REVIEW ARTICLE   **Open Access**

NPR1 as a transgenic crop protection strategy in horticultural species

Katchen Julliany P. Silva[1], Nasser Mahna[1,2], Zhonglin Mou[3,4] and Kevin M. Folta[1,4]

Abstract

The NPR1 (NONEXPRESSOR OF PATHOGENESIS RELATED GENES1) gene has a central role in the long-lasting, broad-spectrum defense response known as systemic acquired resistance (SAR). When overexpressed in a transgenic context in Arabidopsis thaliana, this gene enhances resistance to a number of biotic and abiotic stresses. Its position as a key regulator of defense across diverse plant species makes NPR1 a strong candidate gene for genetic engineering disease and stress tolerance into other crops. High-value horticultural crops face many new challenges from pests and pathogens, and their emergence exceeds the pace of traditional breeding, making the application of NPR1-based strategies potentially useful in fruit and vegetable crops. However, plants overexpressing NPR1 occasionally present detrimental morphological traits that make its application less attractive. The practical utility of NPR-based approaches will be a balance of resistance gains versus other losses. In this review, we summarize the progress on the understanding of NPR1-centered applications in horticultural and other crop plants. We also discuss the effect of the ectopic expression of the A. thaliana NPR1 gene and its orthologs in crop plants and outline the future challenges of using NPR1 in agricultural applications.

Introduction

Plant pathogens challenge profitable production, and in some cases threaten entire industries. Current plant pandemics in banana, citrus, avocado, and cacao mark just a few dire examples where rapid and durable solutions are desperately needed. These visible cases represent just the edge of a much broader problem, as plant pathogens are shuttled worldwide with borderless travel and on winds of weather extremes. At the same time, our arsenal of chemical approaches to quell disease presentation is antiquated and slow to evolve. There also is the desire to limit production inputs, generating savings for farmers and benefits to the environment.

Genetic engineering solutions have proven successful in mitigating the effects of plant disease in the laboratory and a limited number of field trials, in a number of plant species. A series of reports have described the effect of NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1) overexpression on disease progression and symptom presentation. NPR1 is a central regulator of plant defense response. It stands out as a conspicuous target for use in a transgenic context, as a plant gene inappropriately expressed to induce an enhanced response is an intuitive target for engineering disease-tolerant plants.

Overexpression of the Arabidopsis thaliana NPR1 gene (AtNPR1) or its orthologs has been shown to enhance resistance to biotrophic and necrotrophic fungal, viral, and bacterial pathogens in a number of horticultural crop plants, including grape, carrot, tomato, apple, citrus, tobacco, and strawberry. These specialty crop applications occurred after applications in high-acreage agronomic crops like rice, wheat, soybean, peanut, and potato[1–15]. These trials suggest that similar defense mechanisms exist across a range of plant species, making AtNPR1 and its orthologs desirable candidate genes for transgenic manipulation in crops for enhanced disease resistance[3,16–18].

Correspondence: Kevin M. Folta (kfolta@ufl.edu)
[1]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA
[2]Department of Horticultural Sciences, University of Tabriz, Tabriz, Iran
Full list of author information is available at the end of the article

In this review, we provide an overview of reports that have altered disease and pest tolerance by manipulating *NPR1* expression levels, describing the work translated to agronomic crops, and then the reports arising from horticultural crop species.

Plant defense mechanisms

Plants cope with pathogen infection with sophisticated chemical, biochemical, and mechanical barrier systems. These systems combined are the basis of plant innate immunity, and allow plants to survive, grow properly, and produce appreciable crop yields. In contrast to mammals, plants did not evolve mobile defender cells and somatic adaptive immune system. Instead plants rely on the innate immunity of each cell, as well as on systemic signals emanating from infection site to mobilize a defense response[19,20]. Fortunately, pathogen recognition and defense activation occur rapidly. Potentially infecting pathogens must first overcome several physical barriers on the plant surface, such as wax layers, rigid cell walls, cuticular lipids, trichomes, hairs or spines, leaf veins, odd stomatal aperture shapes, and antimicrobial enzymes or secondary metabolites[21,22]. These morphological structures and chemical barriers represent the first line of plant defense. These features play an important primary role in disease resistance by inhibiting or delaying the advance of pathogens into invasive states[23–25]. A successful infection requires breaking initial barriers and efficiently countering a cascade of plant defense responses generated by a suite of mobilized biochemical activities[26].

Pathogen signatures are also recognized by plants at a molecular level. The plant immune system can be summarized in three steps, as described by Jones and Dangl[27] as well as Zipfel[28]. First, pathogen- or microbial-associated molecular patterns (P/MAMPs) are recognized by pattern recognition receptors, resulting in pathogen- or microbial-triggered immunity (P/MTI). Second, virulent effectors are deployed by successful pathogens to interfere with P/MTI, which results in effector-triggered susceptibility. After a given effector is specifically recognized by one of a class of proteins called the nucleotide binding site-leucine-rich repeat proteins, which activate effector-triggered immunity, resulting in disease resistance. This second layer of immunity often culminates with a hypersensitive response (HR), comprised of programmed cell death[29] at the infection site to isolate the pathogen, followed by the upregulation of defense responses such as accumulation of pathogenesis-related (PR) proteins in distal tissues. This response, known as systemic acquired resistance (SAR), protects plants from secondary infection by activating multiple signaling pathways and inducing systematic responses to confront pathogen infection[30,31].

SAR and *PR* genes

SAR is a well-known plant resistance mechanism induced upon pathogen infection. It is activated by the accumulation of the signaling molecule salicylic acid (SA, 2-hydroxybenzoic acid) and the coordinated induction of *PR* genes that encode proteins meeting pathogen attack[32,33]. Systemic accumulation of SA at the onset of SAR has been well characterized in the model plant *A. thaliana*. The key regulator of SAR is the NPR1 protein. It contains BTB/POZ and ankyrin repeat domains, which mediate NPR1 interaction with other proteins and help the protein to associate with the promoters of *PR* genes, activating their expression[34,35]. In the absence of infection, NPR1 is predominantly oligomeric and partitioned to the cytoplasm. During pathogen infection or SA treatment, the NPR1 complex disarticulates, and monomeric proteins are transported into the nucleus. In the nucleus, NPR1 interacts with the TGA family of basic leucine zipper transcription factors[35], which in turn upregulate the transcription of *PR* genes, and help confer resistance to secondary infection[34,36–38].

Use of *AtNPR1* and its orthologs for crop improvement

Heterologous expression of AtNPR1 in agronomic crops

The *AtNPR1* protein triggers the activation of defense genes upon infection, so many groups sought to test the hypothesis that promiscuous expression of the *AtNPR1* gene would result in enhanced resistance to disease in crop plants. The hypothesis was generally tested by placing the *AtNPR1* cDNA downstream of the CaMV 35S promoter. This approach has resulted in some positive effects on disease resistance (Table 1). While the focus of this review is horticultural crops, it is necessary to start with the application of *AtNPR1* in agronomic crops because that is where the first translational experiment was performed.

