Daniela Reis Joaquim de Freitas
(Organizadora)

Producción científica en Ciencias Biológicas

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Ponta Grossa – Paraná – Brasil
Telefone: +55 (42) 3323-5493
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APRESENTAÇÃO

Las Ciencias Biológicas estudian los seres vivos y todas sus relaciones entre sí y con el medio ambiente. Es un campo muy amplio, que engloba diferentes áreas de conocimiento, y que puede ser aplicado en el área de la educación, la investigación, la bioconservación ambiental, la salud, etc.

El trabajo "Producción ciencia en Ciencias Biológicas" está enfocado a discutir la formación del conocimiento en varias áreas que conforman el gran dominio de las Ciencias Biológicas, brindando al lector una visión variada y amplia de lo que se produce en esta área en la actualidad. En este trabajo contamos con seis capítulos compuestos por artículos científicos originales basados en trabajos de investigación.

Los trabajos descritos en este libro abordan temas relacionados con las ciencias de la salud como microbiología, zoología y ecología de especies, botánica, divulgación científica, medio ambiente, biodiversidad y bioconservación. Esta multidisciplinariedad es de gran importancia, ya que la investigación con diferentes perspectivas profesionales tiende a proporcionar una visión más amplia y una mayor aplicabilidad en la vida cotidiana del lector.

Creemos que este trabajo enriquecerá su conocimiento y demostrará que la ciencia puede ser muy placentera. Atena Editora, buscando la calidad, tiene a su disposición un cuerpo editorial compuesto por maestros y doctores formados en las mejores universidades de Brasil, para la revisión de sus obras. Por lo tanto, está asegurado que tiene un trabajo de excelente calidad en sus manos. Esperamos que disfrute de su lectura. ¡Buenos estudios!

Daniela Reis Joaquim de Freitas

SUMÁRIO

CAPÍTULO 5

A PHYLOGENETIC STUDY OF THE MEMBERS OF THE MAPK FAMILY ACROSS VIRIDIPLANTAE

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José Manuel González-Coronel
Departamento de Biología Molecular de Plantas. Instituto de Biotecnología, Universidad Nacional Autónoma de México
Cuernavaca, Morelos, México

Gustavo Rodríguez-Alonso
Departamento de Biología Molecular de Plantas. Instituto de Biotecnología, Universidad Nacional Autónoma de México, Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas. Universidad Autónoma del Estado de Morelos
Cuernavaca, Morelos, México

Ángel Arturo Guevara-García
Departamento de Biología Molecular de Plantas. Instituto de Biotecnología, Universidad Nacional Autónoma de México
Cuernavaca, Morelos, México

ABSTRACT: Protein phosphorylation is regulated by the activity of enzymes generically known as kinases. One of those kinases is Mitogen-Activated Protein Kinases (MAPK), which operate through a phosphorylation cascade conformed by members from three related protein kinase families namely MAPK kinase kinase (MEKK), MAPK kinase (MEK), and MAPK; these three acts hierarchically. Establishing the evolution of these proteins in the plant kingdom is an interesting but complicated task because the current MAPK, MAPKK, and MAPKKK subfamilies arose from duplications and subsequent sub-functionalization during the early stage of the emergence of Viridiplantae. Here, an *in silico* genomic analysis was performed on 18 different plant species, which resulted in the identification of 70 genes not previously annotated as components of the MAPK family. Interestingly, a deeper analysis of the sequences encoded by such genes revealed the existence of putative domains not previously described as signatures of MAP kinases. Additionally, our analysis also suggests the presence of conserved activation motifs besides the canonical TEY and TDY domains, which characterize the MAPK family.
KEYWORDS: Viridiplantae, Phylogeny, MAPK gene family, Novel domains.

INTRODUCTION

Plants have evolved diverse and complex response mechanisms to contend against constantly changing environmental conditions. The perception of these conditions, together with the subsequent transduction and amplification of the generated signals, triggers cellular responses crucial to achieve optimal growth and development. Post-translational modifications (PTMs) constitute a major regulatory mechanism of protein activity [2,3]. PTMs regulate the activation or inhibition of protein activity, change the subcellular localization, alter protein stability, and promote or prevent trans-interactions [2–4]. PTMs are catalyzed by a wide variety of enzymes and are usually reversible [3,5,6]. Particularly, protein kinases regulate processes at the transcriptional,

translational, and post-translational level by catalyzing the addition of a phosphoryl group (PO_3^{2-}) from ATP to a substrate protein in specific amino acid residues usually serine (S), threonine (T) or tyrosine Y 6–8. Phosphorylation reactions depend on the catalytic activity of the kinase and the affinity, or protein-protein interaction capability, for their target proteins [6,7]

Versus other eukaryotic genomes, plants contain a larger number of protein kinases; for example, the *Arabidopsis thaliana* L. genome encodes approximately 1,200 kinases while the rice (*Oryza sativa* L.) genome contains ~1,400 kinase genes [8,9]. On the other hand, approximately 500 and 120 protein kinases are encoded in the *Homo sapiens* L. and *Saccharomyces cerevisiae* Meyen ex E.C. Hansen. genomes, respectively [10]271,853 high-quality sequence reads (5.11-fold coverage of the genome.

The MAPKs belong to the group of serine/threonine protein kinases that are responsible for transforming extracellular *stimuli* into a wide range of cellular responses [6,11]. MAPK signaling cascades are highly conserved in all eukaryotic organisms and are made up of three different gene families MAPK or MPK, MAPK kinase (MKK or MEK), and MAPKK kinases (MPKKK or MEKK) [6,8,12]. These components are sequentially activated by phosphorylation in the activation domain of their substrates [13,14]. The first component of the signaling cascade is the MEKK; it phosphorylates a pair of serine (S) and threonine (T) residues in the S/T-X_{3-5}-S/T domain of MEK proteins. Subsequently, MEKs activate MAPKs by phosphorylation of threonine (T) and tyrosine (Y) residues in the activation domain (T-X-Y) [3,15,16].

The modular and hierarchical arrangement of the MAPK signaling pathways is suitable for amplification and integration of signals at the cellular level [17,18]. Signaling cascades mediated by MAPKs coexist in many cells and are connected and regulated by feedback to at least some degree [3,11,12,17,19,20]. Due to the central role played by the MAP kinases in signal transduction and transmission, it is of great interest to classify the members of these families based on their phylogenetic relationships and their functional characteristics [17,21]. Despite the growing availability of plant genomes, the identification of MPKs has been limited to a small number of species, mainly from monocots [22–24], and dicots [13,16,23,25–30].

