QQS orphan gene and its interactor NF-YC4 reduce susceptibility to pathogens and pests

Mingsheng Qi1,a, Wenguang Zheng2,a, Xuefeng Zhao3, Jessica D. Hohenstein4, Yuba Kandel1, Seth O’Conner2,5, Yifan Wang6, Chuanlong Du6, Dan Nettleton6, Gustavo C. MacIntosh4, Gregory L. Tylka1, Eve S. Wurtele2,6, Steven A. Whitham1 and Ling Li2,5,7,*

1Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, USA
2Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA
3Laurence H. Baker Center for Bioinformatics and Biological Statistics, Iowa State University, Ames, IA, USA
4Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA, USA
5Department of Biological Sciences, Mississippi State University, Starkville, MS, USA
6Department of Statistics, Iowa State University, Ames, IA, USA
7Center for Metabolic Biology, Iowa State University, Ames, IA, USA

Received 22 March 2018; accepted 4 June 2018.
*Correspondence (Tel 1 662-325-7570; fax 1 662-325-7939; email liling@biology.msstate.edu)

Keywords: carbon and nitrogen partitioning, NF-YC4, pathogen, pest, orphan, QQS.

Summary

Enhancing the nutritional quality and disease resistance of crops without sacrificing productivity is a key issue for developing varieties that are valuable to farmers and for simultaneously improving food security and sustainability. Expression of the Arabidopsis thaliana species-specific AtQQS (Qua-Quine Starch) orphan gene or its interactor, NF-YC4 (Nuclear Factor Y, subunit C4), has been shown to increase levels of leaf/seed protein without affecting the growth and yield of agronomic species. Here, we demonstrate that overexpression of AtQQS and NF-YC4 in Arabidopsis and soybean enhances resistance/reduces susceptibility to viruses, bacteria, fungi, aphids and soybean cyst nematodes. A series of Arabidopsis mutants in starch metabolism were used to explore the relationships between QQS expression, carbon and nitrogen partitioning, and defense. The enhanced basal defenses mediated by QQS were independent of changes in protein/carbohydrate composition of the plants. We demonstrate that either AtQQS or NF-YC4 overexpression in Arabidopsis and in soybean reduces susceptibility of these plants to pathogens/pests. Transgenic soybean lines overexpressing NF-YC4 produce seeds with increased protein while maintaining healthy growth. Pull-down studies reveal that QQS interacts with human NF-YC, as well as with Arabidopsis NF-YC, and indicate two QQS binding sites near the NF-YC-histone-binding domain. A new model for QQS interaction with NF-YC is speculated. Our findings illustrate the potential of QQS and NF-YC4 to increase protein and improve defensive traits in crops, overcoming the normal growth-defense trade-offs.

Introduction

Crop plants grow in dynamic environments that abound with challenges. Plants have evolved highly sophisticated immune systems to detect and defend themselves from pathogens/pests (Chisholm et al., 2006; Jones and Dangl, 2006), yet, most economically important crops incur significant yield losses due to diseases (Wulff et al., 2011). Genetic resistance is ideal as an approach to combat disease because it naturally protects crops without additional chemical or mechanical inputs by farmers. To protect crops from a devastating disease, resistant lines are often developed that utilize single genes conferring high levels of resistance to the specific pest or pathogen (Wulff et al., 2011).

Broad-spectrum resistance to multiple pathogens would be an extremely valuable trait. It has been achieved by inducing constitutively active defense responses; however, maintaining constitutive defenses is often energetically costly, impairing plant growth and yield (Bolton, 2009; Heil et al., 2000). For example, after infection by avirulent isolates of powdery mildew that trigger immune responses, seeds of barley have decreased weight and protein content (Smedegaard-Petersen and Stolen, 1981).