The first case of *AtNPR1* overexpression in an agronomic crop was reported by Chern et al.[17], who observed that promiscuous expression of *AtNPR1* in rice led to enhanced resistance to bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*. Disease resistance segregated among individual T1 plants, which approximated *AtNPR1* steady-state transcript levels. As in Arabidopsis, no obvious morphological changes were observed[16]. Agronomic traits of rice *AtNPR1*-transgenic lines were not affected and most plants were fertile.

However, a subsequent report in rice confirmed that while *AtNPR1* overexpression enhanced resistance to *X. oryzae* pv. *oryzae*, it caused detrimental side effects to plant growth[39]. Transgenic rice exhibited lesion-mimic cell death, hydrogen peroxide (H_2O_2) accumulated significantly around lesions, and plants accumulated lower levels of free SA. These phenotypes were heritable and

Table 1 Transgenic crop plants ectopically expressing *AtNPR1*

Plant species	Resistance/tolerance (caused by)	Abnormal phenotype	Reference
Canola	Bacterial disease (*P.syringae*)	a	Potlakayala et al.[44]
Carrot	Sclerotinia rot (*S. sclerotiorum*)Gray mold (*B. cinerea*)Black rot (*A.radicina*)Bacterial leaf blight (*X. hortorum* pv. *carotae*)Powdery mildew (*Erysiphe heraclei*)	a	Wally et al.[7]
Citrus	Citrus canker (*X. citri* ssp. *citri*.)Citrus greening (Huanglongbing)	a	Zhang et al.[9] Dutt et al.[50] Boscariol-Camargo et al.[48]
Cotton	*Verticillium* wilt (*V. dahliae*)*Fusarium* wilt (*F. oxysporum* f. sp. *vasinfectum*)Seedling damping-off (*R. solani*)*Alternaria* leaf spot (*A. alternata*) Nematode (*R. reniformis*)Black root rot (*T. basicola*)	a	Kumar et al.[45] Parkhi et al.[46,47]
Rice	Bacterial blight (*X. oryzae pv. oryzae*)Blast disease (*M. oryzae*)Bakanae disease (*F. verticillioides*) Bacterial foot rot (*E. chrysanthemi*)Sheath blight (*R. solani*)[a]	Lesion-mimic cell deathAccumulation of H_2O_2 around lesionsSusceptibility to the rice yellow mottle virusGrowth retardation, reduced height and smaller seeds, with consequent lower productivity; and development of spontaneous lesions in controlled conditions	Chern et al.[17a] Fitzgerald et al.[39] Quilis et al.[40] Molla et al.[42a]
Plant species	Resistance/tolerance (caused by)	Abnormal Phenotype	Reference
Soybean	Root-knot nematodes (Meloidogyne spp.)Cyst nematode (*H. glycines*)	a	Youssef et al.[13] Matthews et al.[12]
Strawberry	Anthracnose (*C. acutatum*)Crown rot (*C. gloeosporioides*)Powdery mildew (*P. aphanis*) Angular leaf spot (*X. fragariae*)	Shorter plants, reduced canopy size and densityNo production of runners and fruits	Silva et al.[11]
Tobacco	Common cutworm larvae (*S. litura*)Root-rot nematode (*M. incognita*)polyethylene glycol and oxidative stress tolerance	a	Meur et al.[6] Priya et al.[52] Srinivasan et al.[53]
Tomato	Bacterial wilt (*R. solanacearum*)Gray leaf spot (*S. solani*).Bacterial spot (*X. campestris* pv. *vesicatoria*)	Susceptibility to *Fusarium* wilt(*F. oxysporum* f. sp. *lycopersici*)	Lin et al.[3,39]
Wheat	*Fusarium* head blight (*F. graminearum*)	Susceptibility to *Fusarium* seedling blight (*Fusarium* spp.)	Makandar et al.[4a]
Wheat	*Fusarium* head blight (*F. graminearum*)	Susceptibility to *Fusarium* seedling blight (*Fusarium* spp.)	Gao et al.[43]

[a]No abnormal/no reported phenotype

positively correlated with steady-state transcript levels of the *AtNPR1* transgene and endogenous rice defense genes. Quilis et al.[40] also reported that *AtNPR1* has both positive and negative regulatory roles in mediating defense responses in rice against biotic and abiotic stresses. The *AtNPR1* transgene conferred resistance to the fungal pathogens *Magnaporthe oryzae* and *Fusarium verticillioides*, which cause blast and bakanae diseases, respectively, and to the bacterial pathogen *Erwinia chrysanthemi* that causes foot rot disease. The transgenic plants, however, showed susceptibility to rice yellow mottle virus. Ectopic *AtNPR1* expression also resulted in salt and drought stress sensitivity. Unlike Arabidopsis and tobacco[41], rice plants accumulated very high levels of endogenous SA, and these levels did not change after pathogen infection. *AtNPR1* overexpression also affected rice yields under controlled conditions. In the greenhouse, transgenic rice plants overexpressing *AtNPR1* showed slower growth, reduced height, and smaller seeds. In the growth chamber, *AtNPR1* plants developed spontaneous

lesions. Another report in rice showed that green-tissue-specific expression of *AtNPR1* in rice resulted in increased resistance to sheath blight disease (caused by *Rhizoctonia solani*) with no phenotypic abnormalities. This strategy was considered an improvement overexpression from a constitutive promoter, where some deleterious effects were observed[42].

AtNPR1 was also ectopically expressed in wheat in order to assess effects on disease resistance[4]. Transgenic plants exhibited increased resistance to head blight (FHB) caused by *F. graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein.] Petch) and demonstrated stable inheritance of resistance for four generations. In another study, accumulation of the *AtNPR1* transcript positively affected wheat yield[39]. *PR1* transcripts accumulated faster and to higher levels in the transgenic plants. Increased wheat resistance to FHB and *Fusarium* seedling blight (*Fusarium* spp.) was also later reported by Gao et al.[43].

AtNPR1-expressing canola (*Brassica napus* cv. Westar) plants displayed resistance to *Pseudomonas syringae*[44]. The transgenic plants exhibited no abnormal phenotypes, and the *AtNPR1* transcript levels did not correlate with disease resistance.

Constitutive expression also led to effects against other stressors, such as nematodes. The search for increased resistance to nematodes in soybean spurred the examination of several Arabidopsis genes related to SA and JA synthesis and signaling[13]. Transgenic *AtNPR1*-soybean plants were resistant to root-knot nematodes (RKN) (*Meloidogyne* spp.). The number of RKN galls per plant was reduced in transgenic plants. In another study, Matthews et al.[12] generated plants with resistance to soybean cyst nematode (SCN) (*Heterodera glycines*). Promiscuous *AtNPR1* overexpression resulted in the lowest SCN cyst index, suggesting that this gene is a strong candidate for plant genetic engineering to confer nematode resistance.

