NPR1 as a transgenic crop protection strategy in horticultural species

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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 potato1–15. 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 resistance16–18.
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. 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. 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. A successful infection requires breaking initial barriers and efficiently countering a cascade of plant defense responses generated by a suite of mobilized biochemical activities.

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 as well as Zipfel. 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 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.

**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. 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. 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, which in turn upregulate the transcription of PR genes, and help confer resistance to secondary infection.

**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., 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. 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. Transgenic rice exhibited lesion-mimic cell death, hydrogen peroxide (H2O2) accumulated significantly around lesions, and plants accumulated lower levels of free SA. These phenotypes were heritable and
positively correlated with steady-state transcript levels of the AtNPR1 transgene and endogenous rice defense genes. Quilis et al. 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, 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

| Plant species | Resistance/tolerance (caused by) | Abnormal phenotype | Reference |
|---------------|----------------------------------|-------------------|-----------|
| Canola        | Bacterial disease (P.syringae)    | a                 | Potlakayala et al. |
| Carrot        | Sclerotina rot (S. sclerotiorum)Gray mold (B. cinerea)Black rot (A. radicina)Bacterial leaf blight (X. oryzae pv. oryzae)Powdery mildew (Erysiphe heracle) | a | Wally et al. |
| Citrus        | Citrus canker (X. citri ssp. citri)Citrus greening (Huanglongbing) | a | Zhang et al. |
| Cotton        | Verticillium wilt (V. dahliae)Fusarium wilt (F. oxysporum f. sp. vasinfectum)Seedling damping-off (R. solani)Alt ermaria leaf spot (A. alternata)Nematode (R. reniformis)Black root rot (T. basicola) | a | Kumar et al. |
| Rice          | Bacterial blight (X. oryzae pv. oryzae)Bacterial leaf blight (M. oryzae)Bake naka disease (F. verticillioides)Bacterial foot rot (E. chrysanthemi)Sheath blight (R. solani) | Lesion-mimic cell deathAccumulation of H2O2 around lesionsSusceptibility to the rice yellow motte virusGrowth retardation, reduced height and smaller seeds, with consequent lower productivity, and development of spontaneous lesions in controlled conditions | Chern et al. Fitzgerald et al. McGlade et al. Qulis et al. Molla et al. |
| Soybean       | Root-knot nematodes (Meloidogyne spp.)Cyst nematode (H. glycines) | a | Yousef et al. Matthews et al. |
| 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. |
| Tobacco       | Common cutworm larvae (S. litura)Root-rot nematode (M. incognita)Polyethylene glycol and oxidative stress tolerance | a | Meur et al. Priya et al. Srinivasan et al. |
| 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.46 |

*No abnormal/no reported phenotype

Table 1 Transgenic crop plants ectopically expressing AtNPR1
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	extsuperscript{22}.

*AtNPR1* was also ectopically expressed in wheat in order to assess effects on disease resistance	extsuperscript{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	extsuperscript{39}. *PRI* 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.	extsuperscript{43}.

*AtNPR1*-expressing canola (*Brassica napus* cv. Westar) plants displayed resistance to *Pseudomonas syringae* spp.). 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	extsuperscript{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.	extsuperscript{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	extsuperscript{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	extsuperscript{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	extsuperscript{45–47}. *AtNPR1*-expressing cotton lines were resistant to the fungi *V. dahliae*	extsuperscript{36}, *F. oxysporum* f. sp. *vasiuesfectum*, *R. solani*, and *A. alternata* and to the nematode, *Rotylenchulus reniformis*	extsuperscript{47}. The transgenic plants displayed normal growth and development but showed typical discoloration symptoms. *AtNPR1*-expressing cotton also showed resistance to *Thielaviopsis basiocola*, the causal agent of black root rot	extsuperscript{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 *LIPIDOXYGENASE1*.

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.	extsuperscript{9} showed that *AtNPR1* in “Duncan” grapefruit and “Hamlin” sweet orange conferred increased resistance to citrus canker (caused by *X. citri* sp. *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 Boscarel-Camargo et al.	extsuperscript{48}. The most conspicuous effect was an increase in the accumulation of enhanced disease susceptibility1 (*EDSI*), *PRI* and *PR2* transcripts 12–24 h after inoculation by *Xcc*. This induction was more noticeable for *PRI*, 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 *EDSI* 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	extsuperscript{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.

