacobs T. MsERK1: a mitogen-activated protein kinase from a flowering plant. Plant Cell. 1993; 5:87-96
11. Gupta V, Willits MG, Glazebrook J. Arabidopsis thaliana EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jasmonic acid signaling by SA. Molecular Plant Microbe Interaction. 2000; 13:503-511.
12. Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K. Various abiotic stresses rapidly activate Arabidopsis MAP kinases ATMPK4 and ATMPK6. Plant Journal. 2000; 24:655-65.
13. Ichimura K, Shinozaki K, Tena G, Sheen J, Henry Y, Champion A. Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends in Plant Science. 2002; 7:301-8.
14. Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, *et al*. 1998. Phosphorylation of the MAP kinase ERK2 promotes its homo dimerization and nuclear translocation. Cell. 1998; 93:605-15.
15. Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M. Phosphorylation of the MAP kinase ERK2 promotes its homo dimerization and nuclear translocation. Cell. 1998; 93:605-15
16. Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell. 1993; 72:427-41.
17. Kloek AP, Verbsky ML, Sharma SB, Schoelz JE, Vogel J, Klessig DF, *et al*. Resistance to Pseudomonas syringae conferred by an Arabidopsis thaliana coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. Plant Journal. 2001; 26:509-522.
18. Lawton KA, Potter SL, Uknes S, Ryals J. Acquired resistance signal transduction in Arabidopsis is ethylene independent. Plant Cell. 1994; 6:581-588.
19. Li X, Zhang Y, Huang L, Ouyang Z, Hong Y, Zhang H, *et al*. Tomato SlMKK2 and SlMKK4 contribute to disease resistance against Botrytis cinerea. BMC Plant Biology. 2014; 14:166.
20. Luan S. Protein phosphatases in plants. Annual Review of Plant Biology. 2003; 54:63-92.
21. Niki T, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiology. 1998; 39:500-507.
22. Norman C, Vidal S, Palva ET. Interacting signal pathways control defense gene expression in Arabidopsis in response to cell wall-degrading enzymes from Erwinia carotovora. Molecular Plant Microbe Interaction. 2000; 13:430-438.
23. O'Donnell PJ, Jones JB, Antoine FR, Ciardi J. Ethylene dependent salicylic acid regulates an expanded cell death

response to a plant pathogen. Plant Journal. 2001; 25:315-323.
24. Oh CS, Martin GB. Effector-triggered immunity mediated by the Pto kinase. Trends in Plant Science. 2011; 16:132-140.
25. Penninckx IA, Thomma BP, Buchala A, Metraux JP, Broekaert WF. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell. 1998; 10:2103-2113.
26. Penninckx I, Eggermont K, Terras FRG, Thomma GP, De Samblanx GW, Broekaert WF, *et al*. Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. Plant Cell. 1996; 8:2309-2323.
27. Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johansen B, *et al*. Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. Cell. 2000; 103:1111-1120.
28. Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, *et al*. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci. 2000; 97:11655-11660.
29. Stafstrom JP, Altschuler M, Anderson DH. Molecular cloning and expression of a MAP kinase homologue from pea. Plant Molecular Biology. 1993; 22:83-90
30. Takekawa M, Tatebayashi K, Saito H. Conserved docking site is essential for activation of mammalian MAP kinase kinases by specific MAP kinase kinase kinases. Molecular Cell biology. 2005; 18:295-30.
31. Vidal S, Ponce I, Denecke J, Palva ET. Salicylic acid and the plant pathogen Erwinia carotovora induce defense genes via antagonistic pathways. Plant Journal. 1997; 11:115-123.
32. Wang H, Liu Y, Bruffett K, Lee J, Hause G, Walker JC, *et al*. Haplo-insufficiency of MPK3 in MPK6 mutant background uncovers a novel function of these two MAPKs in Arabidopsis ovule development. Plant Cell Online. 2008; 20:602-13.
33. Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell. 2007; 19:63-73.
34. Xie D, Feys BF, James S, Neito-Rostro M, Turner JG. COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science. 1998; 280:1091-1094.
35. Xu Y, Chang PLC, Liu D, Narasimhan ML, Kashchandra GR, Hasegawa PM, *et al*. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell. 1994; 6:1077-1085.
36. Zeng Q, Chen JG, Ellis BE. AtMPK4 is required for male-specific meiotic cytokinesis in Arabidopsis. Plant Journal. 2011; 67:895-906.