Heterologous expression of *AtNPR1* in horticultural crops

The *AtNPR1* gene also triggered resistance to several tropical diseases in tomato[3]. Transgenic tomato plants with the highest *AtNPR1* transcript accumulation exhibited enhanced resistance to bacterial wilt (caused by *Ralstonia solanacearum*), *Fusarium* wilt (caused by *F. oxysporum*. sp. *lycopersici*), gray leaf (caused by *Stemphylium solani*), and bacterial spot (caused by *X. campestris* pv. *vesicatoria*). Plants exhibited normal morphologies and horticultural traits for at least four generations. Lines with medium or low levels of *AtNPR1* expression showed similar or slightly lower resistance to *Fusarium* wilt when compared to wild-type plants.

AtNPR1-expressing carrot plants were resistant to multiple pathogens[7]. Transgenic lines were phenotypically normal compared to non-transformed controls. Three necrotrophic foliar pathogens, *Sclerotinia*

sclerotiorum, *Botrytis cinerea*, and *Alternaria radicina*, a root pathogen, *A. radicina*, a foliar infecting bacteria, *X. hortorum* pv. *carotae*, and a biotrophic fungus, *Erysiphe heraclei*, were tested. Disease symptoms were reduced in all treatments. The best results were observed for *X. hortorum* and *E. heraclei*, which had disease reduced by 80% and 90%, respectively. Transcripts corresponding to carrot *PR* genes were not elevated in *AtNPR1*-overexpressing carrot plants, suggesting enhanced resistance did not correlate with constitutive induction of SAR.

Other examples of broad-spectrum resistance have been described in cotton[45–47]. *AtNPR1*-expressing cotton lines were resistant to the fungi *V. dahliae*[46], *F. oxysporum* f. sp. *vasinfectum*, *R. solani*, and *A. alternata* and to the nematode, *Rotylenchulus reniformis*[47]. The transgenic plants displayed normal growth and development but showed typical discoloration symptoms. *AtNPR1*-expressing cotton also showed resistance to *Thielaviopsis basicola*, the causal agent of black root rot[45]. The transgenic plants displayed significantly higher shoot and root weights, longer shoots, and eventually improved yields. They also exhibited faster and stronger induction of many *PR* genes in roots, including *PR1* and *LIPOXYGENASE1*.

Perennial fruit tree crops, such as citrus, have also been transformed with *AtNPR1* and their growth and development are largely unaffected, while conferring tolerance to disease. Zhang et al.[9] showed that *AtNPR1* in "Duncan" grapefruit and "Hamlin" sweet orange conferred increased resistance to citrus canker (caused by *X. citri* ssp. *citri*.). Basal levels of SA were not altered in any of the tested lines, and the SAR marker gene in citrus, *PR2*, was not constitutively expressed, suggesting that *AtNPR1* ectopic expression did not induce a constitutive defense response. A similar effect was shown in *AtNPR1*-overexpressing sweet orange lines by Boscariol-Camargo et al.[48]. The most conspicuous effect was an increase in the accumulation of *enhanced disease susceptibility1* (*EDS1*), *PR1* and *PR2* transcripts 12–24 h after inoculation by *Xcc*. This induction was more noticeable for *PR1*, which showed a 21,000-fold increase (likely due to low transcript levels in the non-induced plant) in transgenic and non-transgenic lines in relation to the non-inoculated control plants. The authors proposed that *AtNPR1* induces resistance to *Xcc* through a priming mechanism, essentially increasing *EDS1* and *PR* transcript accumulation to a higher level after pathogen inoculation.

Ectopic expression of *AtNPR1* under the control of the bi-directional dual promoter complex with enhanced promoter activity[49] resulted in sweet orange lines with tolerance to Huanglongbing (also called citrus greening; caused by *Liberibacter asiaticus*). An equivalent outcome was witnessed when a phloem specific *A. thaliana SUC2* (*AtSUC2*) promoter was implemented. The transgenic plants had fewer disease symptoms and a few lines

remained symptom-free after 36 months in a field with high disease pressure and highly symptomatic controls[50].

The diploid strawberry (*F. vesca* L. Hawaii 4') has also been engineered with a 35S::*AtNPR1* cassette. Transgenic plants showed increased resistance to anthracnose (caused by *C. acutatum*), crown rot (caused by *C. gloeosporioides*), powdery mildew (caused by *P. aphanis*), and angular leaf spot (caused by *X. fragariae*). The disadvantage was an increased instance of undesirable traits observed during plant growth and development. The transgenic plants were shorter than non-transformed controls, and canopy size and density were reduced. In addition, all lines formed flowers but most did not produce runners, and fruit production was low[11]. These findings suggest that this strategy, while holding promise against disease, would benefit from a more nuanced expression approach, perhaps overexpressing the transcript in leaves or fruits only.

Constitutive expression of *AtNPR1* transcripts has also been investigated for effects on plant response to insect feeding[6]. Transgenic tobacco plants expressing *AtNPR1* were challenged with the herbivore *Spodoptera litura*, the common cutworm larvae. *AtNPR1*-tobacco plants were generally more resistant to herbivore feeding and early larval population growth. The increased resistance to *S. litura* was further investigated and a correlation with increased induction of serine protease inhibitors was observed. These compounds can slow down the digestion of ingested plant tissues in the insect gut, severely affecting larval growth[51]. *AtNPR1*-tobacco plants were also more resistant to the root-knot nematode (*M. incognita*)[52]. High *AtNPR1* transcript levels correlated with a 50–60% reduction in root galls and eggs-masses in infected roots and resulted in constitutive expression of *PR* genes. Another study reported that *AtNPR1*-tobacco displayed enhanced oxidative stress tolerance and did not suffer from polyethylene glycol stress[53].

Heterologous expression of *AtNPR1* in various horticultural crops shows the potential to generate transgenic lines with increased broad-spectrum disease and pest resistance in most cases. Although poor public acceptance of genetically engineered plant products remains a challenge, the public may view cisgenic/intragenic approaches more favorably. While the Arabidopsis gene has shown promise in these trials, the native NPR1 may have a more intimate association with the inductive resistance mechanisms and provide better responses without the collateral effects.

AtNPR1 orthologs in agronomic crops

NPR1 appears to be functionally conserved across an array of agronomically relevant crops, spanning a diversity from rice to coconut palm[54]. Orthologs of *AtNPR1* have been cloned and characterized in many crop plants. While tremendous potential exists in horticultural crops, the first breakthrough and best characterization have been on large-scale commodity crops like rice, which is where early translational work began[1]. The closest match, *Os*NPR1/NH1, shares 46% identity and 60% similarity with the *At*NPR1 protein. The true orthology was confirmed when complementing the Arabidopsis *npr1* mutant[8]. NH1 overexpression conferred resistance to the bacterial pathogen *X. oryzae pv. oryzae* and constitutively high accumulation of *PR* transcripts[1], similar to what was observed upon *AtNPR1* overexpression in rice[17]. This finding suggests that NPR1-mediated disease resistance signaling pathways are similar in rice and Arabidopsis. Some negative phenotypes were reported. *NH1* plants displayed smaller stature, had lower fresh weight, and narrower or smaller leaves[1]. Under controlled conditions, transgenic plants had delayed growth and old leaves tended to senesce faster compared to wild-type plants. They also failed to develop additional tillers, spontaneously developed lesion-mimic spots, and showed more obvious HR-like responses after being challenged with *X. oryzae* pv. *oryzae*. NH1 may mediate antagonistic crosstalk between SA- and JA-dependent pathways in rice. Enhanced herbivore susceptibility in transgenic plants was also observed. Together with *NH1*, two additional *AtNPR1* homologous genes were found in rice (*OsNPR2/NH2* and *OsNPR3/NH3*). They were found to be induced by rice bacterial leaf blight (caused by *X. oryzae* pv. *oryzae*), rice blast (caused by *Magnaporthe grisea*), and the defense-related compounds benzothiadiazole (BTH; a synthetic inducer of SAR), methyl jasmonate (MeJA), and ethylene (ET). Chern et al.[55] illustrated through coexpression analyses that *NH1* and *NH3* may have a common role in rice immunity. However, they have different co-expression patterns with *negative regulator of resistance*(*NRR*) *homolog1* (*RH1*) and *RH2* genes, as *NH1* co-expresses with *RH1* and *RH2* while *NH3* exhibits contrasting expression patterns.