Members of the MAP kinase family have 11 canonical domains with an activation domain between domains VII and VIII. The activation domain of MAP kinases contains a pair of threonine and tyrosine residues, which are phosphorylated by a MEK protein [31]. Angiosperm MAPKs are classified into four groups (A-D) on the basis of their activation domain [16].

The molecular activity of MAP kinases has been seen in *Arabidopsis*. For example, the MEKK1-MKK4/5-MPK3/6 module participates in flagellin-triggered immune response [32]; and the MEKK1-MEK1/2-MPK4/6 module is activated in response to different types of stress [33]. The module MEKK4-MEK4/5-MPK3/6 was initially identified as a regulator of stomata development [34], and later suggested to participate in the regulation of root and embryo development [35]. In this work, MAPKs from seven species not previously analyzed were identified: *Amaranthus hypochondriacus* L., *Azolla filiculoides* [Lam.], *Isoetes echinospora*

Durieu, *Marchantia polymorpha* L., *Pinus taeda* L., and *Ostreococcus tauri* C. Courties & M.-J. Chrétiennot-Dinet, *Salvinia cucullata* [Bory.]; to provide a list of members of the MAPK family of each of the 18 analyzed species. A total of 70 novel sequences of MAPK proteins were obtained. In addition to a detailed study of the members of the MAP kinase family of the aforementioned species, a comparative analysis was also perfomed by adding the genes that code for the members of such families in representative species of the different plant lineage, including a chlorophyta (*Chlamydomonas reinhardtii* PA Dang.), a lycophyte (*Selaginella moellendorffii* P. Beauv.), two bryophytes (*Physcomitrella patens* [Hedw.] Bruch & Schimp., and *Sphagnum fallax* H. Klinggr.), a gymnosperm (*Picea abies* [L.] H. Karst.), and 00/00/00 0:00:00four angiosperm species: *Vitis vinifera* L., *Beta vulgaris* L., *Brachypodium distachyon* [L.] P. Beauv. and *Amborella trichopoda* Baill.

MATERIALS AND METHODS

Identification of MAPK genes in Viridiplantae

A BLASTP was carried out using the sequences of the MAPK proteins of *Arabidopsis thaliana* L. as a query to find putative orthologs in *Amaranthus hypochondriacus* L., *Amborella trichopoda* Baill., *Chlamydomonas reindhardtii* P.A. Daung., *Physcomitrella patens* [Hedw.] Bruch & Schimp., *Selaginella moellendorffii* P. Beauv., *Sphagnum fallax* H. Klinggr. (https://phytozome.jgi.doe.gov/pz/portal.html), *Azolla filiculoides* [Lam.], and *Salvinia cucullata* [Bory.] (https://www.fernbase.org/), *Marchantia polymorpha* L. (https://marchantia.info), *Oryza sativa* L. (http://www.plantgdb.org/OsGDB/), *Ostreococcus tauri* C. Courties & M.-J. Chrétiennot-Dinet. (https://genome.jgi.doe.gov/Ostta4/Ostta4.home.html), *Pinus taeda* L., *Picea abies* [L.] H. Karst. (http://congenie.org/), and *Vitis vinífera* L. (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) databases; considering an expected value (e-value) of $1x10^{-35}$ as a threshold. An additional search was carried out using hidden Markov models using HMMER 3.0 software [36]. Subsequently, the presence of the serine/threonine protein kinase domain (PF00069) was corroborated in the retrieved sequences.

The putative members of the MAPK family must contain the characteristic sequence [L/I/V/M]-[TS]-X-X-[L/I/V/M]-X-T-[K/R]-[W/Y]-Y-R-X-P-X-[L/I/V/M]-[L/I/V/M] including the T-X-Y activation domain [16,23]. The NCBI Conserved Domain Database (http://blast.ncbi.nlm.nih.go), ProtParam online software from the ExPASy suite (https://web.expasy.org/protparam/), and the InterProScan database (https://www.ebi.ac.uk/interpro/search/sequence-search) were used to validate the presence of domains in each of the sequences.

Multiple sequence alignment, phylogenetic, and gene structure analysis

Multiple sequence alignment was generated using the iterative refined method E-INS-i from MAFFT online software (Multiple Alignment using Fast Fourier Transform; https://mafft.cbrc.jp/alignment/server/) [37]huge numbers of biological sequences are available and the

need for MSAs with large numbers of sequences is increasing. To extract biologically relevant information from such data, sophistication of algorithms is necessary but not sufficient. Intuitive and interactive tools for experimental biologists to semiautomatically handle large data are becoming important. We are working on development of MAFFT toward these two directions. Here, we explain (i, and visualized with Jalview software v2.11.1.3 [38]. The IQtree 1.6.6 (http://www.iqtree.org/) and ProtTest (http://darwin.uvigo.es) software were used to determine the evolution substitution model of proteins to be used for the construction of the phylogenetic tree [39,40]. The phylogenetic analysis was performed using the complete sequence of each protein with the IQtree v1.6.6 software. The maximum-likelihood algorithm was used. The tree topology was statistically tested with the bootstrap method with 1,000 iterations. The tree topology was visualized in the iTOL software v5.7 [41].

Identification of novel domains and protein structure prediction

MEME-suite software v5.2.0 [42] was used to identify the canonical and novel MAPK domains. MEME was run with default parameters and a predefined motif length of eight to 12–15 residues. PhosphoSVM software [43] was used to predict phosphorylation of the novel domains. The protein logos were performed with WebLogo software v2.8.2 [44]

The protein structure was predicted with the I-Tasser software v5.1 [1] and visualized in the PyMol software v2.4.1 (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.). The MPK6 crystal structure (PDB id: 5ci6) [45] was used as a template for structure modelling. Figure composition was performed using the InkScape software v1.0 (https://inkscape.org/es/).

