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ARTICLE ADDENDUM

NRC proteins - a critical node for pattern and effector mediated signaling

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ABSTRACT

Plants are constantly exposed to numerous diverse microbes and pests. They lack an adaptive immune system and rely on innate immunity to perceive and ward off potential pathogens. The plant immune system enables plants to overcome invading microorganisms, and can be defined as highly successful in this regard. Nevertheless, specialized pathogens are able to overcome structural barriers, preformed defenses, innate immunity and are a persistent threat to crop and food supplies worldwide. The rapidly growing world population results in massive demands for agricultural products and reliable crop yields. Therefore, the ability to precisely manipulate plant immunity to resist diverse diseases holds significant promise for enhancing crop production.

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Plant immunity is conventionally depicted as a two-tiered system. The first line of plant defense is formed by pattern recognition receptors (PRRs), located at the cell surface, that recognize microbe-associated molecular patterns (MAMPs) and trigger pattern-triggered immunity (PTI).[1] Successful pathogens are able to overcome plant PTI using secreted effectors that suppress PTI, leading to plant susceptibility. Concomitantly, plants evolved the second line of defense based on cytoplasmic immune receptors that recognize effectors (Avrs) and deploy effector-triggered immunity (ETI).[2,3] However, accumulating evidence from the last decade blurs the distinction between the two systems and reveals significant overlap and diversity in immune signaling networks.[4–6]

Blurred distinction

MAMPs are traditionally defined as highly conserved moieties within a class of microbes, essential for microbial fitness,[7,8] while effectors are considered to be species, race or strain specific and confer virulence.[9,10] However, several MAMPs and effectors refuse to follow these rules, creating exceptions to this man-made distinction. For instance, the necrosis-induced peptide 1 (NEP1) and NEP1-like proteins are effectors required for virulence, but they are conserved among bacteria, fungi and oomycetes.[4,5] Other examples of widespread effectors required for pathogen virulence are extracellular protein 6 (Ecp6) and Ecp6 conserved orthologs – LysM effectors, harpin effectors and crinkler effectors that are widely conserved in fungi, Gram-negative bacteria and oomycetes, respectively.[4,5] On the other hand, we find examples of MAMPs with a very narrow known distribution such as Ax21 and Pep-13 that are present in only a few *Xanthomonas* strains and *Phytophthora* species, respectively.[4,5] Additionally, MAMPs such as flagellin, lipopolysaccharide, peptidoglycan, chitin, EIX and PWL (pathogenicity toward weeping lovegrass) contribute to pathogen virulence.[4,5]

The distinction between the outcomes of PTI and ETI can also be confusing. ETI is frequently noted as leading to a more rapid, sustainable and stronger immune response, culminating in cell death known as the hypersensitive response (HR).[2,11] Nonetheless, we know of several MAMPs that are able to induce HR, among them flagellin, CBEL and EIX.[12–15] Meanwhile, we can find weak ETI responses like the ones mediated by RPS4 and Ve1, which recognize *Pseudomonas* and *Verticillium* effectors, respectively.[16,17]

PTI is mediated by cell surface PRRs that can be classified, based on their domains, into different categories. The largest category is that of extracellular leucine-rich repeat (eLRR) containing receptors, and can be further divided into receptor like proteins and kinases (eLRR-RLP/K).[18] ETI is frequently mediated by cytoplasmic receptors, which are primarily nucleotide binding leucine rich repeat (NLR) proteins.[19] However, there are several examples of eLRR-RLP type cell surface receptors that can recognize effectors and mediate ETI, such as Cf2, Cf4, Hcr9-4E, Cf5, Cf9, Ve1, l and LepR3/RLM2,[20–27] and even an eLRR-RLK – I-3.[28] Given the gradient of responses that can be induced by either MAMP or effector recognition, it is now becoming clear that the responses governed by diverse plant immune receptors are integrated in diverse manners.[6]

Convergent immune signaling

Despite the diversity of pathogen components recognized and strength of immune responses, there are commonalities in defense signaling outputs, indicating that recognition of both MAMPs and effectors utilize similar signaling networks. PTI induces rapid activation of MAPK cascades which leads

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/kpsb.

to the activation of downstream signaling pathways.[29] Unsurprisingly, several effectors target MAPK cascade components in order to disrupt PTI.[30–34] More interestingly, there are cases of ETI leading to activation of the same PTI-MAPK cascades.[35] In addition, PTI/ETI share other signaling pathways including ROS signaling, Ca^{2+} signaling, hormone signaling and substantial overlapping transcriptional regulation, resulting in plant immune responses- as recently reviewed by Peng et al.[36] These evidences demonstrate a certain level of convergence in PTI/ETI signaling.