A role for *NH1* against rice blast disease was demonstrated using a genome-wide analysis of BTH-responsive genes[56], BTH-inducible blast resistance was compromised in plants where *NH1* transcripts were suppressed, and enhanced in *NH1*-overexpressing rice plants. This study also showed that photosynthetic activities are reduced by SA signaling. The transcripts corresponding to photosynthesis-related genes and chloroplastic ribosomal genes decrease in abundance when the SA signaling pathway is activated. *NH1*-overexpressing rice plants also showed enhanced resistance to *M. oryzae* and increased steady-state transcript levels of defense genes including *PR-1a*[57].

The mustard (*Brassica juncea*) NPR1, *BjNPR1*, was cloned for assessing defense induction patterns upon chemical treatment and powdery mildew infection[58]. The

amino acid sequence of *Bj*NPR1 shows 98% identity to *B. napus* NPR1 (*Bn*NPR1) and 69% identity to AtNPR1. The protein bears all the important functional domains, including the ankyrin repeats and the BTB-POZ domains. *BjNPR1* was constitutively expressed at low levels but was strongly induced by exogenous application of SA and upon *E. cruciferarum* infection. Moreover, complementation tests of *BnNPR1* in Arabidopsis *npr1* mutants showed restored SA-dependent induction of *PR-1* genes and enhanced basal defense, as well as provided systemic acquired resistance against *P. syringae*[44]. Additionally, *AtNPR1* and *BnNPR1* overexpression in transgenic *B. napus* effectively enhanced basal resistance against *P. syringae* with no obvious developmental abnormalities[44], verifying the functional conservation of NPR1 between *A. thaliana* and *B. napus*.

Liu et al.[59] used a virus-induced gene silencing (VIGS) system to test the role of the tobacco *required for MLA12 resistance1*, *EDS1*, and *NPR1* homologous genes in N-mediated resistance to tobacco rattle virus (TRV). Silencing of these genes compromised N function, suggesting that these genes play essential roles in the N-mediated resistance pathway. Further, characterization of cotton NPR1 (*GhNPR1*) suggested that this *AtNPR1* ortholog is critical for activation of plant defense responses[60]. The predicted amino acid sequence exhibited 52.98%, 52.32% and 54.98% similarity to NPR1 from *A. thaliana*, *B. juncea* and *N. tabacum*, respectively. The GhNPR1 protein also contains an ankyrin repeat domain and a BTB/POZ domain, which are highly conserved among all NPR1 proteins and involved in protein–protein interactions[60]. Transcripts of *GhNPR1* could be markedly induced by SA, MeJA, and ET treatment, and by inoculation of *F. oxysporum* f. sp. *vasinfectum* and *X. campestris* pv. *malvacearum*, suggesting that *GhNPR1* is involved in response to biotic and abiotic stresses and may be critical for activation of defense responses in cotton. The soybean *GmNPR1-1* and *GmNPR1-2* genes are orthologous to *AtNPR1* and can enhance broad-spectrum resistance in soybean[61]. Both *GmNPR1* genes complemented the Arabidopsis *npr1-1* mutant and the transgenic plants were able to show induction of *PR* genes following 2,6-dichloroisonicotinic acid treatment and *P. syringae* infection. In addition, soybean plants showed activation of SAR following infection, suggesting the importance of *GmNPR1* for disease resistance in soybean.

An ortholog of *AtNPR1* has been cloned and characterized from peanut (*Arachis hypogeae*) that is mainly expressed in the roots and leaves[62]. While its overexpression in peanut is yet to be performed, co-overexpression of *BjNPR1* and a *defensin* homolog from *Trigunella foenum-graecum* (*Tfgd*) in peanut under control of the 35S promoter lead to resistance to *Aspergillus flavus*, a pathogen that can lead to production of dangerous mycotoxins such as aflatoxin. There was neither mycelial growth in the transgenic plants nor aflatoxin accumulation in their seeds. The transgenic plants also demonstrated varied levels of resistance to *Cercospora arachidicola* with reduced number of spots and delayed onset of disease[14]. This was a novel approach in overexpression of *AtNPR1* orthologs together with other resistance-related genes to obtain more resistance toward pathogens.

AtNPR1 orthologs in horticultural crops

Apple *AtNPR1* orthologs were cloned from *Malus pumila* (*MpNPR1*) or *Malus hupehensis* (*MhNPR1*) and overexpressed in apple (*Malus × domestica*)[5,63,64]. Constitutively high levels of *MpNPR1* enhanced resistance to three important diseases: fire blight (caused by *E. amylovora*), apple scab (caused by *Venturia inaequalis*), and cedar apple rust (caused by *Gymnosporangium juniperi-virginianae*)[5,63,64]. The transgenic plants did not exhibit detrimental morphological or developmental phenotypes, but they did constitutively upregulate *PR* genes. Increased transcript levels of *MhNPR1* in Fuji apple enhanced resistance to powdery mildew (caused by *P. leucotricha*)[64]. In transgenic tobacco *MhNPR1* induced expression of *PR* genes and contributed to salt and osmotic stress tolerance in addition to enhanced resistance to *B. cinerea*[65]. The tolerance to abiotic stress contrasted with what was observed in rice using the *AtNPR1* gene, which negatively affected tolerance to dehydration and salt stress[66]. Table 2 presents this example along with effects and outcomes of *AtNPR1* ortholog overexpression in various crops.

AtNPR1 orthologs were also identified in grapevine (*Vitis vinifera*)[2]. The increased accumulation of *PR1* and *PR2* transcripts by *VvNPR1* both in *N. benthamiana* and *V. vinifera* strongly suggested that *VvNPR1* is also a component of the SA defense signaling pathway in grapevine. *VvNPR1.1* and *VvNPR1.2* are constitutively expressed, but their transcript levels may be increased with BTH treatment. These genes are functional when overexpressed in *N. benthamiana*, triggering the accumulation of *PR1* and *PR2* transcripts. To gain further information on *VvNPR1* activity in grape, the authors transiently expressed *VvNPR1.1* or *AtNPR1* in *V. vinifera* leaves. Interestingly, both *VvNPR1.1* and *AtNPR1* induced a stronger response to *P. viticola* inoculation. Later, Bergeault et al.[67] provided further information about the *Vv*NPR1 proteins. *Vv*NPR1.1 and *Vv*NPR1.2 showed 52% and 37% identity to the AtNPR1 protein, respectively. All functional domains identified in AtNPR1 were conserved in the *Vv*NPR1 proteins.

