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The diverse roles of NB-LRR proteins in plants

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Abstract

Plant innate immunity relies on specialised immune receptors that can detect and defend against a wide variety of microbes. The first group of receptors comprises the transmembrane pathogen- or pattern-recognition receptors (PRRs), which respond to slowly evolving pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs). The second group of immune receptors is formed by the polymorphic disease resistance (R) proteins that detect microbe-derived effector proteins. Most R proteins are members of the nucleotide binding leucine-rich repeat (NB-LRR) class. Although this class comprises one of the biggest protein families in plants, relatively few have been functionally characterised to date. The question rises whether all NB-LRRs function as immune receptors, or that they might have alternative functions. The answer is: yes, they do have alternative functions that are different from the immune receptor function. This review summarises the current knowledge about non-immune receptor signal transduction functions of NB-LRRs in plants.
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Keywords: Resistance protein; Drought tolerance; Development; Shade avoidance; Photomorphogenesis; Disease resistance; Innate immunity; Defence signalling; Immune receptor

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1. Introduction

Innate immunity in animals and plants is mediated by specialised immune receptors by which plants can detect

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and defend themselves against a wide variety of microbes. In animals these receptors are generally referred to as pathogen- or pattern-recognition receptors (PRRs) as they recognise so-called pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs), which are highly conserved molecules of microbes. This system differs from the adaptive immunity in that the receptors are germ line-encoded. The type of immune receptors that have been identified in animals and plants so far, show similarities in structure and function [1,2]. However, in plants many immune receptors are not regarded as typical PRRs, but are referred to as plant disease resistance (R) proteins as they directly or indirectly recognise race- or strain-specific pathogen-derived proteins, called effectors, which are generally much less conserved [3]. Only a few typical PRRs have been identified in plants so far, of which the best studied examples are the receptor-like kinases (RLKs) EFR and FLS2 [4–6]. However, further investigation will likely reveal the presence of a larger assemblage. It appears that some immune receptors and immune receptor-like proteins play alternative signalling roles that are different from the immune receptor function. This review focuses on the non-immune receptor functions of intracellularly localised R proteins and their analogues of the nucleotide-binding leucine-rich repeat (NB-LRR) class, not only in signal transduction cascades typically involved in innate immunity, but also in other cellular processes. To place these signalling functions into perspective, we first briefly introduce plant innate immunity and the roles of different types of immune receptors therein.

2. Immune receptors in plant innate immunity

As mentioned above, examples of plant immune receptors that can be regarded as PRRs are FLS2 and EFR from Arabidopsis. FLS2 detects the most conserved domain of bacterial flagellin, represented by the flg22 peptide [7], and EFR detects bacterial EF-Tu [4]. Recognition of these PAMPs/MAMPs triggers a basal defence response termed PAMP-triggered immunity (PTI) [3], which can be regarded as a first line of defence. Specific strains or races of microbes evolved ways to circumvent PTI by secreting specific effectors that suppress this response. As a counteraction, a second line of plant defence evolved, which uses the polymorphic immune receptors referred to as R proteins. These receptors are able to directly or indirectly recognise these effectors and subsequently induce a resistance response that is termed effector-triggered immunity (ETI). The transcriptional reprogramming triggered upon ETI is similar to that during PTI, however, the main difference is that ETI triggers it faster and stronger. ETI often results in programmed cell death, which is also termed the hypersensitive response (HR) [3,8].

Several classes of R proteins exist and a common domain among them is the leucine-rich repeat (LRR) domain [3]. This domain is believed to provide recognition specificity due in large part to its diversifying selection at the solvent-exposed residues [9]. The largest class of R proteins comprises the NB-LRR proteins, which confer resistance to a wide variety of microbes [3]. However, it must be noted that plant genomes contain hundreds of genes that code for NB-LRR proteins [10–12], and relatively few have been functionally characterised. Other types of R proteins that have been identified contain an extracellular LRR domain. These proteins are either member of the RLK or the receptor-like protein (RLP) family. In addition to their LRR domain, both classes contain a transmembrane domain, but while the RLKs contain a cytoplasmic serine/threonine kinase domain, the RLPs do not contain a recognisable cytoplasmic signalling domain [3].