Helper NRCs as signaling convertors

In the classical gene-for-gene model proposed by Harold Flor in 1942, an *R* gene typically encodes an NLR receptor that detects and responds to an effector (*Avr*) gene product.[37,38] More recently, a diversity of recognition mechanisms have been revealed.[3] One additional model demonstrates that NLRs can function in pairs, with one NLR functioning as a sensor-for effector detection (s-NLR), and the other as a helper-initiating immune signaling (h-NLR),[39,40] (Figure 1,. One specific NLR family in *Solanaceae*, termed NLR required for cell death (NRC), emerges as a key family of h-NLR required signaling downstream of multiple s-NLRs.[41] Recent publications indicates NRCs function not only as h-NLRs mediating s-NLR signaling, but also as h-NLRs mediating MAMP and effector signaling sensed by diverse eLRR-receptors. NRC2 and NRC3 from *N. benthamiana* were shown to be involved in defense responses mediated by the eLRR-RLP Cf4, which recognizes the Avr4 effector from *Cladosporium fulvum*.[42] We have recently described tomato NRC4 as associated with an eLRR-RLP required for perception of a fungal MAMP – EIX (LeEIX2) and with an eLRR-RLK required for perception of bacterial flagellin (FLS2), enhancing defense responses mediated by them.[43] We have further shown that NRC4 is required for LeEIX2 and likely also FLS2-mediated defense responses. Furthermore, NRC4's N-terminal coiled-coil domain is sufficient to mediate the association with LeEIX2 and can enhance EIX and flagellin- elicited defense responses as efficiently as the full length protein.[43] Earlier works have indicated that tomato NRC1 may be involved in defense responses sensed by the eLRR-RLPs Cf4, Cf9, Ve1 and LeEIX2 as well.[44] Keeping with the sensor-helper model, the eLRR receptors – Cf4, Cf9, Ve1, LeEIX2 and FLS2 can be defined as sensors, detecting effectors and MAMPs. In this context, eLRR-RLP/K act as sensor (s-RLP/K), while NRCs keep their role as an h-NLR (Figure 1). **We propose that taken together, these data essentially position NRCs as a key signaling node required for the initiation of signaling sensed in both PTI and ETI immune pathways (Figure 1).**

In accordance with this concept, we have recently shown that DNA manipulation (using CRISPR-cas9 editing) of tomato NRC4 leads to significant enhancement of immunity. NRC4 CRISPRed plants, encoding a 67 aa truncated variant,