Another functional characterization of *VvNPR1.1* and *VvNPR1.2*, including complementation of the Arabidopsis *npr1* mutant, confirmed *VvNPR1.1* as a functional ortholog of *AtNPR1*[68]. Transgenic grapevine plants

Table 2 Transgenic crop plants ectopically expressing *AtNPR1* orthologs

Plant species	AtNPR1 ortholog	Defensive activity (caused by)	Abnormal phenotype	Reference
Apple	MpNPR1	Resistance to fire blight (E. amylovora)Resistance to apple scab (V. inaequalis)Resistance to cedar apple rust (G, junipe.ri-virginianae)Resistance to powdery mildew (P. leucotricha)Expression of PR genes	a	Malnoy et al.[5] Malnoy et al.[63] Chen et al.[64]
Apple	MhNPR1	Resistance to powdery mildew (P. leucotricha)Expression of PR genes	a	Chen et al.[64]
Canola	BnNPR1	Resistance to P. syringaeExpression of PR genes	a	Potlakayala et al.[44]
Crabapple	MhNPR1	Tolerance to saltTolerance to osmotic stressResistance to fungus (Botrytis cinerea)	a	Zhang et al.[65,66]
Grapevine	VvNPR1.1VvNPR1.2	Resistance to downy mildew (P. viticola)Resistance to powdery mildew (E. necator)Expression of PR proteins	Loss of apical dominanceDeath during acclimatization	Le Henanff et al.[2a] Bergeault et al.[67]
Lily	LhSorNPR1	Enhanced resistance to P. syringae in tomato and Arabidopsis	a	Wang et al.[78]
Mustard	BjNPR1	Powdery mildew (E. cruciferarum).	a	Meur et al.[58]
Peanut	BjNPR1	Resistance to Aspergillus flavusResistance to Cercospora arachidicola	a	Sundaresha et al.[14]
Potato	StoNPR1	Resistance to Verticillium dahliaeExpression of PR genes	a	Deng-wei et al.[15]
Rice	NH1	Resistance to bacterial blight (X. oryzae pv. oryzae)Resistance to rice blast disease (M. oryzae)hypersensitive -like response after pathogen challengeInduction of benzothiadiazole, methyl jasmonate and ethylene defense moleculesExpression of PR genes	Smaller plantsLower fresh weights and narrower or smaller leavesTendency to precocious leaves senescenceNo development of additional tillersSpontaneously develop lesion-mimic spotsEnhanced herbivore susceptibilityReduction of photosynthetic activities	Chern et al.[1] Yuan et al.[8] Sugano et al.[56] Feng et al.[57a]
Tobacco	NgNPR3	Resistance to A. alternataResistance to P. solanacearumResistance to Potato Virus Y (PVY)	a	Zhang et al.[10]
Wheat	ScNPR1	Rye (Secale cereale cv Jingzhouheimai) version confers resistance to Fusarium Head Blight	a	Yu et al.[79]

[a]No abnormal/no reported phenotype

overexpressing *VvNPR1.1* exhibited enhanced resistance to powdery mildew and induced accumulation of PR proteins. However, a loss of apical dominance related to *VvNPR1.1* overexpression was observed in all independent transformants. When ectopically expressed in the Arabidopsis *npr1-2* mutant, *VvNPR1.1* complemented the mutation, restoring normal plant growth, increasing SA concentration, and enhancing resistance to virulent *P. syringae* pv. *maculicola* infection.

With a similar approach, Zhang et al.[69] demonstrated that overexpressing *VaNPR1.1*, an ortholog of *AtNPR1* in *V. aestivalis* cv. Norton, in Arabidopsis *npr1-1* mutant plants restored the accumulation of the *PR-1* transcript, although not completely. The results also showed that overexpression of *VaNPR1.1* in Arabidopsis plants increased tolerance to salinity, but not drought.

Complementation analyses also confirmed the functional conservation of *AtNPR1* orthologs in other plant species. Shi et al.[70] isolated the *AtNPR1* ortholog from cacao (*Theobroma cacao*) and tested *TcNPR1* for complementation of the Arabidopsis *npr1-2* mutant. *TcNPR1* presented similar functions as *AtNPR1* and was able to partially complement the Arabidopsis *npr1-2* mutation. Like AtNPR1, the TcNPR1 protein was translocated to the nucleus after SA treatment and participated in the induction of *PR* gene expression, confirming its likely parallel role in defense response in cacao.

Similarly, two *AtNPR1*-like genes from banana (*MNPR1A* and *MNPR1B*) were isolated and overexpressed in Arabidopsis *npr1-2* mutant plants[71]. Overexpression of both genes restored the resistance of Arabidopsis *npr1-2* mutants to the biotrophic oomycete *Hyaloperonospora arabidopsidis*, the nectrotrophic fungus *B. cinerea*, and the hemi-biotrophic bacterial pathogen *P. syringae*. While the two genes possess different sequences, they are functionally indiscernible in complementation analyses.

Barsalobres-Cavallari et al.[72] isolated the orthologous gene of *AtNPR1* from *Coffea arabica* (*CaNPR1*), representing a promising candidate for engineering resistance in coffee. Typical features of NPR1 proteins were found in *Ca*NPR1, including the BTB/POZ domain, an ankyrin repeat domain, and a nuclear localization signal. Surprisingly, transcript levels of *CaNPR1* were strongly activated by SA treatment but were not altered after pathogen infection.

The homologous genes, *GhNPR1* and *GhTGA2* from *Gladiolus hybridus*, one of the most economically important orchids, were isolated and functionally characterized[73]. Overexpression of *GhNPR1* in an Arabidopsis *npr1* mutant restored its basal resistance to *P. syringae* pv. *tomato* and silencing of *GhNPR1* resulted in enhanced susceptibility to *Curvularia* leaf spot caused by *Curvularia gladioli*.

Deng-wei et al.[15] reported cloning of a *Solanum tovrum* (a wild eggplant) *NPR1* (*StoNPR1*) gene, which was responsive to salicylic acid and *Verticillium dahliae*. They overexpressed *StoNPR1* in *V. dahliae* sensitive potato plants and the transgenic lines demonstrated more resistance to this pathogen in comparison to wild-type and RNAi expressing lines. Furthermore, the expression of *isochorismate synthase1* (*ICS1*) and *PR1a* genes was discernably higher in *StoNPR1* overexpressing lines.

Other *AtNPR1* orthologs have been cloned and characterized from different crops. However, their functional analysis has yet been performed using gain or loss of function tests. For example, in avocado (*Persea americana*) three out of five *AtNPR1*-like genes showed a possible role in plant defense[74]. In papaya (*Carica papaya*), four homologous genes of *AtNPR1* were identified and their expression pattern in different tissues was evaluated[75].