RESULTS AND DISCUSSION

Identification and nomenclature

MAP kinases are of paramount importance for plant development and stress response [3]. The partial redundancy of this protein superfamily, the high sequence similarity among homologs, and the expansion of the gene family in diverse plant species, have hindered the evolutionary studies of MAPKs. To provide a comprehensive phylogenetic analysis of MAPK proteins, a genome-wide identification of MAPK genes was performed for 18 selected species, spanning the major clades of Viridiplantae. Our analysis includes chlorophyta (*C. reindhardtii*, and *O. tauri*), bryophytes (*M. polymoprpha*, *P. patens*, and *S. fallax*), lycophyte (*S. moellendorffii*), seedless vascular plants (*A. filiculoides*, *I. echinospora*, and *S. cucullata*), gymnosperms (*P. taeda* and *P. abies*) and angiosperms. For the last clade we include the basal angiosperm *Amborella trichopoda*, as well as monocotyledonous (*B. dystachion*, and *O. sativa*) and dicotyledoneous species (*A. hypochondriacus*, *A. thaliana*, *B. vulgaris*, and *V. vinifera*). MAPK genes were identified through a combined approach of

homology identification and hidden Markov models (Table 1).

	Specie	Lineage	MAPKs gene number
1	*Arabidopsis thaliana* L.	Angiosperm (Eudicot)	20
2	*Amaranthus hypochondriacus* L.	Angiosperm (Eudicot)	12
3	*Beta vulgaris* L.	Angiosperm (Eudicot)	7
4	*Vitis vinifera* L.	Angiosperm (Eudicot)	14
5	*Brachypodium distachyon* [L.] P. Beauv.	Angiosperm (Monocot)	14
6	*Oryza sativa* L.	Angiosperm (Monocot)	15
7	*Amborella trichopoda* Baill.	Angiosperm (Basal)	8
8	*Picea abies* [L.] H. Karst.	Gymnosperm	11
9	*Pinus taeda* L.	Gymnosperm	12
10	*Azolla filiculoides* [Lam.]	Pteridophyta	15
11	*Salvinia cucullata* [Bory.]	Pteridophyta	14
12	*Isoetes echinospora* Durieu.	Lycophyta	8
13	Selaginella moellendorffii	Lycophyta	5
14	*Sphagnum fallax* H. Klinggr.	Bryophyta	8
15	*Marchantia polymorpha* L.	Bryophyta	6
16	*Physcomitrella patens* [Hedw.] Bruch & Schimp.	Bryophyta	11
17	*Chlamydomonas reindhardtii* P. A. Dang.	Algae	6
18	*Ostreococcus tauri* C. Courties & M.-J. Chrétiennot-Dinet	Algae	3

Table 1. Number of MAPK genes present per genome (specie).

A total of 189 genes were identified: 119 previously described and 70 not previously annotated as member of the MAPK family. Although previous efforts to reconstruct the evolutionary history of MAPK genes have been made, ortholog identification on early divergent plant clades has remained elusive [13]. Moreover, independent studies in species such as *A. thaliana*, *O. sativa*, and *P. trichocarpa*, has led to different nomenclature and classification system for MAPKs [16].

Here, a nomenclature system was stablished in which each of the genes were named using a two-letter code corresponding to the first letter from the genus and species. This two-letter code remained in: Af (*Azolla filiculoides*), Ah (*Amaranthus hypochondriacus*), At (*Arabidopsis thaliana*), Bv (*Beta vulgaris*), Bd (*Brachypodium distachyon*), Cr

(*Chlamydomonas reindhardtii*), Ie (*Isoetes echinospora*), Mp (*Marchantia polymorpha*), Ot (*Ostreoccocus tauri*), Os (*Oryza sativa*), Pp (*Physcomitrella patens*), Pa (*Picea abies*), Pt (*Pinus taeda*), Sc (*Salvinia cucullata*), Sf (*Sphagnum fallax*), Sm (*Selaginella moellendorffii*), and Vv (*Vitis vinífera*); to distinguish *Amborella trichopoda* from *A. thaliana*, the second letter of both genus and species was added to the first one (i.e., Amtr). Next to this letter code the acronym MPK (from **M**itogen-activated **P**rotein **K**inase) was included along with a and a number referring to its most likely ortholog in *Arabidopsis thaliana* L. [46]. Likewise, when two or more of the identified sequences have the same putative ortholog in *Arabidopsis*, they were distinguished by adding a letter in alphabetical order. This is the case of the *P. taeda* sequences PITA_000030510, PITA_000007088 and PITA_000001460, which, were putative orthologs of AtMPK5 and were renamed as PtMPK5a, PtMPK5b and PtMPK5c, respectively.

PHYLOGENETIC ANALYSIS OF MAP KINASES

A multiple sequence alignment of the retrieved MAP kinases sequences was built and used to reconstruct the molecular phylogeny of MAP kinases. The alignment shows a high degree of conservation in the sequences that correspond to the 11 characteristic MAPK domains; moreover, all analyzed sequences contain the TXY-activation domain. A maximum likelihood tree was constructed for the MAP kinase-retrieved sequences; the tree was rooted with the *Saccharomyces cerevisiae* Fus3 protein that was selected as an outgroup given its similarity to Viridiplantae MAPKs. Previous studies classified the *Arabidopsis* MAPK genes into four groups (A, B, C and D) according to their sequence similarity and the presence of the TDY or TEY phosphorylation motifs [16]. The resulting ML tree topology displays five well-supported clades with bootstrapping values >90.0% out of 1000 replicates; thus, we support the previous suggestion to consider another group to classify MAP kinases (A-E); the existence of an additional group F has been refuted [47] (Figure 1).

Figure 1. Maximum-likelihood tree reconstructed from amino acid sequences. The tree was rooted with the Fus3 protein from *Saccharomyces cerevisiae*. Bootstrap values from 1,000 replicates are indicated at nodes with support values >49%. Each MAPK group is indicated with a distinctive color. Different species are indicated with shapes and colors.

The MAPK group A contains sequences retrieved from pteridophytes, gymnosperms and angiosperms. Surprisingly, no group A MAPKs were identified in the lycophytes, bryophytes or algae species analyzed. This observation suggests that group A MAPKs arose in the Euphyllophyte clade after the separation from the lycophytes, which occurred ca. 420 million years ago (MYA) [48,49] (Figure 1). AtMPK3 and AtMPK6 both belong to group A MAPKs and are the two most widely studied MAPK proteins. Together with their putative orthologs in other plant species, these species have been involved in responses to biotic and abiotic stresses [20,50,51]. Members of group B have been involved in cell division and in the responses to biotic and abiotic stress; in particular, the loss of function mutant *mpk4* of *A. thaliana* leads to a constitutive phenotype of systemic acquired resistance [52,53]. Some members such as AtMPK13, another group B MAPK, are activated through the cell cycle, and they are located specifically in the phragmoplast during telophase [54].