3. What are plant NB-LRRs?

Plant NB-LRRs contain a C-terminal LRR domain, a varying N-terminal effector domain, and a central NB domain. The latter is proposed to act as a nucleotide-dependent molecular switch regulating the conformation and signalling activity of these proteins [13]. The NB domain in plant NB-LRRs is called the NB-ARC domain, after nucleotide binding domain of apoptotic protease activating factor 1 (APAF-1), R proteins and *Caenorhabditis elegans* Death-4 (CED-4) [14]. This domain specifically binds and hydrolyses ATP [15,16]. Based on the occurrence of this particular type of NB domain, NB-ARC proteins form a specific subgroup within the superfamily called 'signal transduction ATPases with numerous domains' (STAND) [17]. Another STAND subgroup that is most closely related to the NB-ARC subgroup (based on NB domain structure) contains a number of animal NB-LRR proteins that are also implicated in innate immunity [18,19]. The N-terminal domain, found in plant NB-LRRs, can either be a Toll/Interleukin-1 receptor (TIR) or non-TIR domain, the latter frequently containing predicted coiled-coil (CC) motifs [12,20]. Based on this, these proteins can be subdivided into the TIR-NB-LRR and CC-NB-LRR class. These N-terminal domains are not related to those occurring in the animal NB-LRRs. For a more detailed review about the structure and function of plant NB-LRRs, including comparisons with the structurally related animal NB-LRR proteins we direct the reader to three recent reviews [13,21,22].

4. Non-immune receptor functions of NB-LRRs in defence signalling

4.1. NRC1

Of the functionally characterised plant NB-LRRs, most were identified as immune receptors that confer dominant resistance to a specific strain or race of a pathogen [3]. However, some NB-LRRs have been identified as innate immunity signalling components downstream of immune receptors. One example is tomato *NRC1* (NB-LRR protein