The tobacco (*Nicotiana glutinosa*) *NPR1* ortholog, *NgNPR3*, activates discrete signal transduction pathways. Zhang et al.[10] showed that transgenic plants overexpressing this gene displayed enhanced resistance to *A. alternata*, *P. solanacearum*, and potato virus Y in a dose-dependent manner. The induction of *PR* genes after pathogen infection was higher in resistant plants and no obvious developmental defects were observed. Expression of the endogenous gene is induced by defense molecules (SA, INA, H_2O_2, and MeJA) and several pathogens (*P. solanacearum*, *P. parasitica*, *R. solani*, and *A. alternata*). The authors also reported that the NgNPR3 protein accumulates in the nucleus in response to SAR activators. The *Ng*NPR3 protein contains an ankyrin repeat domain and a BTB/POZ domain, which are motifs highly conserved among AtNPR1 and AtNPR1-like proteins. Taken together, these observations suggest a role for *NgNPR3* in induced defense responses in tobacco.

In addition to agronomic and horticultural applications, *AtNPR1* orthologs have been isolated and shown to confer effects in trees. Shao et al.[76] isolated two *AtNPR1* homologous genes (*PtNPR1.1* and *PtNPR1.2*) from poplar (*Populus deltoids*). The *PtNPR1.1* and *PtNPR1.2* genes expressed in different tissues and responded to SA and MeJA with different time courses. While a *Pt*NPR1.1-GFP fusion protein was localized to the cytoplasm after being expressed in Arabidopsis mesophyll protoplasts, the authors concluded that these genes are promising candidates for engineering resistance to broad-spectrum pathogens in poplar. In sugarcane, an *AtNPR1* homolog (*ScNPR1*) was cloned and identified. *ScNPR1* was upregulated after SA or *Ustilago scitaminea* infection, but downregulated in response to MeJA or ET. Moreover, a higher accumulation of *ScNPR1* transcripts was found in the leaf and sheath tissues of resistant cultivars to *Ustilago scitaminea*[77].

Conclusion and prospects

High-value horticultural crop plants are constantly threatened by pests and pathogens. The emergence of epidemics, such as greening disease in citrus and laurel wilt in avocado, is a prominent reminder that new strategies are needed for disease tolerance. The loss of fumigants like methyl bromide and the projected phase-out of other fumigants leave fewer options for horticultural crop plant producers, meaning that genetic protection may be more feasible (and environmentally sustainable) than chemical protection.

There is significant interest in understanding the fundamental mechanisms of plant disease resistance, and the NPR1 protein has a well-defined role in this process. Whether through traditional breeding of *NPR1* alleles that affect disease presentation or defense gene expression, or transgenic installation of NPR1-modulating factors, this gene stands central in many designs for enhanced tolerance to biotic (and sometimes abiotic) challenges. There is potential for identification of specific alleles that could potentially be more active in various species, or perhaps pyramiding NPR1-related genes into a common genetic background. These approaches could speed molecular breeding efforts.

But all of these potential solutions must be approached conservatively. SAR induction is a complex system that involves multiple physiological and biochemical mechanisms and a substantial suite of genes. Constitutive activation of defense responses can exert deleterious effects on plant growth and development, and can impinge important processes relevant to crop production. Overexpression of the *AtNPR1* gene or its orthologs has been shown to enhance disease resistance in numerous plant species, including those with substantial commercial value. However, the same overexpressors also presented other negative attributes, such as yield loss, which could be an unacceptable trade-off to its implementation. Thus, understanding the physiological and molecular mechanisms of how resistance is elicited, and under which condition the resistance expression becomes deleterious for fitness, would greatly help crop disease resistance improvement.

Author details
[1]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA. [2]Department of Horticultural Sciences, University of Tabriz, Tabriz, Iran. [3]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA. [4]Graduate Program in Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL 32611, USA

References
1. Chern, M., Fitzgerald, H. A., Canlas, P. E., Navarre, D. A. & Ronald, P. C. Overexpression of a rice NPR1 homolog leads to constitutive activation of defense response and hypersensitivity to light. *Mol. Plant Microbe Interact.* **18**, 511–520 (2005).
2. Le Henanff, G. et al. Characterization of Vitis vinifera NPR1 homologs involved in the regulation of pathogenesis-related gene expression. *BMC Plant Biol.* **9**, 54 (2009).
3. Lin, W.-C. et al. Transgenic tomato plants expressing the Arabidopsis NPR1 gene display enhanced resistance to a spectrum of fungal and bacterial diseases. *Transgenic Res.* **13**, 567–581 (2004).
4. Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N. & Shah, J. Genetically engineered resistance to Fusarium head blight in wheat by expression of Arabidopsis NPR1. *Mol. Plant Microbe Interact.* **19**, 123–129 (2006).
5. Malnoy, M., Jin, Q., Borejsza-Wysocka, E. E., He, S. Y. & Aldwinckle, H. S. Overexpression of the apple MpNPR1 gene confers increased disease resistance in malus × domestica. *Mol. Plant Microbe Interact.* **20**, 1568–1580 (2007).
6. Meur, G. et al. Constitutive expression of Arabidopsis NPR1 confers enhanced resistance to the early instars of Spodoptera litura in transgenic tobacco. *Physiol. Plant* **133**, 765–775 (2008).
7. Wally, O., Jayaraj, J. & Punja, Z. Broad-spectrum disease resistance to necrotrophic and biotrophic pathogens in transgenic carrots (Daucus carota L.) expressing an Arabidopsis NPR1 gene. *Planta* **231**, 131–141 (2009).
8. Yuan, Y. et al. Functional analysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. *Plant Biotechnol. J.* **5**, 313–324 (2007).
9. Zhang, X. et al. Over-expression of the Arabidopsis NPR1 gene in citrus increases resistance to citrus canker. *Eur. J. Plant Pathol.* **128**, 91–100 (2010).
10. Zhang, Y. et al. Identification of a novel NPR1-like gene from Nicotiana glutinosa and its role in resistance to fungal, bacterial and viral pathogens. *Plant Biol.* **12**, 23–34 (2010).
11. Silva, K., Brunings, A., Peres, N., Mou, Z. & Folta, K. The Arabidopsis NPR1 gene confers broad-spectrum disease resistance in strawberry. *Transgenic Res.* **24**, 1–12 (2015).
12. Matthews, B. et al. Arabidopsis genes, AtNPR1, AtTGA2 and AtPR-5, confer partial resistance to soybean cyst nematode (Heterodera glycines) when overexpressed in transgenic soybean roots. *BMC Plant Biol.* **14**, 96 (2014).
13. Youssef, R. M. & Matthews, B. F. Expression of Arabidopsis genes *AtNPR1* and *AtTGA2* in transgenic soybean roots of composite plants confers resistance to root-knot nematode (*Meloidogyne incognita*). *Int. J. Curr. Biotechnol.* **1**, 15–25 (2013).
14. Sundaresha, S. et al. Co-overexpression of Brassica juncea NPR1 (BjNPR1) and Trigonella foenum-graecum defensin (Tfgd) in transgenic peanut provides comprehensive but varied protection against Aspergillus flavus and Cercospora arachidicola. *Plant Cell Rep.* **35**, 1189–1203 (2016).
15. Deng-wei, J., Min, C. & Qing, Y. Cloning and characterization of a Solanum torvum NPR1 gene involved in regulating plant resistance to Verticillium dahliae. *Acta Physiol. Plant* **36**, 2999–3011 (2014).
16. Cao, H., Li, X. & Dong, X. Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. *Proc. Natl Acad. Sci. USA* **95**, 6531–6536 (1998).
17. Chern, M.-S. et al. Evidence for a disease-resistance pathway in rice similar to the NPR1-mediated signaling pathway in Arabidopsis. *Plant J.* **27**, 101–113 (2001).
18. Friedrich, L. et al. NIM1 overexpression in Arabidopsis potentiates plant disease resistance and results in enhanced effectiveness of fungicides. *Mol. Plant Microbe Interact.* **14**, 1114–1124 (2001).
19. Chisholm, S. T., Coaker, G., Day, B. & Staskawicz, B. J. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* **124**, 803–814 (2006).