Groups A and B are sister clades according to the tree topology; this observation together with its absence in algae species suggests that these MAPK groups were the last to be acquired (Figure 1). Group C MAPKs are less characterized than MAPKs from groups A and B, although the expression and activity of AtMPK7 (group C) is regulated by the circadian cycle [55]. Group D makes up the largest group of MAPK proteins including 61 sequences. This is characterized by the sequence TDY in its activation domain; in

addition, group D MAPKs have an extended C-end compared to proteins from the groups A-C. In *Arabidopsis*, the AtMPK8 protein from group D is involved in seed germination and dormancy [56].

Finally, members of group E are known as **M**apk-**h**omologous **k**inases (MHKs) and may contain the TEY or TDY sequence and even some non-canonical motif in the activation domain [16]. It remains a matter of debate whether group E MAPKs constitute functional MAPKs given their similarity to cyclin-dependent kinases (CDKs), even though their biochemical properties are largely unknown.

MAPK ANALYSIS

Of the genomes under study, the angiosperm clade has the highest number of MAPK members per family, with *A. thaliana* having the highest number of genes encoding MAPKs (20) followed by rice (*O. sativa*) and grape (*Vitis vinifera*) with 15 and 14 genes, respectively. In addition, species *A. filiculoides* and *S. cucullata* in the pteridophytes clade have 15 and 14 genes, respectively. The large number of MAPK genes in Azolla and Salvinia might be a result of whole genome duplication events in the Salviniales [57]. On the other hand, *O. tauri* in the algae group has the lowest number of genes of the analyzed species (3), while *C. reindhardtti* has 6. Bryophytes have an increase in the number of MAPK genes with respect to that of algae. The members of this clade, *P. patens*, *S. fallax*, and *M. polymorpha* contain 11, 8, and 6 genes, respectively. The MAPK gene family expansion in bryophytes is congruent with the expansion of gene families in land plants upon terrestrialization and might be associates with plant facing new types of stress, such as dehydration, gravity, exposure to ultraviolet light, etc. after land colonization [58,59] (Figure 1; Table 1).

ANALYSIS OF MOTIFS AND DOMAINS CONSERVED IN THE MAPK FAMILY

MAPKs are characterized by the presence of multiple domains. Eight domains, named I-V, VIa, VIb, and VII are distributed towards the N-terminal preceding the activation domain. Towards the C-terminal end of the activation domain, there are five other domains previously described as typical of MAPKs. Domain VIII, IX, and X precede the CD domain; finally, the XI domain is localized around 30 amino acids after the CD domain (Table 2) [31,33]. We used the MEME's bioinformatics software to address domain conservation among the identified MAPK sequences.

Domain	Consensus sequence
I	[V/P]-[I/V]-G-[K/R]-G-[S/A]-Y-G-[V/I]-V-C-S-A
II	E-X-V-A-I-K-K-I-X-[N/D]-[A/V/I]-F-[E/D]-[N/H]-X_2-D-A
III	R-[T/I]-L-R-E-[I/L]-K-L-L-R-[H/L]-[L/M]-[R/D]
IV	P-X-[R/K]-X_2-F-X-D-[V/I]-Y
V	V-[F/Y]-E-L-M-[E/D]-[T/S]-D-L-H-Q-[V/I]-I-[K/R]
VIa	[F/Y]-F-L-Y-Q-[L/I/M]-L-R-[G/A]-L-K-Y
VIb	H-[S/T]-A-N-[V/I]-[L/F/Y]-H-R-D-K-L-P-[K/S]-N-[L/I]-L-[A/L]-N
VII	C-[D/K]-L-K-I-[C/A]-D-F-G-L-A-R-[V/T]
VIII	[V/A]-T-R-W-Y-R-A-P-E-L-[L/C]-[L/G]-[S/N]
IX	A-I-D-[I/V/M]-W-S-[V/I]-G-C-I-F-[A/M]-E-[L/I/M]-[L/M]
X	P-[L/I]-F-P-G-X_3-[V/L]-X-Q-L-X-L-[I/M]-T-[D/E]
XI	F-D-P-X_2-R-[I/P]-[T/S]-[A/V]-X-[E/D]-A-L-X-[H/D]-P-Y-[F/L]
CD	L-H-D-X_2-D-E-P

Table 2. Consensus sequences of the conserved domains in MAPKs.

Of the 90 sequences analyzed that belong to angiosperms, 83 present all the canonical domains. The remaining seven (2 sequences from *A. trichopoda* and 5 from *V. vinifera*) lack at least one domain: VvMPK1 and VvMPK7 (domains I-IV), VvMK5a and VvMPK5b (III-V and VIa), VvMPK20 (I -III), AmtrMPK5 (IV), and AmtrMPK16a (XI). The fact that the vast majority of sequences belonging to angiosperms contain all the canonical domains could reflect a bias because this clade is the most studied. Most of the sequences of the analyzed angiosperm species lack multiple domains. In *P. taeda*: PtMPK5a (III-V, and VIa), PtMPK5b (III-V, VIa, and IX), PtMPK5c (III-V, VIa, and IX-X), PtMPK11 (I -III), PtMPK16 (II-V, and VIa), and PtMPK20 (IX-X). In *P. abies*: we note PaMPK2 (I-VII), PaMPK4a (V), PaMPK7a (VIb and VII-XI), PaMPK7b and PaMPK10 (III, V, and VIa), PaMPK11 (I-III, and V), and PaMPK16 (IX and XI). Eight of these sequences belong to group E MAPKs, which include MHKs and whose inclusion as MAPKs, as already mentioned, has been debated due to their sequence similarity to CDKs [12]. Whether group E proteins function as MAPKs has been poorly studied, although an ortholog of these proteins in the fungus *Ustilago maydis* is activated by phosphorylation by a MAPKK and contains the activation motif TXY [60]. This observation supports the hypothesis that group E MAPKs should be regarded as a subfamily of MAPKs. In fern species, the AfMPK6b and AfMPK16b sequences of *A. filiculoides* lack domain I and domain IX, respectively. The AfMPK4d sequence lacks domains IV and IX. On the other hand, in *S. cucullata*, the ScMPK6b sequence lacks the V,

VIa and VIb domains; the ScMPK16c sequence lacks domain XI. Domains III, IV and VII are absent in the ScMPK2 sequence. In the case of *I. echinospora*, the sequence IeMPK4 lacks I-V domains while IeMPK7 and IeMPK11 lacks domain XI.

