***In silico* analysis and molecular investigation of the nuclear shuttle protein-interacting kinase 1 (NIK1) in six Solanaceous species**

Mehdi Safaeizadeh^{1*}, Nachelli Malpica²

¹Department of Plant Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

²Zürich-Basel Plant Science Center, Department of Environmental Sciences, University of Basel, 4056 Basel, Switzerland

Author contact information:

* To whom correspondence should be addressed: Mehdi Safaeizadeh

Author contact information:

First author:

Assistant Prof. Dr. Mehdi Safaeizadeh

Ma_Safaei@sbu.ac.ir

Mehdi.Safaeizadeh@gmail.com

Mehdi.Safaeizadeh@unibas.ch

Second author:

Dr. Nachelli Malpica

Nachelli.Malpica@gmail.com

Abstract

The first layer of innate immunity in plants is initiated through the perception of microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs). MAMP/DAMP perception initiates downstream defense responses, a process which ultimately leads to pattern triggered immunity (PTI). In Arabidopsis, the nuclear shuttle protein-interacting kinase 1 (NIK1), among other PRRs, is one of the most important central components of PTI signaling and kinase signaling cascade, since it is involved in the plant antiviral

response against geminiviruses. Despite the characterization of the structure and function of the NIK1 receptors made by some groups, studies related to NIK1 importance in the current gene-editing era are missing. By simple *in silico* analysis, in this study we investigated the NIK1 homologues from six Solanaceous plant species including: tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), *Solanum pennellii*, eggplant (*Solanum melongena*), pepper (*Capsicum annum*), and *Nicotiana benthamiana*. The phylogenetic analyses of different NIK1 proteins from Arabidopsis and six Solanaceous plants revealed nine different clades. As expected, we found that these NIK1 orthologs have similar genomic structures suggesting a similar function. We could identify that SotubNIK1, SolyNIK1, SopenNIK1, CANIK1, NibenNIK1, SmeNIK1 have the highest sequence homology with AtNIK1. Additionally, the conserved protein kinase domain (PKD) that is present in NIK1 from *Arabidopsis thaliana* was bioinformatically analyzed and found in other species. As this highly conserved NIK1 region is present in several crops of economic importance, its potential is highlighted as a possible target site for gene editing, to develop crops tolerant to geminiviral infections.

Key words: cell signaling, NIK1, phylogenetic analysis, protein structure, signal transduction, *Arabidopsis thaliana*, gene editing, geminiviruses

Introduction

Plants and animals are always at risk of infections from various micro-organisms. There are several observed similarities between the plant and animal immune systems^{1,2}, but as plants lack specialized mobile immune cells, as well as the adaptive immune system that is present in animals, they largely depend on their innate immune system to defend themselves against potential infections³.

Plants are equipped with different arrays of defense strategies⁴. In a co-evolutionary arm race between plants and pathogen interactions, as a first line of defense against microbial pathogens, plants perceive microbes via microbe-associated molecular patterns (MAMPs) by plasma membrane-

resident immune receptors which are called pattern-recognition receptors (PRRs). These receptors are located on the cell surface^{4,5}. As a consequence of MAMPs perception, a signaling cascade is initiated that finally leads to pattern-triggered immunity PTI⁴, where global transcriptional changes and cell re-programming take place⁸. Interference with this network can paralyze the adequate response upon pathogen infection⁶⁻⁷.

Besides PTI, there is another layer of the immune system called effector triggered immunity (ETI), which is initiated by the activation of nucleotide-binding leucine-rich repeat (NLR) proteins. These receptors detect the presence of pathogen-derived effectors within plant cells, but the underlying mechanisms of recognition are still poorly understood⁹. Overlapping responses between PTI and ETI have been observed, although ETI has been shown to be stronger than PTI leading to plant cell death. Like the plant innate immune system shares a common ancestor, the better understanding of the defense mechanisms in the model plant *Arabidopsis thaliana* offers an opportunity to formulate adequate defense strategies against crop diseases¹⁰.

A. thaliana is able to detect the presence of a variety of MAMPs including bacterial elicitors like flagellin¹¹, the elongation factor EF-Tu¹¹, peptidoglycans¹², lipopolysaccharides¹³, bacterial cold shock proteins⁴, bacterial superoxide dismutase¹³, conserved bacterial peptides⁴, bacterial outer membrane vesicles¹⁴ and more recently bacterial RNAs¹⁵⁻¹⁶. However, despite the identification of some of these elicitor receptors¹⁷, many of them remain still unknown.