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. 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. 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. AtNPR1-tobacco plants were also more resistant to the root-knot nematode (M. incognita). 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.

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 crops. 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. 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. The closest match, OsNPR1/NH1, shares 46% identity and 60% similarity with the AtNPR1 protein. The true orthology was confirmed when complementing the Arabidopsis npr1 mutant. NH1 overexpression conferred resistance to the bacterial pathogen X. oryzae pv. oryzae and constitutively high accumulation of PR transcripts, similar to what was observed upon AtNPR1 overexpression in rice. 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. Under controlled conditions, transgenic plants had delayed growth and old leaves tended to senescce 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 cross-talk 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. illustrated through co-expression 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) homolog 1 (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. 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.

The mustard (Brassica juncea) NPR1, BjNPR1, was cloned for assessing defense induction patterns upon chemical treatment and powdery mildew infection. The
The amino acid sequence of BnNPR1 shows 98% identity to B. napus NPR1 (BnNPR1) and 69% identity to AtNPR1. The protein bears all the important functional domains, including the ankyrin repeats and the BTB-POZ domains. BnNPR1 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. Additionally, AtNPR1 and BnNPR1 overexpression in transgenic B. napus effectively enhanced basal resistance against P. syringae with no obvious developmental abnormalities, 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 resistance 1, EDS 1, 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. 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. 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. 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 hypogaea) that is mainly expressed in the roots and leaves. While its overexpression in peanut is yet to be performed, co-overexpression of BjNPR1 and a defensin homolog from Trigonella 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 arachidica with reduced number of spots and delayed onset of disease. 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 punilla (MpsNPR1) or Malus hupehensis (MhNPR1) and overexpressed in apple (Malus domestica). Constitutively high levels of MpsNPR1 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 juniperovirginianae). 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). 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. 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. 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). 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. provided further information about the VvNPR1 proteins. VvNPR1.1 and VvNPR1.2 showed 52% and 37% identity to the AtNPR1 protein, respectively. All functional domains identified in AtNPR1 were conserved in the VvNPR1 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. 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) | a                   | Malnoy et al. 5, Malnoy et al. 63, Chen et al. 64 |
| Apple         | MhNPR1          | Resistance to powdery mildew (P. leucotricha) | a                   | Chen et al. 64 |
| Canola        | BnNPR1          | Resistance to P. syringae | a                   | Potlakayala et al. 14 |
| Crabapple     | MhNPR1          | Tolerance to salt stress | a                   | Zhang et al. 65, 66 |
| Grapevine     | VAPR1.1/VvNPR1.2 | Resistance to powdery mildew (E. necator) | a                   | Le Henaff et al. 73, Bergeault et al. 67 |
| Lily          | LhsNPR1         | 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 flavus | a                   | Sundaresha et al. 14 |
| Potato         | StoNPR1         | Resistance to Verticillium dahliae | a                   | Deng-wei et al. 55 |
| Rice           | NH1             | Resistance to bacterial blight (X. oryzae pv. oryzae) | a                   | Chern et al. 1, Yuan et al. 8, Sugano et al. 56, Feng et al. 27a |
| Tobacco       | NgNPR3          | Resistance to A. alternata | a                   | Zhang et al. 16 |
| Wheat          | ScNPR1          | Rye (Secale cereale cv Jingzhouheima) version confers resistance to | a                   | Yu et al. 79 |

*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 transormants. 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 npr1-1 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 plants71. 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 CaNPR1, 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 hybrids, one of the most economically important orchids, were isolated and functionally characterized73. 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 discernibly 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 defense74. In papaya (Carica papaya), four homologous genes of AtNPR1 were identified and their expression pattern in different tissues was evaluated75.

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, H2O2, 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 NgNPR3 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 (PtNPRI.1 and PtNPRI.2) from poplar (Populus deltoides). The PtNPRI.1 and PtNPRI.2 genes expressed in different tissues and responded to SA and MeJA with different time courses. While a PtNPRI.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 scitaminea77.
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.

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Conflict of interest

The authors declare that they have no conflict of interest.

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