The lack of several domains in the angiosperm and fern sequences is due to the fact that the genomes of these species are still fragmented, in the databases. Some of the ORFs that are found as hits might come from fragmented sequences and therefore the sequences corresponding to certain domains are missing. In the bryophytes *M. polymorpha*, *S. fallax*, and *P. patens*, we see MpMPK3 and MpMPK5 (III-V, and VIa), MpMPK4b (III and IV), PpMPK4b (II-IV, VIa, and IX), PpMPK4c (III-V, VIa, and IX), PpMPK15 (III, IV, VIa, and IX), PpMPK16a, and PpMPK16b (IX). For their part, the three sequences identified here from the alga *O. tauri* lack domain IV. Thus, the results of this analysis allow us to suggest that the presence of the activation motif (T-X-Y) together with five domains (II, III, V, and VII) located towards the N-terminal end of the activation domain and present in all the analyzed sequences are sufficient to identify MAP kinases from any plant species. In addition to this, the presence of domains VIII-XI in most Viridiplantae MAPK sequences suggests that these domains are of critical importance for maintaining either the function or structure of these proteins.

MAPK proteins contain a T-X-Y motif whose phosphorylation leads to MAPK activation [16]. The Viridiplantae multiple sequence alignment shows that all analyzed species contain the canonical activation domain in the form of T-E-Y (98 sequences), T-D-Y (77 sequences), and T-S-Y (1 sequence). The alignment also shows the presence of non-canonical activation domains including M-E-Y (2 sequences), T-E-M (2 sequences), T-H-E (4 sequences), T-H-L (1 sequence), T-H-Q (1 sequence), T-K-T (2 sequences), T-Q-M (1 sequence), and T-S-Y (1 sequence). The distribution of these activation domains on the MAPK phylogeny shows that the T-E-Y domain is present in MAPKs from groups A, B, C, and E, while the T-D-Y is present on the D and E groups. The activation domain contained in group E sequences exhibits the highest variability, which is especially evident on Bryophyta including T-D-Y, T-H-E, T-K-T, and T-Q-M variants (Table 3).

	Motif	Group A	Group B	Group C	Group D	Group E
Activation domain sequence	TEY	27	40	28	-	3
	TDY	-	-	-	58	9
	MEY	-	OsMPK5 BdMPK4a	-	-	-
	TDG	-	-	PaMPK7a	-	-
	TEM	-	-	-	-	PaMPK5 PaMPK7b

Activation domain sequence						
	THE	-	-	-	-	PpMPK4b PpMPK4c PtMPK5b PtMPK5c
	THL	-	-	-	-	PtMPK16b
	THQ	-	-	-	-	IeMPK16b
	TKT	-	-	-	-	IeMPK16c MpMPK3
	TQM	-	-	-	-	PamK10
	TSY	-	-	OtMPK2	-	-
	Total	27	42	30	58	23

Table 3. Distribution of the different activation domains in MAPK proteins.
(**Bd**) *B. dystachion*; (**Ie**) *I. echinospora*; (**Mp**) *M. polymorpha*; (**Os**) *O. sativa*; (**Ot**) *O. tauri;* (**Pa**) *P. abies*; (**Pt**) *P. taeda.*

In addition to the 11 characteristic domains, MAP kinases also contain the sequence (L/H)-D-X_2-D-E-P known as the common docking domain (CD domain) [20]. Our results show that the CD domain is present in most proteins from the A, B, and C MAPK group; although it is also present in a few sequences from the groups D and E, it is generally absent in proteins from these clades. The CD includes a motif involved in recognition and binding to MAPK substrates, as well as in protein-protein interaction with MEKs. The adjacent aspartate (D) and glutamate (E) residues in the CD, are essential for interaction with the basic residues lysine (K) and arginine (R), located in the binding site of MEK proteins [61]. It remains to be experimentally demonstrated whether the absence of the CD domain has any impact on the function of these group D and E MAPKs either for interaction and phosphorylation by MEKs or in MAPKs substrate specificity.

Identification of novel and distinctive domains for each group of MAPKs

The domain analysis of the retrieved sequences suggests the existence of six novel domains, named 12-17 (Figure 2). The presence of these domains might predict the group to which a MAPK protein belongs facilitating MAPK identification during genome or transcriptome annotation. The consensus sequence of such domains consists of 12-17 amino acid residues (Table 4).

Domain	Consensus sequence
12	G-N-X-F-E-V-[T/S]-X-K-Y
13	Y-X-[M/L]-W-[Q/R]-[T/S]-X-F-E-I-D-T-K-Y
14	H-[P/K]-D-I-V-E-[I/V/K]-[K/L]-[I/H/N]-[I/K]-[M/L]-L-P
15	M-L-X-F-[D/N]-P-X_2-R-I-[T/S]
16	E-[L/V]-[I/L]-G-[T/S]-P-X-[E/D]-X-D-L-X-F-[L/I/V]
17	A-[R/K]-[R/K]-Y-[L/I/V]-X_2-[L/M]-[R/P]-X_3-[P/R/K]-X-[P/S]

Table 4. Consensus sequences of the novel domains discovered in MAPKs.

Domain 12 is located approximately 15 residues before domain I from group A and B proteins. It is also present in OtMPK13 from *O. tauri*. Domain 13 is also located towards the N-terminus end of domain I but is only present in the proteins from group C therefore providing a signature for group C MAPKs. Domain 14 is located between domains II and III and typifies proteins from group D. Domains 15–17 are distributed towards the C-terminal end and, if present, they are always located before the CD domain between the canonical domains X and XI. Domain 15 is present in proteins from the B and E group with the sole exception of PtMPK16b from group E. Except VvMPK3 and VvMPK6 that belong to group A, domain 16 is only located in group B MAPKs. Domain 17 is present in most proteins except those in groups C and E. Interestingly, domain 17 is also present in the Fus3 kinase of *S. cerevisiae* this prompts the hypothesis that this domain was present in the ancestral MAPK sequences and was lost in groups C and E (Figure 2).