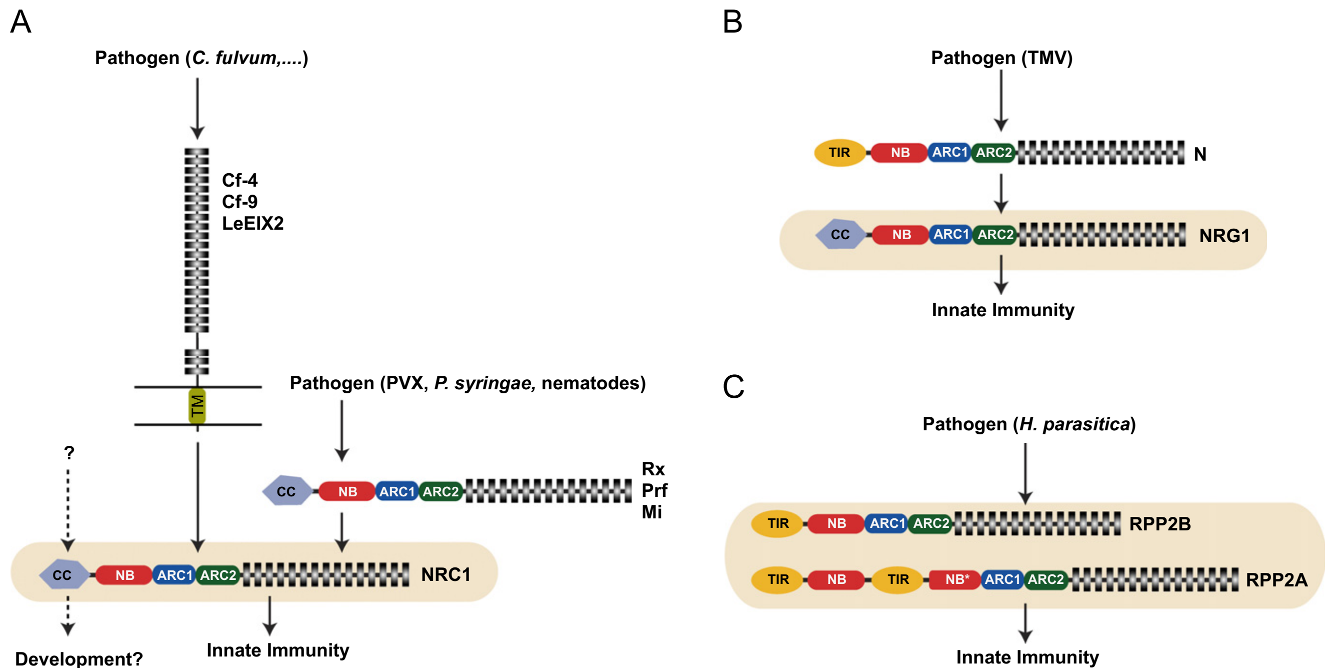

Fig. 1. NB-LRRs with non-immune receptor signalling functions in innate immunity. (A) The NB-LRR protein NRC1 is required for Cf-4 (an extracellular RLP-type immune receptor)-mediated HR and resistance to *Cladosporium fulvum* secreting the Avr4 effector. NRC1 is also required for the HR mediated by the RLPs Cf-9 and LeEIX2, as well as for the HR mediated by the NB-LRRs Rx, Prf and Mi. Silencing of NRC1 results in a reduction in plant growth indicating a possible role in development (dashed arrow). (B) The NB-LRR protein NRG1 is required for N-mediated resistance to tobacco mosaic virus, which suggests a signalling role downstream of the N resistance protein. (C) The RPP2B protein requires the unusual NB-LRR protein RPP2A for the induction of resistance to *Hyaloperonospora parasitica* Cala2. Both proteins might physically interact in a 'resistosome' complex to detect a pathogen-derived effector, or one of the proteins functions downstream of the other in the initiated defence signalling cascade.
The different protein domains are indicated. Subdomains of the NB-ARC domain are indicated as NB, ARC1 and ARC2. The LRR domains are shown as black repeat structures. Note that the N-terminal NB-ARC domain of RPP2A lacks the ARC1 and ARC2 subdomains and the C-terminal NB-ARC domain lacks the pre-P-loop and P-loop motifs (indicated with an asterisk).

required for HR-associated cell death 1) that was identified in a virus-induced gene silencing (VIGS) screen as required for Cf-4-mediated HR [23]. This CC-NB-LRR is also required for Cf-4-mediated resistance to *Cladosporium fulvum* secreting the Avr4 effector, and the HR triggered by multiple immune receptors including both RLPs and NB-LRRs (i.e. Cf-4, Cf-9, LeEIX2, Rx, Prf and Mi) (Fig. 1A) [24]. In contrast to suppression of resistance to *C. fulvum*, *NRC1* silencing did not affect Rx-, Pto/Prf- and N-mediated resistance to potato virus X (PVX), *Pseudomonas syringae*, and tobacco mosaic virus (TMV), respectively. However, the HR in *Rx*- or *Pto*-transgenic *Nicotiana benthamiana* plants induced by transient expression of their cognate effectors was significantly reduced by silencing of *NRC1*. This could indicate that NRC1 functions in a pathway that is specific for the HR, but not for disease resistance to PVX, *P. syringae* or TMV, and that the HR triggered by Cf-4 contributes largely to the resistance to *C. fulvum*.

Mitogen-activated protein kinase (MAPK) cascades are activated by a number of biotic and abiotic stresses [25]. Cf-4-mediated recognition of Avr4 induces the activation of three tomato MAPKs (LeMPK1, LeMPK2 and LeMPK3) that are important for HR and/or resistance to *C. fulvum* [26]. Silencing of the MAPKK-encoding gene *MEK2* in *N. benthamiana* decreased the HR triggered by a constitutively active NRC1 mutant that has a mutation in the MHD-motif of the NB-ARC domain [13]. This indicates that NRC1 induces the activation of an MAPK cascade involving MEK2 [24]. Transient expression of a constitutively active mutant form of tomato MEK2 or MAPKKKα in *N. benthamiana* resulted in an HR that was not compromised by silencing of *NRC1*, confirming that NRC1 acts upstream of an MAPK cascade. MEK2 is also activated upon TMV-infection of tobacco that carries *N* and is required for resistance to this virus [27]. Moreover, MEK2 orthologues in other plant species are also activated in response to pathogen attack and are required for both the HR and resistance [25]. As mentioned, NRC1 signals through MEK2; however, silencing of *NRC1* did not affect N-mediated resistance to TMV. Therefore, it is possible that NRC1 functions as a signal integrator downstream of immune receptors to specifically induce the HR, but none of the other resistance responses. Further studies are needed to test this hypothesis. The finding that an NB-LRR functions downstream of an RLP (e.g. Cf-4, Cf-9 and LeEIX2) resembles the putative downstream regulatory function (s) of the human NB-LRR protein NOD2 in Toll-like receptor (TLR) 2-mediated innate immune signalling [28,29].