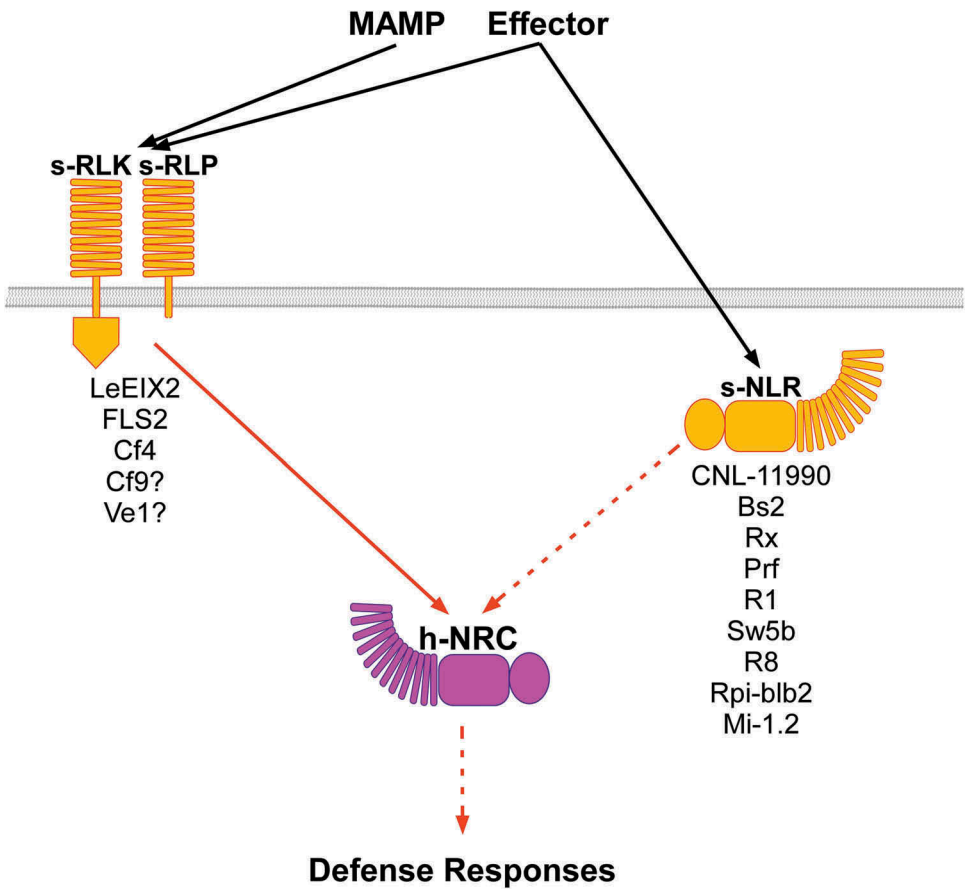

Figure 1. Schematic overview of plant defense signaling mediated by the NRC helper NLR (h-NRC). Perception of MAMPs or effectors by extracellular or intracellular immune receptors act as pathogen sensors (s-RLK, s-RLP, s-NLR) and lead to initiation of immune-signaling by a family of helper NLRs (h-NRC).