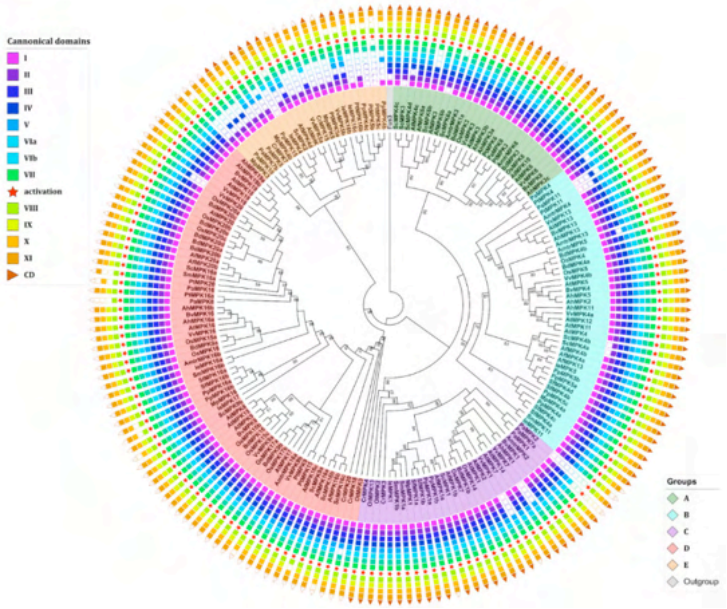

Figure 2. Phylogenetic analysis and identification of novel domains in MAPKs. The analysis of novel domains was performed with the MEME suite[42]. The distribution of the novel domains is group specific and could, therefore, facilitate MAPK identification and annotation. Sequence nomenclature follows a two-letter code to indicate genus and species (see text).

The conservation and distribution of these novel MAPK domains in a group-specific manner could suggest that they are involved in either maintaining the structure of MAPKs or in fine-tuning the selectivity towards substrates or protein interactors. This hypothesis is supported by homology structure prediction of selected MAPKs, which shows that domains 12, 13, 15, 15, and 17 are exposed to the solvent and could therefore provide an interaction surface. Meanwhile domain 14 is in a pocket (Figure 3). Moreover, a computational prediction of phosphorylation sites suggests that domains 15 and 16 contain amino acid residues susceptible to phosphorylation, whether these residues are indeed phosphorylated or if the identified novel domains are of relevance for the MAPKs structure and function remains to be experimentally demonstrated and extends beyond the scope of this study.

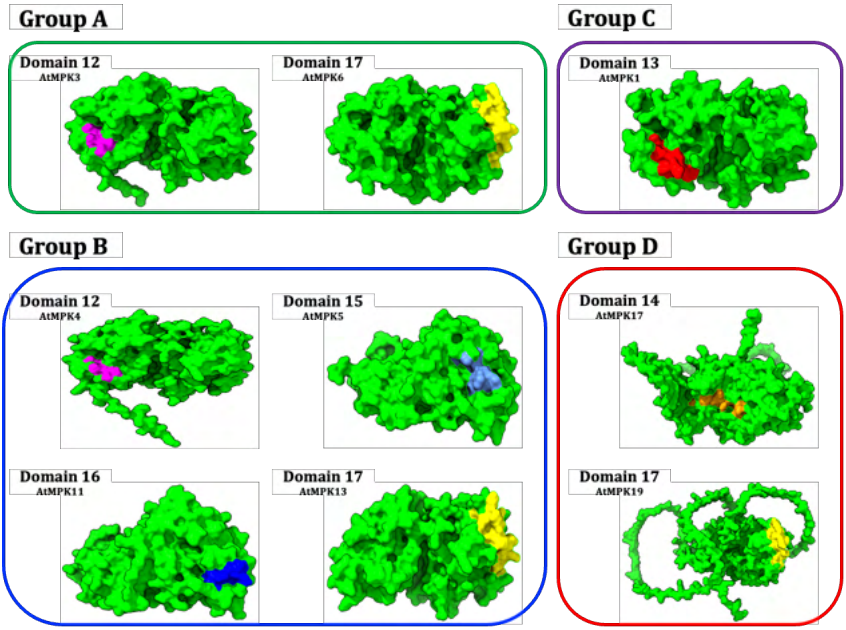

Figure 3. Molecular structure of plant MAPKs showing putative localization of the novel domains. The protein structures were obtained from homology modelling and visualized in PyMol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.). Each domain is presented as a sequence logo, obtained from the multiple sequence alignment of all the retrieved sequences [44]graphical representations of the patterns within a multiple sequence alignment. Sequence logos provide a richer and more precise description of sequence similarity than consensus sequences and can rapidly reveal significant features of the alignment otherwise difficult to perceive. Each logo consists of stacks of letters, one stack for each position in the sequence. The overall height of each stack indicates the sequence conservation at that position (measured in bits, and highlighted in the protein structure with a distinctive color.

CONCLUSIONS

MAPKs are among the oldest signal transduction pathways. In plants, they are involved in the regulation of various physiological processes such as hormone signaling [15,46] and respond to different types of stress [6,16–18,20]. Several members of the MEK family can act as convergence points of a great variety of signals and stimuli with an extraordinary high substrate specificity towards a MAPK. MAPKs on the other hand seem to function as divergence points [26]. Despite their involvement in several biological processes and the previous efforts to elucidate the evolutionary paths of MAPKs diversification in plants such convergence has been limited by the intrinsic properties of the MAPK signaling cascade components, i.e., its functional redundancy, high similarity at the amino acid level, gene family expansion and contraction in several plant taxa, and the availability of sequenced plant genomes.

Here, the phylogenetic relationships between MAPKs from 18 Viridiplantae species were analyzed. The analyzed species were selected to span the major Viridiplantae clades

ranging from Chlorophyta, i.e., green algae, up to angiosperms. This allows for a comprehensive and genome wide exploration of MAPKs across Viridiplantae, and the consequent reconstruction of gene of gene phylogenies to assess the diversification of the MAPK signaling cascades in plants. The need to implement a heuristic nomenclature system has become more evident with the identification of a large number of MAPK family genes. In addition, the system proposed by Hamel *et al*. (2006) named MAPK genes according to their Arabidopsis orthologs and signature sequences that typify MAPK genes and has been extended. The proposed nomenclature might be used as a practical tool to aid in the identification of novel MAPK genes in additional plant genomes and even during genome annotation projects. The reconstructed ML trees exhibit a well resolved and supported topology; therefore, it is useful to recognize the most likely Arabidopsis ortholog of the retrieved MAPK sequences.