Plant PRRs are subdivided by the nature of their ligand-binding ectodomain¹ and include receptor-like kinases (RLKs) and receptor-like proteins (RLPs) which lack a cytoplasmic kinase domain. RLKs which are involved in ligand binding, possess a variable extracellular domain, a single transmembrane domain and an intracellular kinase domain¹. Genome-wide investigation in *Arabidopsis thaliana* showed that RLKs are part of the gene family that shares a common ancestor with the animal receptor tyrosine kinases (RTKs), and receptor serine and threonine kinases (RSKs). Data has shown that more than 600 RLKs constitute 2.5% of all the protein-coding genes in

76 *Arabidopsis*^{19a}. The motifs making up the extracellular part of RLKs vary greatly among members,
77 the most common being leucine-rich repeats (LRRs) (235 of the 610 RLKs contain 1–32 LRRs in
78 their extracellular domain)^{19a}. LRR-RLKs have a role in many different important signaling
79 processes such as immunity²⁰, hormone signaling²¹, and development²². The structural plasticity of
80 LRR-containing proteins allows them to bind to many distinct ligands, including peptides, lipids,
81 hormones and other biochemical compounds¹.

82 The family of LRR-RLKs has been divided into 13 groups (LRR-RLK I to LRR-RLK XIII)^{19b}.
83 Recent phylogenetic studies showed that the somatic embryogenesis receptor kinases (SERKs)
84 together with NIK1 and NIK2 receptors, belong to clade 1 of the five clades described for the LRR-
85 RLK II group of receptor-like kinases in the plant kingdom³⁵. The SERK family, including SERK1,
86 SERK2, BAK1/SERK3, and BAK1-LIKE1/SERK4 (BKK1/SERK4) is involved in plant
87 development and disease resistance and they serve as co-receptors in brassinosteroids and immune
88 signaling (Li et al., 2002, Nam and Li, 2002, Chinchilla et al., 2007). The SERK proteins have a
89 small extracellular domain consisting of 4.5–5 LRRs. They distinguish themselves from other LRR-
90 RLK II members by the presence of a serine-proline rich (SPP) motif in their extracellular domain²³.
91 Phylogenetic analysis of SERK proteins in *A. thaliana* and tomato indicates that SERKs constitute a
92 highly conserved protein family²⁴⁻²⁵. Despite a partial redundancy in their functions, each member
93 of the SERK family performs a very specific subset of signaling roles and each member has a specific
94 function²⁴⁻²⁵. The functional specificity of this highly homologous protein family is still not fully
95 understood, and there are many questions regarding the evolution and functional properties of many
96 of its members²⁴. The short amino acid motifs present in their extracellular domains are highly
97 conserved²⁴⁻²⁵ and these are important for their interaction with other receptor kinases²⁴⁻²⁵.
98 BAK1/SERK3, one of the best characterized members of this family, is known to act as a coreceptor
99 of the Brassinosteroid insensitive (BRI-1) receptor, involved in cell elongation and tolerance to
100 environmental stress, and of other several PRRs related to innate immunity like FLS2, PEPR1,
101 PEPR2 and EF-TU receptor (EFR)²⁷.

In *Arabidopsis* the nuclear shuttle protein (NSP)- interacting kinase 1 (NIK1) has been identified as an LRR-RLK II receptor involved in the recognition of geminiviruses. One of its most important features is the phosphorylation of the threonine (T) residue 474 present in its kinase domain that is fundamental for its activation^{25 36} to promote the inhibition of the viral and host mRNA translation. The model proposes that the phosphorylation of threonine leads to the phosphorylation of the downstream component ribosomal protein L10A (RPL10), which interacts in the nucleus with the L10-interacting MYB domain-containing protein (LIMYB), to down regulate genes associated with the translational machinery of the host. This one also leads to the translational inhibition of the virus and an increased tolerance to geminiviruses by the infected plants^{37,37b}. As a counter-defense, the viral nuclear shuttle protein (NSP) binds to the kinase domain of NIK1 to inhibit T474 phosphorylation and activation, favoring viral infection. Interestingly, in recent years Santos et al., 2009, reported a gain-of-function mutation in the NIK1 kinase domain (T474D) from *Arabidopsis* that leads to the hyperactivation of NIK1 kinase activity and resistance against geminiviral infection when expressed in tomato (Brustolini et al.,2015).