displayed intensified defense responses when challenged with EIX, and presented a higher resistance to *B.cinerea*.[43] Our work, together with previous publications,[41–44] positions NRC as a signaling funnel for multiple PTI and ETI sensor-receptors, demonstrating that CRISPR editing of NRCs could potentially result in agriculturally improved *Solanaceae* varieties possessing resistance to a broad spectrum of pathogens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Boutrot F, Zipfel C. Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu Rev Phytopathol. 2017;55:257–286. doi:10.1146/annurev-phyto-080614-120106.
2. Jones JD, Dangl JL. The plant immune system. Nature. 2006;444:323–329. doi:10.1038/nature05286.
3. Cui H, Tsuda K, Parker JE. Effector-triggered immunity: from pathogen perception to robust defense. Annu Rev Plant Biol. 2015;66:487–511. doi:10.1146/annurev-arplant-050213-040012.
4. Thomma BP, Nurnberger T, Joosten MH. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell. 2011;23:4–15. doi:10.1105/tpc.110.082602.
5. Vidhyasekaran P., editor. PAMP signaling in plant innate immunity. In: PAMP signals in plant innate immunity. signaling and communication in plants. Dordrecht: Springer; 2014. p. 17–161.
6. Wu CH, Derevnina L, Kamoun S. Receptor networks underpin plant immunity. Science. 2018;360:1300–1301. doi:10.1126/science.aat2623.
7. Nurnberger T, Brunner F. Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. Curr Opin Plant Biol. 2002;5:318–324.
8. Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell. 1997;91:295–298.
9. Chisholm ST, Coaker G, Day B, Staskawicz BJ. Host-microbe interactions: shaping the evolution of the plant immune response. Cell. 2006;124:803–814. doi:10.1016/j.cell.2006.02.008.
10. Bent AF, Mackey D. Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. Annu Rev Phytopathol. 2007;45:399–436. doi:10.1146/annurev.phyto.45.062806.094427.
11. Tsuda K, Katagiri F. Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr Opin Plant Biol. 2010;13:459–465. doi:10.1016/j.pbi.2010.04.006.
12. Naito K, Taguchi F, Suzuki T, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y. Amino acid sequence of bacterial microbe-associated molecular pattern flg22 is required for virulence. Mol Plant Microbe Interact. 2008;21:1165–1174. doi:10.1094/MPMI-21-9-1165.
13. Hann DR, Rathjen JP. Early events in the pathogenicity of Pseudomonas syringae on Nicotiana benthamiana. Plant J. 2007;49:607–618. doi:10.1111/j.1365-313X.2006.02981.x.
14. Khatib M. LC, Esquerré-Tugayé M.-T., Bottin A., Rickauer M. The CBEL elicitor of Phytophthora parasitica var. nicotianae activates defence in Arabidopsis thaliana via three different signalling pathways. New Phytol. 2004;162:501–510. doi:10.1111/j.1469-8137.2004.01043.x.
15. Ron M, Avni A. The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. Plant Cell. 2004;16:1604–1615. doi:10.1105/tpc.022475.
16. Van Esse HP, Fradin EF, De Groot PJ, De Wit PJ, Thomma BP. Tomato transcriptional responses to a foliar and a vascular fungal pathogen are distinct. Mol Plant Microbe Interact. 2009;22:245–258. doi:10.1094/MPMI-22-3-0245.
17. Wirthmueller L, Zhang Y, Jones JD, Parker JE. Nuclear accumulation of the Arabidopsis immune receptor RPS4 is necessary for triggering EDS1-dependent defense. Curr Biol. 2007;17:2023–2029. doi:10.1016/j.cub.2007.10.042.
18. Liang X, Zhou JM. Receptor-Like Cytoplasmic Kinases: central Players in Plant Receptor Kinase-Mediated Signaling. Annu Rev Plant Biol. 2018;69:267–299. doi:10.1146/annurev-arplant-042817-040540.
19. Dodds PN, Rathjen JP. Plant immunity: towards an integrated view of plant-pathogen interactions. Nat Rev Genet. 2010;11:539–548. doi:10.1038/nrg2812.
20. Catanzariti AM, Do HT, Bru P, De Sain M, Thatcher LF, Rep M, Jones DA. The tomato I gene for Fusarium wilt resistance encodes an atypical leucine-rich repeat receptor-like protein whose function is nevertheless dependent on SOBIR1 and SERK3/BAK1. Plant J. 2017;89:1195–1209. doi:10.1111/tpj.13458.
21. Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JD. The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell. 1996;84:451–459.
22. Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JD. Characterization of the tomato Cf-4 gene for resistance to Cladosporium fulvum identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. Plant Cell. 1997;9:2209–2224.
23. Westerink N, Brandwagt BF, De Wit PJ, Joosten MH. Cladosporium fulvum circumvents the second functional resistance gene homologue at the Cf-4 locus (Hcr9-4E) by secretion of a stable avr4E isoform. Mol Microbiol. 2004;54:533–545. doi:10.1111/j.1365-2958.2004.04288.x.
24. Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD. The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell. 1998;10:1915–1925.
25. Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JD. Isolation of the tomato Cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. Science. 1994;266:789–793.
26. Larkan NJ, Lydiate DJ, Parkin IA, Nelson MN, Epp DJ, Cowling WA, Rimmer SR, Borhan MH. The Brassica napus blackleg resistance gene LepR3 encodes a receptor-like protein triggered by the Leptosphaeria maculans effector AVRLM1. New Phytol. 2013;197:595–605. doi:10.1111/nph.12043.
27. Fradin EF, Zhang Z, Juarez Ayala JC, Castroverde CD, Nazar RN, Robb J, Liu CM, Thomma BP. Genetic dissection of Verticillium wilt resistance mediated by tomato Ve1. Plant Physiol. 2009;150:320–332. doi:10.1104/pp.109.136762.
28. Catanzariti AM, Lim GT, Jones DA. The tomato I-3 gene: a novel gene for resistance to Fusarium wilt disease. New Phytol. 2015;207:106–118. doi:10.1111/nph.13348.
29. Meng X, Zhang S. MAPK cascades in plant disease resistance signaling. Annu Rev Phytopathol. 2013;51:245–266. doi:10.1146/annurev-phyto-082712-102314.
30. Zhang Z, Wu Y, Gao M, Zhang J, Kong Q, Liu Y, Ba H, Zhou J, Zhang Y. Disruption of PAMP-induced MAP kinase cascade by a