Orthology-based nomenclature systems provide functional insights for each MAPK clade [15,27]; thus, some of the novel domains identified in this work could be correlated to substrate, activator, or inhibitor specificity as well as to protein-protein interactions. It is likely that some of the conclusions reached, or the number of identified sequences in some of the analyzed species, might need to be adjusted as new genome versions are updated or reannotated. Also, the inclusion of more genomes specifically from plant clades that are still underrepresented such as lycophyte or gymnosperm might help to refine the reconstructed phylogenies. International collaborative projects such as the 10KP will overcome this gap in the near future [62].

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REFERENCES

1. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc. 2010;5(4):725–38.

2. Olsen JV, Mann M. Status of Large-scale Analysis of Post-translational Modifications by Mass Spectrometry. Molecular & Cellular Proteomics. 2013;12(12):3444–52.

3. Bigeard J, Hirt H. Nuclear Signaling of Plant MAPKs. Front Plant Sci. 2018;9:469.

4. Bigeard J, Colcombet J, Hirt H. Signaling Mechanisms in Pattern-Triggered Immunity (PTI). Molecular Plant. 2015;8(4):521–39.

5. Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K. Various abiotic stresses rapidly activate Arabidopsis MAP kinases ATMPK4 and ATMPK6. Plant J. 2000;24(5):655–65.

6. Mishra NS, Tuteja R, Tuteja N. Signaling through MAP kinase networks in plants. Archives of Biochemistry and Biophysics. 2006;452(1):55–68.

7. Schweighofer A, Hirt H, Meskiene I. Plant PP2C phosphatases: emerging functions in stress signaling. Trends in Plant Science. 2004;9(5):236–43.

8. The Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature. 2000;408(6814):796–815.

9. Dardick C, Chen J, Richter T, Ouyang S, Ronald P. The Rice Kinase Database. A Phylogenomic Database for the Rice Kinome. Plant Physiol. 2007;143(2):579–86.

10. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The Sequence of the Human Genome. Science. 2001;291(5507):1304–51.

11. Colcombet J, Hirt H. *Arabidopsis* MAPKs: a complex signalling network involved in multiple biological processes. Biochemical Journal. 2008;413(2):217–26.

12. Dóczi R, Ökrész L, Romero AE, Paccanaro A, Bögre L. Exploring the evolutionary path of plant MAPK networks. Trends in Plant Science. 2012;17(9):518–25.

13. Hamel L-P, Nicole M-C, Sritubtim S, Morency M-J, Ellis M, Ehlting J, et al. Ancient signals: comparative genomics of plant MAPK and MAPKK gene families. Trends in Plant Science. 2006;11(4):192–8.

14. Kersten B, Agrawal GK, Durek P, Neigenfind J, Schulze W, Walther D, et al. Plant phosphoproteomics: An update. Proteomics. 2009;9(4):964–88.

15. Wu P, Wang W, Li Y, Hou X. Divergent evolutionary patterns of the MAPK cascade genes in Brassica rapa and plant phylogenetics. Hortic Res. 2017;4(1):17079.

16. (Kazuya Ichimura et al.) MG, Ichimura K, Shinozaki K, Tena G, Sheen J, Henry Y, et al. Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends in Plant Science. 2002;7(7):301–8.

17. Chardin C, Schenk ST, Hirt H, Colcombet J, Krapp A. Review: Mitogen-Activated Protein Kinases in nutritional signaling in Arabidopsis. Plant Science. 2017;260:101–8.

18. Luo J, Wang X, Feng L, Li Y, He J-X. The mitogen-activated protein kinase kinase 9 (MKK9) modulates nitrogen acquisition and anthocyanin accumulation under nitrogen-limiting condition in Arabidopsis. Biochemical and Biophysical Research Communications. 2017;487(3):539–44.

19. Tena G, Asai T, Chiu W-L, Sheen J. Plant mitogen-activated protein kinase signaling cascades. Current Opinion in Plant Biology. 2001;4(5):392–400.

20. Jonak C. Complexity, Cross Talk and Integration of Plant MAP Kinase Signalling. Current Opinion in Plant Biology.2002;5(5):415–24.

21. Popescu SC, Popescu GV, Bachan S, Zhang Z, Gerstein M, Snyder M, et al. MAPK target networks in Arabidopsis thaliana revealed using functional protein microarrays. Genes & Development. 2009;23(1):80–92.

22. Rao KP, Richa T, Kumar K, Raghuram B, Sinha AK. In Silico Analysis Reveals 75 Members of Mitogen-Activated Protein Kinase Kinase Kinase Gene Family in Rice. DNA Research. 2010;17(3):139–53.

23. Wang J, Pan C, Wang Y, Ye L, Wu J, Chen L, et al. Genome-wide identification of MAPK, MAPKK, and MAPKKK gene families and transcriptional profiling analysis during development and stress response in cucumber. BMC Genomics. 2015;16(1):386.

24. Chen L, Hu W, Tan S, Wang M, Ma Z, Zhou S, et al. Genome-Wide Identification and Analysis of MAPK and MAPKK Gene Families in Brachypodium distachyon. Wu K, editor. PLoS ONE. 2012;7(10):e46744.

25. Kong F, Cao M, Li N, Sun B, Sun M, Mao Y. Genome-Wide Identification, Phylogeny, and Expressional Profiles of the Mitogen-Activated Protein Kinase Kinase Kinase (MAPKKK) Gene Family in Pyropia yezoensis. Front Mar Sci. 2020;7:193.

26. Liu Y, Zhang D, Wang L, Li D. Genome-Wide Analysis of Mitogen-Activated Protein Kinase Gene Family in Maize. Plant Mol Biol Rep. 2013;31(6):1446–60.

27. Çakır B, Kılıçkaya O. Mitogen-activated protein kinase cascades in Vitis vinifera. Front Plant Sci [Internet]. el 22 de julio de 2015 [citado el 8 de noviembre de 2021];6. (http://journal.frontiersin.org/Article/10.3389/fpls.2015.00556/abstract)

28. Jiang M, Chu Z. Comparative analysis of plant MKK gene family reveals novel expansion mechanism of the members and sheds new light on functional conservation. BMC Genomics. 2018;19(1):407.

29. Singh A, Nath O, Singh S, Kumar S, Singh IK. Genome-wide identification of the MAPK gene family in chickpea and expression analysis during development and stress response. Plant Gene. 2018;13:25–35.