Since viral diseases cause important economical loses in solanaceous plants²⁷⁻²⁸ and other important crops like beans, manioc and maize, the identification of the genes which can have a role in antiviral defense in plants becomes necessary. Despite the extensive research done on signaling receptors and proven participation of NIK1 in signaling cascades against viral infection, there is few published data on the phylogenetic analysis of NIK1 in solanaceous plants and in other crops.

In the current publication, the phylogenetic analysis of NIK1 at the amino-acid level in *Arabidopsis thaliana* and six important solanaceous plants is shown. After performing multiple sequence alignment, domain analysis and protein structure analysis, we could identify six NIK1 orthologs in the following solanaceous plants including tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), *Solanum pennellii*, eggplant (*Solanum melongena*), pepper (*Capsicum annum*), and *Nicotiana benthamiana*. Additionally, the NIK1 kinase domain was also identified in other important crops. The present research could be the base to characterize the function of NIK1 orthologs in

128 Solanaceous plants and other crops of economic importance.

129 **Materials and methods**

130 **Database searches**

131 To identify NIK1-related proteins in the six solanaceous species, the amino acid sequence of
 132 AT5G16000 (NIK1) in *Arabidopsis thaliana* was directly obtained from TAIR
 133 (<https://www.arabidopsis.org/>) and used as query to blast against the six solanaceous species using
 134 the tBLASTN program in the Sol Genomics Network (SGN) (<https://solgenomics.net>), TAIR
 135 (<https://www.arabidopsis.org/>), Phytozome (columbine), and the National Center for Biotechnology
 136 Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) websites to identify possible NIK1 orthologs.
 137 All searches were cross analyzed to compile a list of the best possible candidates. Protein sequences
 138 with more than 90% sequence identity were considered.

139 **Multiple sequence alignment analysis**

140 Multiple alignment analysis of the sequences was performed based on the Clustal Omega
 141 software⁹³. The protein sequence alignment of NIK1 from *Arabidopsis thaliana*, tomato (*Solanum*
 142 *lycopersicum*), potato (*Solanum tuberosum*), *Solanum pennellii*, eggplant (*Solanum melongena*),
 143 pepper (*Capsicum annum*), and *Nicotiana benthamiana* was done, and the alignments were manually
 144 corrected and trimmed to obtain comparable sequences (Figure 1). The protein sequence alignment
 145 of Arabidopsis NIK1 kinase domain was also done using the same software (Figure 6).

146 **Phylogenetic analyses and comparison of the amino acid sequences**

147 The phylogenetic analysis was carried out using MEGA software (Molecular Evolutionary Genetics
 148 Analysis) version X (Kumar *et al.*, 2018). A maximum likelihood phylogenetic tree was constructed
 149 using default parameters and 1000 bootstrap replicates. All branches with bootstrap values < 60 were
 150 collapsed.

151

152 **Structure analysis of NIK1 amino acid sequences and motif investigation**

Using the NPS@SOPMA (<https://npsa-prabi.ibcp.fr/>) with its default settings, the secondary structures of NIK1 proteins including AtNIK1, SotubNIK1, SolyNIK1, SopenNIK1, CANIK1, NibenNIK1, SmeNIK1 were predicted. The investigation of the structure analysis included the percentage of each amino acid and position and the alpha helix number. In addition, beta bridges and random coils were identified. To determine the motifs in the NIK1 amino acid sequences, motif analysis was performed using the MEME tool with motif number set as 10. The other parameters were set as default. The Swiss-Model (<https://www.swissmodel.expasy.org/>), which is based on the automatic ExPASy (Expert Protein Analysis System) (Waterhouse et al., 2018; Waterhouse et al., 2016), was used to determine the 3D models of the protein tertiary structures of AtNIK1, SotubNIK1, SolyNIK1, SopenNIK1, CANIK1, NibenNIK1, SmeNIK1.

Results

Identification and phylogenetic analysis of the NIK1 proteins in six Solanaceous plants

Taking the NIK1 gene AT5G16000.1 from *Arabidopsis thaliana* (AtNIK1) as a reference, we could identify a total of 66 NIK1 homologs with top hits in the six studied solanaceous plants including: 11 from *Arabidopsis thaliana*, 10 from potato (*Solanum tuberosum*), 10 from tomato (*Solanum lycopersicum*), nine from *Solanum pennellii*, six from eggplant (*Solanum melongena*), seven from pepper (*Capsicum annum*), and 13 from *Nicotiana benthamiana*. (Supplementary Dataset S1). All the proteins showed the highest sequence similarity with AtNIK1. The amino acid sequences among the solanaceous plants were highly conserved, suggesting that NIK1 orthologs exist in Solanaceous plants. To better observe the relatedness among AtNIK1 and its possible orthologs in *Solanaceae*, an un-rooted phylogenetic tree was constructed with the full-length sequences of the selected proteins through a maximum likelihood phylogenetic analysis. (Supplementary Dataset S2). The found homologues were named according to the corresponding species as shown in Figure 2.