4.2. NRG1

In a VIGS screen, the *N. benthamiana* CC-NB-LRR, *N requirement gene 1* (*NRG1*), was identified as being required for N-mediated resistance to tobacco mosaic virus (TMV) [30] (Fig. 1B). N is a TIR-NB-LRR immune receptor from tobacco that recognises the viral helicase protein p50 [31,32]. NRG1 is not required for resistance mediated by the CC-NB-LRRs Rx or Prf, and therefore might be specific for N. This in contrast to NRC1 that appears to be required for the function of multiple immune receptors. The difference in specificity is not surprising as NRG1 and NRC1 are not orthologous and are in fact only distantly related [24]. Co-expression of p50 and N *in planta* induces oligomerisation of N [33], the first example of oligomerisation among plant NB-LRR proteins. This is in contrast to animal systems, in which oligomerisation of the NB-LRRs, but also of the NB-ARC proteins, APAF-1 and CED-4, has been observed frequently [34]. Oligomerisation of these proteins is mediated by the NB domain, but the stoichiometry of many of these complexes have not been resolved, as these interactions were mostly identified by co-immunoprecipitation techniques. The same is true for N, but in this case the oligomerisation is mainly, if not only, mediated by the N-terminal TIR domain. It is not clear whether the NB domain also participates in this interaction [33]. NB-ARC proteins for which the stoichiometry of their protein complexes have been determined are CED-4 and APAF-1 [35,36]. Activation of APAF-1 triggers the formation of a ring-shaped complex with seven APAF-1 molecules, called the apoptosome, and activation of CED-4 triggers the formation of a homo-tetramer. The human NB-LRR IPAF, forms hetero-oligomers with other NB-LRRs, and seems to be an important constituent of the NAIP5 inflammasome (containing the NB-LRR NAIP5) that mediates innate immunity against *Legionella pneumophila* [37,38]. It has been suggested that N might form hetero-oligomers with NRG1; however, no interaction was found between these proteins, and silencing of *NRG1* did not affect the oligomerisation of N [30].

4.3. RPP2A and RPP2B

In Arabidopsis, two NB-LRR proteins are required for race-specific resistance to the oomycete pathogen *Hyaloperonospora parasitica* race Cala2: RPP2A and RPP2B (Fig. 1C) [39]. While RPP2B is a typical TIR-NB-LRR protein, RPP2A is a TIR-NB-LRR-like protein that is unique in that it has two TIR-NB modules that are both truncated in their NB-ARC. The most N-terminal NB-ARC domain lacks the ARC subdomains and the C-terminal one lacks the pre-P-loop and P-loop motifs that are required for ATP-binding in other NB-LRRs [13]. The core NB subdomain in the first module could be complemented for function by the ARC subdomains of the C-terminal module, but such complementation has not been described before. Thus, if this complementation does not take place it is unlikely that this protein would be able to trigger an RPP2B-independent defence response if a mutation in RPP2A would have been introduced that in other NB-LRRs lead to constitutive activation of defence (e.g. mutation in MHD-motif). This constitutive defence triggered by such NB-LRR mutants depends on a functional NB-ARC domain [15,40,41]. Sinapidou and coworkers [39] present models how RPP2A and RPP2B could function cooperatively to confer resistance to the *H. parasitica* strain Cala2. These models are based on direct interaction of one or both proteins with the cognate effector of the pathogen, or binding of one or both proteins to a virulence target that is manipulated by the effector. In case both proteins directly or indirectly sense the presence of the effector, both proteins can be regarded as immune receptors. However, if one of the two is a constituent of the RPP2 'resistosome' that does not play a direct role in the effector perception, or even functions further downstream in RPP2 defence signalling, this protein cannot be regarded as an immune receptor and could function similar to NRC1, for example. Biochemical studies should be performed to elucidate the exact biochemical relationship between RPP2A and RPP2B in resistance to *H. parasitica*.