30. Zhang S, Xu R, Luo X, Jiang Z, Shu H. Genome-wide identification and expression analysis of MAPK and MAPKK gene family in Malus domestica. Gene.2013;531(2):377–87.

31. Hirt H. Multiple roles of MAP kinases in plant signal transduction. Trends in Plant Science. 1997;2(1):11–5.

32. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu W-L, Gomez-Gomez L, et al. MAP kinase signalling cascade in Arabidopsis innate immunity. Nature. 2002;415(6875):977–83.

33. Teige M, Scheikl E, Eulgem T, Dóczi R, Ichimura K, Shinozaki K, et al. The MKK2 Pathway Mediates Cold and Salt Stress Signaling in Arabidopsis. Molecular Cell.2004;15(1):141–52.

34. 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*. The Plant Cell. 2007;19(1):63–73.

35. López-Bucio JS, Dubrovsky JG, Raya-González J, Ugartechea-Chirino Y, López-Bucio J, de Luna-Valdez LA, et al. Arabidopsis thaliana mitogen-activated protein kinase 6 is involved in seed formation and modulation of primary and lateral root development. Journal of Experimental Botany. 2014;65(1):169–83.

36. Johnson LS, Eddy SR, Portugaly E. Hidden Markov model speed heuristic and iterative HMM search procedure. BMC Bioinformatics. 2010;11(1):431.

37. Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics. 2019;20(4):1160–6.

38. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009;25(9):1189–91.

39. Abascal F, Zardoya R, Posada D. ProtTest: selection of best-fit models of protein evolution. Bioinformatics. 2005;21(9):2104–5.

40. Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 2016;44(W1):W232–5.

41. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Research. 2019;47(W1):W256–9.

42. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Research. 2009;37(Web Server):W202–8.

43. Dou Y, Yao B, Zhang C. PhosphoSVM: prediction of phosphorylation sites by integrating various protein sequence attributes with a support vector machine. Amino Acids.2014;46(6):1459–69.

44. Crooks GE, Hon G, Chandonia J-M, Brenner SE. WebLogo: A Sequence Logo Generator. Genome Res. 2004;14(6):1188–90.

45. Wang B, Qin X, Wu J, Deng H, Li Y, Yang H, et al. Analysis of crystal structure of Arabidopsis MPK6 and generation of its mutants with higher activity. Sci Rep. 2016;6(1):25646.

46. Nicole M-C, Hamel L-P, Morency M-J, Beaudoin N, Ellis BE, Séguin A. MAP-ping genomic organization and organ-specific expression profiles of poplar MAP kinases and MAP kinase kinases. BMC Genomics. 2006;7(1):223.

47. Mohanta TK, Arora PK, Mohanta N, Parida P, Bae H. Identification of new members of the MAPK gene family in plants shows diverse conserved domains and novel activation loop variants. BMC Genomics. 2015;16(1):58.

48. Raven JA, Edwards D. Roots: evolutionary origins and biogeochemical significance. Journal of Experimental Botany. 2001;52(suppl 1):381–401.

49. Dolan L, Scheres B. Root pattern: Shooting in the dark? Seminars in Cell & Developmental Biology.1998;9(2):201–6.

50. Takahashi F, Yoshida R, Ichimura K, Mizoguchi T, Seo S, Yonezawa M, et al. The Mitogen-Activated Protein Kinase Cascade MKK3–MPK6 Is an Important Part of the Jasmonate Signal Transduction Pathway in *Arabidopsis*. The Plant Cell. 2007;19(3):805–18.

51. Kovtun Y, Chiu W-L, Tena G, Sheen J. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proceedings of the National Academy of Sciences. 2000;97(6):2940–5.

52. Lewis TS, Shapiro PS, Ahn NG. Signal Transduction through MAP Kinase Cascades. En: Advances in Cancer Research [Internet]. Elsevier; 1998 [citado el 22 de noviembre de 2021]. p. 49–139. (https://linkinghub.elsevier.com/retrieve/pii/S0065230X08607654)

53. Rasmussen MW, Roux M, Petersen M, Mundy J. MAP Kinase Cascades in Arabidopsis Innate Immunity. Front Plant Sci [Internet]. 2012 [citado el 8 de noviembre de 2021];3. (http://journal.frontiersin.org/article/10.3389/fpls.2012.00169/abstract)

54. Calderini O, Bogre L, Vicente O, Binarova P, Heberle-Bors E, Wilson C. A cell cycle regulated MAP kinase with a possible role in cytokinesis in tobacco cells. Journal of Cell Science. 1998;111(20):3091–100.

55. Schaffer R, Landgraf J, Accerbi M, Simon V, Larson M, Wisman E. Microarray Analysis of Diurnal and Circadian-Regulated Genes in Arabidopsis. The Plant Cell. 2001;13(1):113–23.

56. Zhang W, Cochet F, Ponnaiah M, Lebreton S, Matheron L, Pionneau C, et al. The MPK 8- TCP 14 pathway promotes seed germination in Arabidopsis. Plant J.2019;100(4):677–92.

57. Li F-W, Brouwer P, Carretero-Paulet L, Cheng S, de Vries J, Delaux P-M, et al. Fern genomes elucidate land plant evolution and cyanobacterial symbioses. Nature Plants. 2018;4(7):460–72.

58. Panchy N, Lehti-Shiu M, Shiu S-H. Evolution of Gene Duplication in Plants. Plant Physiol. 2016;171(4):2294–316.

59. Van de Peer Y, Mizrachi E, Marchal K. The evolutionary significance of polyploidy. Nat Rev Genet. 2017;18(7):411–24.

60. Garrido E, Voß U, Müller P, Castillo-Lluva S, Kahmann R, Pérez-Martín J. The induction of sexual development and virulence in the smut fungus *Ustilago maydis* depends on Crk1, a novel MAPK protein. Genes Dev. 2004;18(24):3117–30.

61. Tanoue T, Adachi M, Moriguchi T, Nishida E. A conserved docking motif in MAP kinases common to substrates, activators and regulators. Nat Cell Biol. 2000;2(2):110–6.

62. Cheng S, Melkonian M, Smith SA, Brockington S, Archibald JM, Delaux P-M, et al. 10KP: A phylodiverse genome sequencing plan. GigaScience (https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giy013/4880447).

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