To evaluate and investigate the phylogenetic relationship among them, phylogenetic analysis was investigated in depth. As illustrated in the Figure 2, phylogenetic analysis with bootstrap values of 0

to 100 showed that 66 NIK1-related proteins were found to be divided into eight different clades (Clade I to VIII). A glance at the phylogenetic tree shows that the highly similar sequences with AtNIK1 are present in clades I and II, being clade I the largest clade. Clade I is subdivided in two clusters, each of six sequences. Clade I is comprised of 12 sequences including: two from *A. thaliana*, two from *S. tuberosum*, two from *S. lycopersicum*, two from *S. pennellii*, two from *S. melongena*, two from *C. annuum*, and two from *N. benthamiana*.

Clade II is composed of 7 sequences subdivided in two clusters where, cluster 1 contains the NIK1 sequence from *A. thaliana*, and cluster 2 the sequences from *Nicotiana benthamiana*, potato, tomato and chili pepper (two from *N. benthamiana*, one from *S. tuberosum*, one from *S. lycopersicum*, one from *S. pennellii*, and one from *C. annuum*).

Clade III consists of eight members and has two clusters. The first cluster includes AtCIK2 and AtCIK3, while the second cluster contains one sequence from *S. tuberosum*, one from *S. lycopersicum*, one from *S. melongena*, one from *S. pennellii*, one from *C. annuum*, and one from *N. benthamiana*.

The fourth clade has 10 members. Cluster 1 includes one sequence from *A. thaliana* (AtNIK3) and the rest of the nine sequences, which belong to cluster 2, include one sequence from *S. tuberosum*, one from *S. lycopersicum*, one from *S. melongena*, two from *S. pennellii*, one from *C. annuum*, and three from *N. benthamiana*.

Clade V has five sequences. While AtCIK4 is represented in one cluster, four other members of this clade, comprising the second cluster, include one sequence from *S. lycopersicum*, one from *S. pennellii*, one from *C. annuum*, and one from *N. benthamiana*.

Clade VI consists of 7 sequences. The first cluster comprises two sequences from *S. tuberosum*, one from *S. lycopersicum*, one from *S. melongena*, two from *N. benthamiana*, and cluster 2 comprises one sequence from *A. thaliana*.

202 Clade VII is represented by two clusters. The first cluster consists of four sequences including one
203 from *S. tuberosum*, one from *S. lycopersicum*, one from *S. melongena*, and one from *S. pennellii*.
204 Two members from *A. thaliana* in cluster 2 are related to the SERK1 and SERK2 families.

205 Clade VIII consists of two clusters. The first one comprising sequences from the SERK4 and SERK5
206 families together with BAK1, and a second cluster comprising two sequences from *S. lycopersicum*,
207 two from *S. tuberosum*, one from *S. pennellii*, two from *N. benthamiana* and one from *C. annuum*.

208 **Identification of NIK1 kinase domain in other important species**

209 The results of the multiple alignment of the kinase domain present in *Arabidopsis thaliana* NIK1 also
210 showed that this domain is present in related proteins from other important crops like soy, maize,
211 lupin, pumpkin, rice, cotton, etc. which are also susceptible to geminiviral infection (Figure 6).

212 **Discussion**

213 In innate immunity, co-receptors play an important role in the activation of RLK-mediated signal
214 transduction cascades. NIK1 is regarded as one of the most important co-receptors and its role in
215 antiviral defense has been confirmed²⁶. As solanaceous plants are very sensitive to viral infections
216 including those induced by geminiviruses, a comprehensive analysis of NIK1 orthologs and their
217 biological characterization are necessary, to elaborate possible defense strategies against geminiviral
218 infections. The NIK1 orthologs shown in the present work offer an opportunity to do so. For example,
219 CRISPR/Cas9 has already been used to develop resistance against *Turnip mosaic*
220 *virus* (TuMV) in *A. thaliana* and resistance in cucumber against other viruses by disrupting
221 the function of translation factors (Pyott et al. 2016; Chandrasekaran et al. 2016). Using the
222 same technology, the introduction of a mutation in the NIK1 kinase domain as shown by
223 Brustolini et. al. (2015) could be an alternative to be considered to develop resistance in
224 solanaceous plants and other important crops like maize, rice and cotton, which also contain
225 similar receptors with the same kinase domain. The present study based on the phylogenetic
226 and structural analysis of NIK1 and its orthologs in other species, had the goal to start to pave