5. Do the 'incomplete' TIR-NB-LRR proteins, TX and TN, play a role in innate immunity?

Besides the *TIR-NB-LRRs*, plants also have genes that code for 'incomplete' TIR-NB-LRRs: *TX* and *TN* genes [12,42]. The TX proteins possess an N-terminal TIR domain, but lack the NB-LRR domain, whereas the TN proteins also lack the LRR domain, but contain the NB domain or part thereof. Many of these genes in Arabidopsis are expressed and are not likely to be pseudo-genes. Meyers and co-workers [43] suggest that these proteins could function as adaptor proteins analogous to the TIR-containing adaptors for TLRs in animals, like MyD88. By the homophilic interaction of the TIR domains of MyD88 and TLRs, kinases (e.g. IRAKs) are recruited by the death domain of MyD88. Since the TIR domain of N also mediates homophilic interaction to provide oligomerisation, TIR-TIR interactions between a TIR-NB-LRR and a TX or TN protein is a plausible hypothesis. Whether TX and TN proteins play a role in plant innate immunity is not clear. One report describes the identification and characterisation of the *TX* gene *NRSA-1*, for non-host resistance to *Striga asiatica* 1, derived from the non-host plant marigold (*Tagetes erecta*) [44]. The expression of this gene was upregulated during attempted parasitism by this parasitic weed. Non-host plants, like marigold, seem to mount a defence response against this parasite that involves cell death and the formation of cell wall appositions at the penetration site, which resembles the defence responses to microbial plant pathogens. Haustorial penetration in these non-hosts is arrested at an early stage, preventing the parasite to connect to the vascular tissue [44,45]. *NRSA-1* expression is highly induced in the region

of the roots that is attacked by *S. asiatica*, and systemic upregulation of this gene was also observed in the leaves. Jasmonic acid (JA) treatment also resulted in the induction of *NRSA-1* expression, but wounding or treatment with salicylic acid (SA), paraquat or abscisic acid (ABA) did not [44]. It would be interesting to investigate whether *TX* and *TN* genes are involved in the regulation of plant innate immunity.

6. NB-LRRs with alternative signalling functions

6.1. ADR1

An activation-tagging screen in Arabidopsis, aimed to identify mutants with higher *pathogenesis related* (*PR*)-*1* expression levels, resulted in the retrieval of the dominant mutant *adr1* (activated defence response 1) [46]. In this mutant, the CaMV 35S enhancers were found to be inserted 4 kb upstream of the promoter of the CC-NB-LRR gene *ADR1* (Fig. 2A), resulting in its constitutive expression. The *adr1* mutant shows a constitutive defence phenotype: constitutive expression of defence marker genes, accumulation of SA and enhanced resistance to biotrophic pathogens. This phenotype was abolished by crossing the mutant with a NahG line that depletes SA by converting it to catechol [47], and it also diminished the expression of the tagged *ADR1* gene itself. These results indicate that the specificity of the *ADR1* promoter was not completely abolished by the CaMV 35S enhancers. In wild type Arabidopsis, *ADR1* expression is induced in an incompatible interaction with *P. syringae* expressing AvrB, by treatment with the systemic acquired resistance (SAR)-inducing chemical benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) and by wounding, but not by treatment with JA or ethylene (ET) [46]. The constitutive defence phenotype of the *adr1* mutant is similar to that of *cpr1* (constitutive expressor of PR genes 1) and *cir1* (constitutively induced resistance 1) [48,49]. However, the *adr1* mutant shows a striking additional phenotype that was not observed for the other constitutive defence mutants: a markedly enhanced drought tolerance [50]. The *adr1* plants have a small stature, a phenotype generally observed for mutants that express defence constitutively [48,49,51]. However, this phenotype was not directly linked to the drought tolerance phenotype as conditional over-expression of *ADR1* in wild type plants also leads to drought tolerance. The drought tolerance could also not be explained by a reduced transpiration rate in the mutant, as this was similar to wild type plants. Marker genes that are specifically upregulated upon dehydration were constitutively expressed in the *adr1* mutant already under normal conditions, which could explain the drought tolerance phenotype [50]. One example of such a marker gene is the *DREB2A* gene coding for a dehydration-responsive element binding protein (DREB), which is upregulated by drought, but also by SA and reactive oxygen intermediates (ROI) [50]. Since SA and ROI levels are high in the *adr1* mutant, it could explain the upregulation of *DREB2A*.