20. Dangl, J. L. & Jones, J. D. G. Plant pathogens and integrated defence responses to infection. *Nature* **411**, 826–833 (2001).

21. Agrios, G. N. *Plant Pathology* 5th edn (Elsevier Academic Press, Amsterdam, 2005).

22. Reina-Pinto, J. J. & Yephremov, A. Surface lipids and plant defenses. *Plant Physiol. Biochem.* **47**, 540–549 (2009).

23. Dai, X. et al. TrichOME: a comparative omics database for plant trichomes. *Plant Physiol.* **152**, 44–54 (2010).

24. Peters, K. M. & Berry, R. E. Effect of hop leaf morphology on two spotted spider mite. *J. Econ. Entomol.* **73**, 235–238 (1980).

25. War, A. R. et al. Mechanisms of plant defense against insect herbivores. *Plant Signal Behav.* **7**, 1306–1320 (2012).

26. Dixon, R. A. Natural products and plant disease resistance. *Nature* **411**, 843–847 (2001).

27. Jones, J. D. G. & Dangl, J. L. The plant immune system. *Nature* **444**, 323–329 (2006).

28. Zipfel, C. Early molecular events in PAMP-triggered immunity. *Curr. Opin. Plant Biol.* **12**, 414–420 (2009).

29. Boller, T. & He, S. Y. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science* **324**, 742–744 (2009).

30. Durrant, W. E. & Dong, X. Systemic acquired resistance. *Annu. Rev. Phytopathol.* **42**, 185–209 (2004).

31. Vlot, A. C., Klessig, D. F. & Park, S. W. Systemic acquired resistance: the elusive signal(s). *Curr. Opin. Plant Biol.* **11**, 436–442 (2008).

32. An, C. & Mou, Z. Salicylic acid and its function in plant immunity. *J. Integr. Plant Biol.* **53**, 412–428 (2011).

33. Dong, X. N. P. R. 1 all things considered. *Curr. Opin. Plant Biol.* **7**, 547–552 (2004).

34. Pirnia, S. *Novel Plant DNA Binding Protein: Non-expresser of Pathogenesis Related 1 Gene (NPR1) Involved in Disease Resistance*. Doctoral thesis, Brock University (2016).

35. Zhang, Y., Fan, W., Kinkema, M., Li, X. & Dong, X. Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene. *Proc. Natl Acad. Sci. USA* **96**, 6523–6528 (1999).

36. Fu, Z. Q. & Dong, X. Systemic acquired resistance: turning local infection into global defense. *Annu Rev. Plant Biol.* **64**, 839–863 (2013).

37. Fan, W. & Dong, X. In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. *Plant Cell* **14**, 1377–1389 (2002).

38. Subramaniam, R., Desveaux, D., Spickler, C., Michnick, S. W. & Brisson, N. Direct visualization of protein interactions in plant cells. *Nat. Biotechnol.* **19**, 769–772 (2001).

39. Fitzgerald, H. A., Chern, M.-S., Navarre, R. & Ronald, P. C. Overexpression of (At) NPR1 in rice leads to a BTH- and environment-induced lesion-mimic/cell death phenotype. *Mol. Plant Microbe Interact.* **17**, 140–151 (2004).

40. Quilis, J., Penas, G., Messeguer, J., Brugidou, C. & San Segundo, B. The Arabidopsis AtNPR1 inversely modulates defense responses against fungal, bacterial, or viral pathogens while conferring hypersensitivity to abiotic stresses in transgenic rice. *Mol. Plant Microbe Interact.* **21**, 1215–1231 (2008).

41. Dempsey, D. M. A., Shah, J. & Klessig, D. F. Salicylic acid and disease resistance in plants. *Crit. Rev. Plant Sci.* **18**, 547–575 (1999).

42. Molla, K. A. et al. Tissue-specific expression of Arabidopsis NPR1 gene in rice for sheath blight resistance without compromising phenotypic cost. *Plant Sci.* **250**, 105–114 (2016).

43. Gao, C. S. et al. Inverse effects of Arabidopsis NPR1 gene on fusarium seedling blight and fusarium head blight in transgenic wheat. *Plant Pathol.* **62**, 383–392 (2013).

44. Potlakayala, S. D., DeLong, C., Sharpe, A. & Fobert, P. R. Conservation of non-expressor of pathogenesis-related genes1 function between Arabidopsis thaliana and Brassica napus. *Physiol. Mol. Plant Pathol.* **71**, 174–183 (2007).

45. Kumar, V., Joshi, S., Bell, A. & Rathore, K. Enhanced resistance against Thielaviopsis basicola in transgenic cotton plants expressing Arabidopsis NPR1 gene. *Transgenic Res.* **22**, 359–368 (2013).

46. Parkhi, V., Kumar, V., Campbell, L. A. M., Bell, A. A. & Rathore, K. S. Expression of Arabidopsis NPR1 in transgenic cotton confers resistance to non-defoliating isolates of verticillium dahliae but not the defoliating isolates. *J. Phytopathol.* **158**, 822–825 (2010).

47. Parkhi, V. et al. Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing Arabidopsis NPR1. *Transgenic Res.* **19**, 959–975 (2010).

48. Boscariol-Camargo, R. L., Takita, M. A. & Machado, M. A. Bacterial resistance in AtNPR1 transgenic sweet orange is mediated by priming and involves EDS1 and PR2. *Trop. Plant Pathol.* **41**, 341–349 (2016).

49. Li, Z. T., Jayasankar, S. & Gray, D. Bi-directional duplex promoters with duplicated enhancers significantly increase transgene expression in grape and tobacco. *Transgenic Res.* **13**, 143–154 (2004).

50. Dutt, M., Barthe, G., Irey, M. & Grosser, J. Transgenic citrus expressing an Arabidopsis NPR1 gene exhibit enhanced resistance against huanglongbing (HLB; Citrus Greening). *PLoS ONE* **10**, e0137134 (2015).