227 the way to study NIK1 receptors with the aim to characterize their functions in *A. thaliana*
 228 and solanaceous plants, through experiments that involve NIK1 protein expression or the use
 229 of NIK1 mutant plants in combination with infection trials performed with geminiviruses.

230

231

232

233

234

Figure 1. Multiple amino acid sequence alignment of NIK1 in *Arabidopsis thaliana* and six Solanaceous species including: AtNIK1 in *A. thaliana*; SopenNIK1 in *Solanum tuberosum*; SolyNIK1 in *Solanum lycopersicum*; SopenNIK1 in *Solanum pennellii*; CANIK1 in *Capsicum annum*; SmeNIK1 in *Solanum melongena*; NibenNIK1.1 and NibenNIK1.2 in *Nicotiana benthamiana*. The alignment was carried out by using Clustal Omega.

236

237

238

Figure 2. Phylogenetic analysis of the *Arabidopsis thaliana* AtNIK1 protein sequence and its homologs in six representative solanaceous plants. The species indicated are potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), *Solanum pennellii*, eggplant (*Solanum melongena*), pepper (*Capsicum annum*), and *Nicotiana benthamiana* protein sequences homologs. AtNIK1 protein in *Arabidopsis thaliana* was labelled. Sequences for comparisons were obtained from Solgenomic.net GenBank. The accession numbers and protein names (if available) are given. Analysis was done by maximum likelihood method with 500 bootstrap replicates implemented in MEGAX (Molecular Evolutionary Genetics Analysis) version X.

Figure 3. Conserved protein motif analysis of NIK1 in *Arabidopsis thaliana* and six Solanaceous species including: AtNIK1 in *A. thaliana*; SopenNIK1 in *Solanum tuberosum*; SolyNIK1 in *Solanum lycopersicum*; SopenNIK1 in *Solanum pennellii*; CANIK1 in *Capsicum annum*; SmeNIK1 in *Solanum melongena*; NibenNIK1.1 and Niben NIK1.2 in *Nicotiana benthamiana*. Conserved motifs in the NIK1 proteins were elucidated by MEME website (version 5.0.3). 10 motifs were shown by different colors. Different motifs were represented in different colors in the protein. The details of the conserved motifs 1, 2 and 3 which have the highest amino-acid sequence similarity are shown. The height of the amino acids present in each motif is shown in bits.

Figure 4. 3D structure analysis of NIK1 in *Arabidopsis thaliana* and six Solanaceous species including: AtNIK1 in *A. thaliana*; SopenNIK1 in *Solanum tuberosum*; SolyNIK1 in *Solanum lycopersicum*; SopenNIK1 in *Solanum pennellii*; CANIK1 in *Capsicum annum*; SmeNIK1 in *Solanum melongena*; NibenNIK1.1 and NibenNIK1.2 in *Nicotiana benthamiana*. The structure models were constructed using the Swiss-model.

242

243

Figure 5. Secondary structure analysis of the NIK1 *A. thaliana* and six Solanaceous plants. AtNIK1 in *A. thaliana*; SopenNIK1 in *Solanum tuberosum*; SolyNIK1 in *Solanum lycopersicum*; SopenNIK1 in *Solanum pennellii*; CANIK1 in *Capsicum annum*; SmeNIK1 in *Solanum melongena*; NibenNIK1.1 and NibenNIK1.2 in *Nicotiana benthamiana*. Alpha helix is colored in blue, extended strand is colored in red. Random coil is colored in purple.