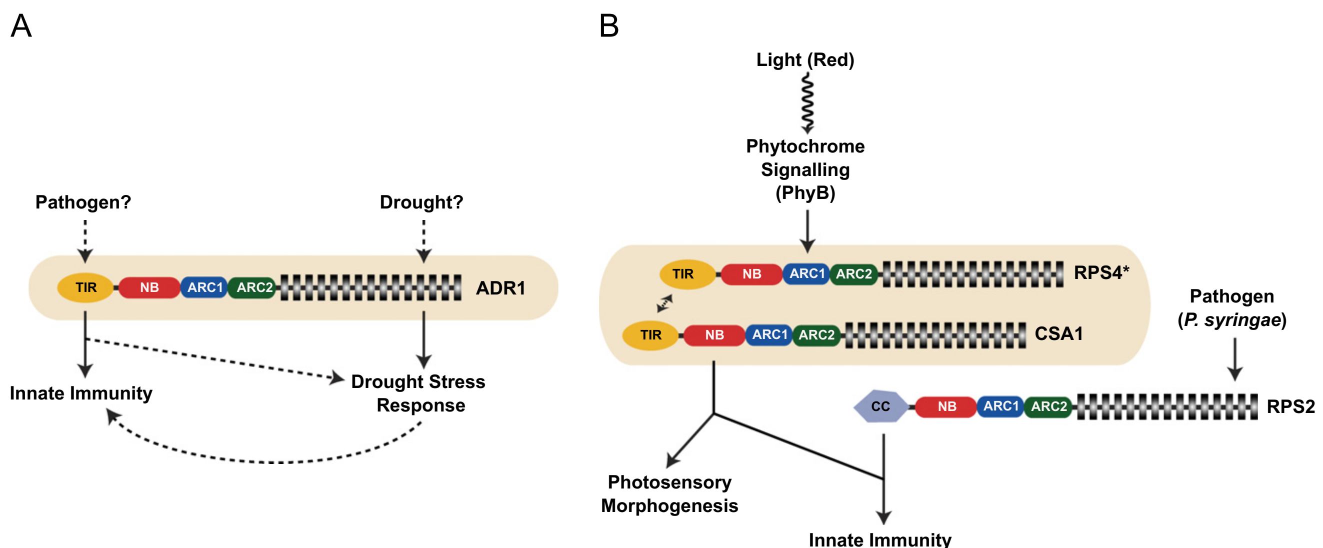

Fig. 2. NB-LRRs with alternative signalling functions. (A) An activation-tagged *adr1* mutant line shows an enhanced drought tolerance, which suggests that ADR1 is involved in the regulation of the drought stress response. However, it is not known whether drought activates this protein in wild type plants (right arrows). The signalling function of ADR1 in the drought stress response might be independent of its potential role in innate immunity. However, it is also possible that this response is part of the ADR1-mediated defence response to a particular microbe, in which activation of drought stress responsive genes is required for resistance to this microbe (middle dashed arrows). (B) An activation-tagged *csa1* mutant has a constitutive shade-avoidance phenotype similar to a *phyB* mutant. The NB-LRR protein CSA1 plays a role in the phytochrome signalling pathway through phyB, which regulates photomorphogenesis. The CSA1 signalling function is dependent on the resistance protein RPS4, which indicates that the latter NB-LRR is also involved in phytochrome signalling. The *csa1* mutant shows a decreased RPS2-mediated resistance to *P. syringae*, similar to the *PhyB* mutant. This suggests a crosstalk between innate immunity and phytochrome signalling pathways (solid line). CSA1 and RPS4 might hetero-oligomerise through their TIR domains (double headed arrow). *The immune receptor function of RPS4 is not indicated here. The different domains of the NB-LRRs are indicated. Subdomains of the NB-ARC domain are indicated as NB, ARC1 and ARC2. The C-terminal LRR domain is shown as a black repeat structure.