51. Heath, R. L. et al. Proteinase inhibitors from Nicotiana alata enhance plant resistance to insect pests. *J. Insect Physiol.* **43**, 833–842 (1997).

52. Priya, D. B., Somasekhar, N., Prasad, J. & Kirti, P. Transgenic tobacco plants constitutively expressing Arabidopsis NPR1 show enhanced resistance to root-knot nematode, Meloidogyne incognita. *BMC Res. Notes* **4**, 231 (2011).

53. Srinivasan, T., Rajesh Kumar, K. R., Meur, G. & Kirti, P. B. Heterologous expression of Arabidopsis NPR1 (AtNPR1) enhances oxidative stress tolerance in transgenic tobacco plants. *Biotechnol. Lett.* **31**, 1343–1351 (2009).

54. Nic-Matos, G., Narváez, M., Peraza-Echeverría, S., Sáenz, L. & Oropeza, C. Molecular cloning of two novel NPR1 homologue genes in coconut palm and analysis of their expression in response to the plant defense hormone salicylic acid. *Genes Genomics* **39**, 1007–1019 (2017).

55. Chern, M. et al. Interaction specificity and coexpression of rice NPR1 homologs 1 and 3 (NH1 and NH3), TGA transcription factors and negative regulator of resistance (NRR) proteins. *BMC Genomics* **15**, 461 (2014).

56. Sugano, S. et al. Role of OsNPR1 in rice defense program as revealed by genome-wide expression analysis. *Plant Mol. Biol.* **74**, 549–562 (2010).

57. Feng, J.-X. et al. Involvement of OsNPR1/NH1 in rice basal resistance to blast fungus Magnaporthe oryzae. *Eur. J. Plant Pathol.* **131**, 221–235 (2011).

58. Meur, G., Budatha, M., Dutta Gupta, A., Prakash, S. & Kirti, P. B. Differential induction of NPR1 during defense responses in Brassica juncea. *Physiol. Mol. Plant Pathol.* **68**, 128–137 (2006).

59. Liu, Y., Schiff, M., Marathe, R. & Dinesh-Kumar, S. P. Tobacco Rar1, EDS1 and NPR1/NIM1 like genes are required for N-mediated resistance to tobacco mosaic virus. *Plant J.* **30**, 415–429 (2002).

60. Zhang, Y. et al. Molecular cloning and characterization of GhNPR1, a gene implicated in pathogen responses from cotton (Gossypium hirsutum L.). *Biosci. Rep.* **28**, 7–14 (2008).

61. Sandhu, D., Tasma, I. M., Frasch, R. & Bhattacharyya, M. K. Systemic acquired resistance in soybean is regulated by two proteins, Orthologous to Arabidopsis NPR1. *BMC Plant Biol.* **9**, 105 (2009).

62. Wu, Q. et al. Molecular cloning and characterization of NPR1 gene from Arachis hypogaea. *Mol. Biol. Rep.* **41**, 5247–5256 (2014).

63. Malnoy, M., Borejsza-Wysocka, M. M., Aldwinckle, H. S. & Jin, Q. L. & HeS. Y. Transgenic apple lines over-expressing the apple gene MpNPR1 have increased resistance to fire blight. *Acta Hort.* **704**, 521–526 (2006).

64. Chen, X.-K. et al. Overexpressing MhNPR1 in transgenic Fuji apples enhances resistance to apple powdery mildew. *Mol. Biol. Rep.* **39**, 8083–8089 (2012).

65. Zhang, J. Y. et al. Malus hupehensis NPR1 induces pathogenesis-related protein gene expression in transgenic tobacco. *Plant Biol.* **14**(Suppl 1), 46–56 (2012).

66. Zhang, J.-Y., Qu, S.-C., Qiao, Y.-S., Zhang, Z. & Guo, Z.-R. Overexpression of the Malus hupehensis MhNPR1 gene increased tolerance to salt and osmotic stress in transgenic tobacco. *Mol. Biol. Rep.* **41**, 1553–1561 (2014).

67. Bergeault, K., Bertsch, C., Merdinoglu, D. & Walter, B. Low level of polymorphism in two putative NPR1 homologs in the Vitaceae family. *Biol. Direct.* **5**, 9–9 (2010).

68. Le Henanff, G. et al. Vitis vinifera VvNPR1.1 is the functional ortholog of AtNPR1 and its overexpression in grapevine triggers constitutive activation of PR genes and enhanced resistance to powdery mildew. *Planta* **234**, 405–417 (2011).

69. ZHANG, Y.-m., . & NiX.-l. & QiuW. Characterization of NPR1 genes from Norton and Cabernet sauvignon grapevine. *J. Integr. Agric.* **12**, 1152–1161 (2013).

70. Shi, Z., Maximova, S. N., Liu, Y., Verica, J. & Guiltinan, M. J. Functional analysis of the Theobroma cacao NPR1 gene in Arabidopsis. *BMC Plant Biol.* **10**, 248 (2010).

71. Yocgo, R. E., Creissen, G., Kunert, K. & Chikwamba, R. Two different banana NPR1-like coding sequences confer similar protection against pathogens in Arabidopsis. *Trop. Plant Biol.* **5**, 309–316 (2012).

72. Barsalobres-Cavallari, C. F., Petitot, A. S., Severino, F. E., Maia, I. G. & Fernandez, D. In *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education* Vol. 1 (ed A. Méndez-Vilas) 774 (Formatex Research Center, Badajoz, 2013).

73. Zhong, X. et al. The NPR1 homolog GhNPR1 plays an important role in the defense response of Gladiolus hybridus. *Plant Cell Rep.* **34**, 1063–1074 (2015).

74. Backer, R. et al. Phylogenetic and expression analysis of the NPR1-like gene family from Persea americana (Mill.). *Front. Plant Sci.* **6**, 300 (2015).

75. Peraza-Echeverria, S. et al. The NPR1 family of transcription cofactors in papaya: insights into its structure, phylogeny and expression. *Genes Genomics* **34**, 379–390 (2012).

76. Shao, Y., Zhang, H., He, H., Cheng, B. & Xiang, Y. Molecular cloning and characterization of orthologues of NPR1 gene from poplar. *J. Phytopathol.* **161**, 35–42 (2013).

77. Chen, J.-W. et al. Molecular cloning and expression analysis of a npr1 gene from sugarcane. *Pak. J. Bot.* **44**, 193–200 (2012).

78. Wang, L. et al. Overexpression of LhSorNPR1, a NPR1-like gene from the oriental hybrid lily 'Sorbonne', conferred enhanced resistance to Pseudomonas syringae pv. tomato DC3000 in Arabidopsis. *Physiol. Mol. Biol. Plants* **23**, 793–808 (2017).

79. Yu, G., Zhang, X., Yao, J., Zhou, M. & Ma, H. Resistance against Fusarium head blight in transgenic wheat plants expressing the ScNPR1 gene. *J. Phytopathol.* **165**, 223–231 (2017).