244

245

246

248

16

RVW43297.1 Protein NSP-interacting kinase 3 [Vitis vinifera]
KAF1872979.1 hypothetical protein LaI_00016091 [Lupinus albus]
RXH79724.1 hypothetical protein DVH24_040871 [Malus domestica]
KAB2609660.1 protein trichome birefringence-like 2 [Pyrus ussuriensis x Pyrus communis]
XP_018682745.1 PREDICTED: probable LRR receptor-like serine/threonine-protein kinase At5g45780 isoform X2 [Musa acuminata subsp. malaccensis]
PPD73556.1 hypothetical protein GOBAR_DD29529 [Gossypium barbadense]
KAG4960347.1 hypothetical protein JHK87_036980 [Glycine soja]
EOY01502.1 LRR receptor-like serine/threonine-protein kinase [Theobroma cacao]
KZN06697.1 hypothetical protein DCAR_007534 [Daucus carota subsp. sativus]
XP_022938474.1 protein NSP-INTERACTING KINASE 1-like isoform X2 [Cucurbita moschata]
XP_023550222.1 protein NSP-INTERACTING KINASE 1-like isoform X3 [Cucurbita pepo subsp. pepo]
NP_197104.1 NSP-interacting kinase 1 [Arabidopsis thaliana]
KAG5376762.1 hypothetical protein IGI04_041358 [Brassica rapa subsp. trilocularis]
VAI79141.1 unnamed protein product [Triticum turgidum subsp. durum]
EAAZ42894.1 hypothetical protein OsJ_27488 [Oryza sativa Japonica Group]
XP_014755496.2 probable LRR receptor-like serine/threonine-protein kinase At2g23950 isoform X2 [Brachypodium distachyon]
XP_008664633.1 probable LRR receptor-like serine/threonine-protein kinase At2g23950 isoform X1 [Zea mays]
KAG0523795.1 hypothetical protein BDA96_07G153500 [Sorghum bicolor]
XP_016690348.2 probable LRR receptor-like serine/threonine-protein kinase At2g23950 isoform X1 [Gossypium hirsutum]
RYR76902.1 hypothetical protein Ahy_A01g001415 isoform A [Arachis hypogaea]
GFS36074.1 leucine-rich repeat protein kinase family protein [Actinidia rufa]
XP_004242632.1 probable LRR receptor-like serine/threonine-protein kinase At4g30520 [Solanum lycopersicum]

249

Figure 6. Multiple alignment of the *Arabidopsis thaliana* NIK1 (AT5G16000.1/NP_197104.1) kinase domain with other plant species, which could be mutated (T474D) to gain resistance against geminiviral infection. The T474 residue is indicated by the red rectangle on the yellow region showing a section of the kinase domain present in these proteins.

250
251

252

253

254

255

256

257

258

259

260

261

262

263

References:

1. Couto D, and Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nature Review Immunology* **16**: 537-52.
2. Ronald, P.C., and Beutler, B. (2010). Plant and animal sensors of conserved microbial signatures. *Science* **330**: 1061-1064.
3. Spoel, S.H., and Dong, X. (2012). How do plants achieve immunity? Defence without specialized immune cells. *Nature Review Immunology* **12**: 89-100.
4. Boller, T., and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* **60**: 379-406.
5. Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., Mengiste, T., Zhang, Y., and Zhou, J.M. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host Microbe*. **7**: 290-301.
6. Thatcher, L., Anderson, J., and Singh, K. (2005). Plant defence responses: what have we learnt from arabidopsis. *Functional plant biology* **32**: 1-19.
7. Ulker, B., and Somssich, I.E. (2005). WRKY transcription factors: from DNA binding towards biological function. *Current Opinion in Plant Biology* **7**: 491-8.
8. Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D., Boller, T., and Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* **125**: 749-760.
9. Dangl, J.L., Horvath, D.M., and Staskawicz, B.J., (2013). Pivoting the plant immune system from dissection to deployment. *Science* **341**: 746–51.

10. Sarris, P.F., Cevik, V., Dagdas, G., Jones, J.D., and Krasileva, K.V. (2016). Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. *BMC Biology* **19**: 8.
11. Zipfel, C., and Felix, G. (2005). Plants and animals: a different taste for microbes? *Current Opinion in Plant Biology* **8**: 353-360.
12. Macho, A.P., and Zipfel, C. (2014). Plant PRRs and the Activation of innate immune Signaling. *Molecular Cell* **54**: 263-272.
13. Newman, M.A., Sundelin, T., Nielsen, J.T., and Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Front Plant Science*. **16**: 139.
14. Bahar, O., Mordukhovich, G., Luu, D.D., Schwessinger, B., Daudi, A., Jehle, A.K., Felix, G., and Ronald, P.C. (2016). Bacterial outer membrane vesicles induce plant immune responses. *Molecular Plant Microbe Interactions* **29**: 374-84.
15. Niehl, A., Wyrsh, I., Boller, T., and Heinlein, M. (2016). Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants. *New Phytologist* **211**: 1008-19.
16. Park, Y.S., Lee, B., and Ryu, C.M. (2016). Getting to PTI of bacterial RNAs: Triggering plant innate immunity by extracellular RNAs from bacteria. *Plant Signal Behavior*. **11**: e1198866.
17. Trdá, L., Boutrot, F., Claverie, J., Brulé, D., Dorey, S. and Poinssot, B. (2015). Perception of pathogenic or beneficial bacteria and their evasion of host immunity: pattern recognition receptors in the front line. *Frontiers in Plant Science*. doi: 10.3389/fpls.2015.00219