However, plants that were treated with BTH to induce a strong accumulation of SA could not provide significant drought tolerance, just as the high SA-accumulating mutants mentioned above, *cpr1* and *cir1*. This indicates that *adr1* also might signal through SA-independent pathways. Surprisingly, the *ADR1* gene itself is not upregulated by drought [50], but this does not exclude the possibility that it is involved in regulating the drought stress response (Fig. 2A). There is a significant amount of crosstalk in the signalling pathways activated by biotic and abiotic stresses that is extensively reviewed by Fujita and co-authors [52]. It is possible that the constitutive expression of *ADR1* in the *adr1* mutant pleiotropically activates the drought stress responsive genes, because of a potential crosstalk between drought stress and defence signalling in this mutant. To answer the question whether ADR1 is a true regulator of the drought stress response, an *ADR1* knock-out line should be assessed for its drought tolerance phenotype. Another possibility is that ADR1 is not involved in the drought stress response induced by drought, but that ADR1-mediated activation of drought stress responsive genes is part of a unique defence response against a particular pathogen (Fig. 2A). ADR1 could have an innate immune signalling function downstream of immune receptors, but the possibility that ADR1 has an as yet unrecognised immune receptor function itself can of course not be excluded. Further studies need to be performed to elucidate the signalling role of this CC-NB-LRR protein.

6.2. *PLFOR48*

Another plant NB-LRR protein that might be involved in processes other than innate immunity is the TIR-NB-LRR encoded by *PLFOR48* from sunflower. Four independent transgenic sunflower lines that contain a *PLFOR48* antisense cDNA construct showed severe developmental abnormalities, such as stunted growth and reduced apical dominance [53]. Southern blot analysis with genomic DNA derived from tobacco probed with the sunflower *PLFOR48* cDNA revealed a faint band that could correspond to the orthologous *TIR-NB-LRR* gene from tobacco. Eight independent transgenic tobacco lines, containing the sunflower *PLFOR48* antisense cDNA construct, also showed developmental abnormalities, albeit less severe. These lines exhibited strongly deformed seed pods, but no stunted growth. The *PLFOR48* gene is one of the *TIR-NB-LRR* genes found in a cluster at the genomic *Pl6* locus of sunflower that confers resistance to various downy mildew races (*Plasmopara halstedii*) [53,54]. Because of the strong developmental phenotype of the *PLFOR48* antisense sunflower lines, the role of this gene in resistance to this pathogen could not be investigated. However, because the transgenic tobacco lines did not show severe abnormalities, resistance to *Phytophthora parasitica* var *nicotianae* could be tested. The transgenic lines exhibited a significantly enhanced susceptibility to this pathogen compared to wild type tobacco. This indicates that the tobacco gene homologous to the sunflower *TIR-NB-LRR PLFOR48* could be involved in regulating disease resistance as well as development. The same could be true for the CC-NB-LRR discussed earlier, NRC1 (Fig. 1A). Silencing of this gene in both tomato and *N. benthamiana* by VIGS resulted in a significant growth reduction, while silencing of the *R* gene *Cf-4* had no effect on plant growth [24].

6.3. *CSA1*

An Arabidopsis TIR-NB-LRR that has not been associated directly with plant innate immunity is *constitutive shade avoidance 1* (*CSA1*) (Fig. 2B). This gene was identified in a mutagenesis screen for activation-tagged lines with constitutive shade avoidance [55]. Shade avoidance is a response to competitor plants that is aimed to position the leaves for maximum light capture. It involves elongation of stem-like structures (e.g. hypocotyl and petiole), the upward orientation of leaves (hyponasty) and reduced branching. Light that has passed through a canopy induces this response as it is rich in far-red (FR) light and poor in red (R) light that has been absorbed by chlorophyll. Plants react rapidly to changes in R:FR ratios via their photoreceptors of the phytochrome family. These receptors exist in two photoconvertible forms: the inactive 'Pr' (the R absorbing form) and the active 'Pfr' (the FR absorbing form). Active Pfr translocates into the nucleus where it regulates gene expression by interacting with several transcription factors. The shade-avoidance response is also regulated by the quantity of blue light, which is sensed by the blue light photoreceptors called cryptochromes and phototropins [56–58]. The Arabidopsis *phyB* mutant has a constitutive shade-avoidance phenotype that resembles the *csa1* mutant [55]. In seedlings grown in complete darkness, exposure to light (e.g. continuous red, far-red or blue light) signals a major shift in developmental pattern, including the arrest of hypocotyl growth. In this assay, the *phyB* mutant displayed a reduced response only under red light but not under far-red or blue light, as phyB is most efficiently activated by red light. Exactly the same was observed for the *csa1* mutant, which indicates that this mutant is not generally defective in light regulation of cell growth. No additive effects on the photomorphogenesis phenotypes were observed in a *phyB csa1* double mutant, indicating that the *csa1* mutant impairs phyB signalling. In the *csa1* mutant, the activation tagging T-DNA construct was inserted just downstream of the TIR domain-encoding region. This resulted in constitutive expression of a truncated CSA1 protein only containing a TIR domain and lacking the NB-ARC and LRR domains. Expression of this truncated NB-LRR showed a semi-dominant negative effect. Transgenic lines over-expressing the truncated *CSA1* gene under control of the CaMV 35S promoter in a wild type background pheno-copied the *csa1* mutant. A T-DNA knock-out line without CaMV 35S enhancer