Pseudomonas syringae effector activates plant immunity mediated by the NB-LRR protein SUMM2. Cell Host Microbe. 2012;11:253–263. doi:10.1016/j.chom.2012.01.015.
31. Zhang Z, Liu Y, Huang H, Gao M, Wu D, Kong Q, Zhang Y. The NLR protein SUMM2 senses the disruption of an immune signaling MAP kinase cascade via CRCK3. EMBO Rep. 2017;18:292–302. doi:10.15252/embr.201642704.
32. Zhang J, Shao F, Li Y, Cui H, Chen L, Li H, Zou Y, Long C, Lan L, Chai J, et al. A pseudomonas syringae effector inactivates MAPKs to suppress PAMP-induced immunity in plants. Cell Host Microbe. 2007;1:175–185. doi:10.1016/j.chom.2007.03.006.
33. Wang Y, Li J, Hou S, Wang X, Li Y, Ren D, Chen S, Tang X, Zhou JM. A pseudomonas syringae ADP-ribosyltransferase inhibits arabidopsis mitogen-activated protein kinase kinases. Plant Cell. 2010;22:2033–2044. doi:10.1105/tpc.110.075697.
34. Eschen-Lippold L, Jiang X, Elmore JM, Mackey D, Shan L, Coaker G, Scheel D, Lee J. Bacterial AvrRpt2-like cysteine proteases block activation of the arabidopsis mitogen-activated protein kinases, MPK4 and MPK11. Plant Physiol. 2016;171:2223–2238. doi:10.1104/pp.16.00336.
35. Thulasi Devendrakumar K, Li X, Zhang Y. MAP kinase signalling: interplays between plant PAMP- and effector-triggered immunity. Cell Mol Life Sci. 2018. doi: 10.1007/s00018-018-2839-3.
36. Peng Y, Van Wersch R, Zhang Y. Convergent and Divergent Signaling in PAMP-Triggered Immunity and Effector-Triggered Immunity. Mol Plant Microbe Interact. 2018;31:403–409. doi:10.1094/MPMI-06-17-0145-CR.
37. Flor HH. Inheritance of pathogenicity in Melampsora lini. Phytopathology. 1942;32:653–669.
38. Flor HH. Current status of gene-for-gene concept. Annu Rev Phytopathol. 1971;9:275-+. doi:10.1146/annurev.py.09.090171.001423.
39. Bonardi V, Tang S, Stallmann A, Roberts M, Cherkis K, Dangl JL. Expanded functions for a family of plant intracellular immune receptors beyond specific recognition of pathogen effectors. Proc Natl Acad Sci U S A. 2011;108:16463–16468. doi:10.1073/pnas.1113726108.
40. Collier SM, Hamel LP, Moffett P. Cell death mediated by the N-terminal domains of a unique and highly conserved class of NB-LRR protein. Mol Plant Microbe Interact. 2011;24:918–931. doi:10.1094/MPMI-03-11-0050.
41. Wu CH, Abd-El-Haliem A, Bozkurt TO, Belhaj K, Terauchi R, Vossen JH, Kamoun S. NLR network mediates immunity to diverse plant pathogens. Proc Natl Acad Sci U S A. 2017;114:8113–8118. doi:10.1073/pnas.1702041114.
42. Wu CH, Belhaj K, Bozkurt TO, Birk MS, Kamoun S. Helper NLR proteins NRC2a/b and NRC3 but not NRC1 are required for Pto-mediated cell death and resistance in Nicotiana benthamiana. New Phytol. 2016;209:1344–1352. doi:10.1111/nph.13764.
43. Leibman-Markus M, Pizarro L, Schuster S, Lin ZJD, Gershony O, Bar M, Coaker G, Avni A. The intracellular nucleotide binding leucine-rich repeat receptor - SlNRC4a enhances immune signaling elicited by extracellular perception. Plant Cell Environ. 2018. doi: 10.1111/pce.13347.
44. Gabriels SH, Vossen JH, Ekengren SK, Van Ooijen G, Abd-El-Haliem AM, Van Den Berg GC, Rainey DY, Martin GB, Takken FL, De Wit PJ, et al. An NB-LRR protein required for HR signalling mediated by both extra- and intracellular resistance proteins. Plant J. 2007;50:14–28. doi:10.1111/j.1365-313X.2007.03027.x.