18. Ausubel, F.M. (2005). Are innate immune signaling pathways in plants and animals conserved? *Nature Immunology* **6**: 973-9.
19. Greeff, C., Roux, M., Mundy, J., and Petersen, M. (2012). Receptor-like kinase complexes in plant innate immunity. *Frontiers in plant science* **3**: 209-212.
- a. 19.a -Shiu SH, Bleecker AB. Plant receptor-like kinase gene family: diversity, function, and signaling. *Sci STKE*. 2001 Dec 18;2001(113):re22. doi: 10.1126/stke.2001.113.re22. PMID: 11752632.
- b. 19.b - Shiu, S.H., and Bleecker, A.B. (2001b). Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. *Proc. Natl. Acad. Sci. USA* 98:10763-10768
20. Gómez-Gómez L, Boller T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Mol Cell*. 2000 Jun;5(6):1003-11. doi: 10.1016/s1097-2765(00)80265-8. PMID: 10911994.
21. Li J, Chory J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell*. 1997 Sep 5;90(5):929-38. doi: 10.1016/s0092-8674(00)80357-8. PMID: 9298904.
22. Clark SE: Organ formation at the vegetative shoot meristem. *Plant Cell* 1997, 9:1067–1076.
23. Hecht V, Vielle-Calzada JP, Hartog MV, Schmidt ED, Boutilier K, Grossniklaus U, de Vries SC. The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. *Plant Physiol*. 2001 Nov;127(3):803-16. Erratum in: *Plant Physiol* 2002 Jan;128(1):314. PMID: 11706164; PMCID: PMC129253.

24. Aan den Toorn M, Albrecht C, de Vries S. On the Origin of SERKs: Bioinformatics Analysis of the Somatic Embryogenesis Receptor Kinases. *Mol Plant*. 2015 May;8(5):762-82. doi: 10.1016/j.molp.2015.03.015. Epub 2015 Apr 9. PMID: 25864910.
25. Sakamoto T, Deguchi M, Brustolini OJ, Santos AA, Silva FF, Fontes EP. The tomato RLK superfamily: phylogeny and functional predictions about the role of the LRR-II-RLK subfamily in antiviral defense. *BMC Plant Biol*. 2012 Dec 2;12:229. doi: 10.1186/1471-2229-12-229. PMID: 23198823; PMCID: PMC3552996.
26. Li B, Ferreira MA, Huang M, Camargos LF, Yu X, Teixeira RM, Carpinetti PA, Mendes GC, Gouveia-Mageste BC, Liu C, Pontes CSL, Brustolini OJB, Martins LGC, Melo BP, Duarte CEM, Shan L, He P, Fontes EPB. The receptor-like kinase NIK1 targets FLS2/BAK1 immune complex and inversely modulates antiviral and antibacterial immunity. *Nat Commun*. 2019 Nov 1;10(1):4996. doi: 10.1038/s41467-019-12847-6. PMID: 31676803; PMCID: PMC6825196.
27. Hančinský R, Mihálik D, Mrkvová M, Candresse T, Glasa M. Plant Viruses Infecting Solanaceae Family Members in the Cultivated and Wild Environments: A Review. *Plants (Basel)*. 2020 May 25;9(5):667. doi: 10.3390/plants9050667. PMID: 32466094; PMCID: PMC7284659.
28. Safaeizadeh M, Saidi A, Palukaitis P. Molecular characterization of cucumber mosaic virus isolates infecting tomato in Hamedan and Tehran provinces of Iran. *Acta Virol*. 2015 Jun;59(2):174-8. doi: 10.4149/av_2015_02_174. PMID: 26104334.