sequences in *CSA1* (*csa1-2*) only showed a weak but significant photomorphogenic phenotype, especially when seedlings were grown in the dark and then shifted to red light. *CSA1* is a member of the TIR-NB-LRR-B2 (TNL-B2) family [12], and two other highly related *TIR-NB-LRRs* in this clade are *RPS4*, which confers resistance to *P. syringae* carrying the effector AvrRPS4 [59], and *AT5G44870*, a gene that is regulated by phyA and phyB [60,61]. Null mutants carrying T-DNA insertions in these genes showed the same phenotype as the *csa1-2* knock-out line, indicating that these genes could also play a role in phyB signalling [55]. The authors speculate that CSA1 might normally hetero-oligomerise with RPS4 and AT5G44870 via their TIR domains upon activation of these proteins (Fig. 2B), which could be similar to the oligomerisation of N via its TIR domain [33]. The truncated CSA1 protein, only consisting of the TIR domain, might therefore negatively affect RPS4 and AT5G44870 dominantly by constitutive binding to their TIR domains. In that case, the presence of an increased amount of RPS4 should attenuate the phenotype. Indeed, over-expression of RPS4 in the *csa1* mutant background showed a wild type phenotype in all the photomorphogenesis assays and thus restored the sensitivity of the *csa1* mutant to red light [55]. This suggests that the dominant negative effect on phyB signalling is mediated by interfering with the function of RPS4 and possibly other related TIR-NB-LRRs. A dramatic increase in the levels of expression of the gene encoding the homeodomain transcription factor *HAT4* (also known as *ATHB-2*) is one of the earliest responses to a reduction in the R:FR ratio [62,63]. In the *csa1* mutant this gene was already expressed to higher levels than in the wild type plants under white light (high R:FR ratio) and this could explain the constitutive shade-avoidance phenotype. The authors conclude that CSA1 and RPS4 might mediate or modulate phyB signalling by either acting as early components of the pathway or by modulating the levels or activity of an early step of this signalling cascade [55].

It was previously reported that phytochrome signalling is important for disease resistance, as an Arabidopsis *phyA phyB* double mutant is impaired in PR-gene expression upon application of SA, and displays a reduced RPS2-mediated resistance to *P. syringae* containing *AvrRpt2* [64]. The *phyB* single mutant also shows decreased RPS2-mediated resistance, as well as the *csa1* mutant [55]. These data indicate that there is a crosstalk between phytochrome- and defence signalling (Fig. 2B), and that the NB-LRRs CSA1 and RPS4 might be involved in phyB signalling, possibly independent from their direct roles in innate immunity signalling. Future studies should be performed to shed more light on this.

7. Conclusion

From the papers discussed in this review, a picture emerges where plant NB-LRR proteins can have signalling functions different from pathogen perception (i.e. immune receptor function). Some of these functions consist of a signalling role in innate immunity downstream of immune receptors (Fig. 1), whereas others are involved in signalling cascades important for additional cellular processes, such as drought tolerance, development and photomorphogenesis, which are possibly independent from or only indirectly related to innate immunity (Fig. 2). Since evidence for these non-immune receptor signalling functions remains scarce, more research should be aimed to elucidate the alternative roles of NB-LRR proteins in plants.

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