29. Chinchilla D, Shan L, He P, de Vries S, Kemmerling B. One for all: the receptor-associated kinase BAK1. *Trends Plant Sci.* 2009 Oct;14(10):535-41. doi: 10.1016/j.tplants.2009.08.002. Epub 2009 Sep 10. PMID: 19748302; PMCID: PMC4391746.
30. Hančinský R, Mihálik D, Mrkvová M, Candresse T, Glasa M. Plant Viruses Infecting Solanaceae Family Members in the Cultivated and Wild Environments: A Review. *Plants (Basel).* 2020 May 25;9(5):667. doi: 10.3390/plants9050667. PMID: 32466094; PMCID: PMC7284659.
31. Safaeizadeh M, Saidi A, Palukaitis P. Molecular characterization of cucumber mosaic virus isolates infecting tomato in Hamedan and Tehran provinces of Iran. *Acta Virol.* 2015 Jun;59(2):174-8. doi: 10.4149/av_2015_02_174. PMID: 26104334.
- 33- Voloudakis A.E., Mukherjee S.K., Roy A. (2021) Novel Technologies for Transgenic Management for Plant Virus Resistance. In: Sarmah B.K., Borah B.K. (eds) *Genome Engineering for Crop Improvement. Concepts and Strategies in Plant Sciences.* Springer, Cham. https://doi.org/10.1007/978-3-030-63372-1_7
- 34- Brustolini, O.J.B., Machado, J.P.B., Condori-Apfata, J.A., Coco, D., Deguchi, M., Lariato, V.A.P., Pereira, W.A., Alfenas-Zerbini, P., Zerbini, F.M., Inoue-Nagata, A.K., Santos, A.A., Chory, J., Silva, F.F., and Fontes, E.P.B. (2015). Sustained NIK-mediated antiviral signalling confers broad spectrum tolerance to begomoviruses in cultivated plants. *Plant Biotechnol J.* 13:1300-1311
35. Hosseini, S., Schmidt, E.D.L., and Bakker, F.T. (2020). Leucine-rich receptor-like kinase II phylogenetics reveals five main clades throughout the plant kingdom. *The Plant Journal* 103: 547-560
36. Santos, A.A., Carvalhi, C.M., Florentino, L.H., Ramos, H.J.O., Fontes, E.P.B.

380 (2009). Conserved threonine residues within the A loop of the receptor NIK1 differentially
381 regulate the kinase function required for antiviral signaling. PLoS ONE 2009,4,e5781.

382 37. Zorzatto C, Machado JPB, Lopes KGV, Nascimento KJT, Pereira WA, Brustolini OJ,
383 Reis PA, Calil IP, Deguchi M, Sachetto-Martins G et al. (2015). NIK1- mediated translation
384 suppression functions as a plant antiviral immunity mechanism. Nature 520: 679-682

385 38. Pyott D.E., Sheehan E., and Molnar A. (2016). Engineering of CRISPR/Cas9-
386 mediated potyvirus resistance in transgene-free Arabidopsis plants. Mol Plant Pathol.
387 17:1276-88

388
389 39. Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M,
390 Sherman A, Arazi T and Gal-On A. (2016). Development of broad virus resistance in
391 non-transgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol. 17: 1140-
392 53. doi: 10.1111/mpp.12375. Epub 2016 Apr 21.

393
394 40. J. Li, J. Wen, K.A. Lease, J.T. Doke, F.E. Tax, J.C. Walker (2002). BAK1,
395 an *Arabidopsis* LRR receptor-like protein kinase, interacts with BRI1 and modulates
396 brassinosteroid signaling. Cell, 110 pp. 213-222

397
398 41. K.H. Nam, J. Li (2002). BRI1/BAK1, a receptor kinase pair mediating
399 brassinosteroid signaling. Cell, 110 pp. 203-212

400

42.D. Chinchilla, C. Zipfel, S. Robatzek, B. Kemmerling, T. Nurnberger, J.D. Jones, G. Felix, T. Boller (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence Nature, 448 pp. 497- 500

37b. Carvalho CM, Santos AA, Pires SR, Rocha CS, Saraiva DI, Machado JP, Mattos EC, Fietto LG, Fontes EPB. Regulated nuclear trafficking of rpL10A mediated by NIK1 represents a defense strategy of plant cells against virus. PLoS Pathog. 2008c; 4:e1000247. [PubMed: 19112492]

38. Santos AA, Lopes KVG, Apfta JAC, Fontes EPB (2010). NSP-interacting kinase, NIK: a transducer of plant defence signalling. J. Exp. Bot. 61:3839–3845. [PubMed: 20624762]