Also, in addition to being resistant to a variety of pathogens, Arabidopsis mutants with constitutively active immunity such as the defense no death (dnd1) and constitutive PR gene expression (cpr) lines display a dwarfed morphology (Bowling et al., 1997; Clarke, 1998; Clough et al., 2000; Genger et al., 2008). Silencing the expression of MAP kinase 4 (MPK4) in soybean, which constitutively activates salicylic acid (SA)-based defenses, results in plants that develop spontaneous necrosis and are severely stunted, in addition to being more resistant to pathogens (Liu et al., 2011). In contrast, mutants with suppressed immune systems exhibit increased fitness under pathogen-free conditions, growing taller and producing more seeds than wild-type (WT) plants (Heil and Baldwin, 2002). This ‘growth-defense trade-off’ phenomenon is the current paradigm, and novel strategies are needed to maximize crop fitness while enhancing broad-spectrum defense. Genes that limit the growth of diverse plant pathogens/pests without impairing crop growth and yield could provide new traits for breeding resilient crops (Huot et al., 2014). Each sequenced species, prokaryote or eukaryote, contains protein-coding genes that are unique to that particular species (orphan genes) (Arendsee et al., 2014; Carvunis et al., 2012;
Gollery et al., 2006; Wissler et al., 2013). Little is known about the functional significance of the vast majority of orphan genes (Arendsee et al., 2014). Qua Quine Starch (QQS, At3g30720) is an Arabidopsis thaliana orphan gene, encoding a small protein of only 59 aa with no known functional/structural motifs. It has no homology with proteins of genes of other species, including those of A. lyrata and A. halereii (Li et al., 2009, 2015). QQS regulates carbon and nitrogen partitioning to starch and protein in Arabidopsis, and also in the leaves and seeds of transgenic QQS-expressing soybean, corn and rice (Li and Wurtele, 2015b; Li et al., 2009, 2015). Transgenic plants with perturbed QQS expression have altered starch and protein accumulation but normal development and morphology. Overexpression of QQS increases protein content and decreases starch content, while down-regulation of QQS decreases protein and increases starch (Li and Wurtele, 2012, 2015a,b; Li et al., 2009, 2015).

QQS interacts with the Nuclear Factor Y subunit C4 protein (NF-YC4; At5g63470) (Li et al., 2015), a subunit of the heterotrimeric NF-YA/NF-YB/NF-YC transcription factor, which is conserved across eukaryotes (Laloum et al., 2013; Nardini et al., 2013). NF-Ys comprise a large family in plant species, with a total of 30 NF-Ys (10 NF-YCs) in Arabidopsis (Laloum et al., 2013; Li et al., 2015; Petroni et al., 2012). Although AtNF-YC4-QQS interaction has been demonstrated by several methods in vitro and in vivo (Li et al., 2015), it has not been ruled out that QQS does not interact with other plant NF-YCs, or indeed other plant molecules. However, overexpression of AtNF-YC4 in Arabidopsis mimics the QQS-overexpression phenotype, increasing protein and decreasing starch (Li et al., 2015). Similar to the consequence of QQS overexpression, the overexpression of NF-YC4 does not impact morphology/yield (Li and Wurtele, 2015b; Li et al., 2009, 2015).

One major function of orphan genes may be to increase the survival of organisms in new environments (Arendsee et al., 2014; Carvunis et al., 2012; Lacombe et al., 2010; Li et al., 2009; Luhua et al., 2013; Wissler et al., 2013). Reflective of this general concept about orphan genes and because the level of QQS mRNA is responsive to multiple abiotic and biotic stresses (Arendsee et al., 2014; Li and Wurtele, 2015b; Li et al., 2009), we postulated that QQS might play a role in plant responses to pathogens/pests, in addition to its established role in carbohydrate and protein composition. A function for QQS in plant defense would be consistent with our hypothesis that this orphan gene improved Arabidopsis’ ability to adapt to changing biotic stresses (Arendsee et al., 2014).

To determine whether QQS and NF-YC4 might function in plant defense, we examined existing and new transcriptomic data from Arabidopsis subjected to varying biotic conditions or with perturbed QQS levels, identifying that expression of QQS and NF-YC4 is altered in response to biotic stresses, and changes in QQS expression can alter expression of genes involved in plant responses to pathogens/herbivores/abiotic stresses. These results led us to evaluate the susceptibility to pathogens/pests of Arabidopsis lines that had overexpression/down-regulation/loss-of-function of QQS or NF-YC4 (Li and Wurtele, 2015b; Li et al., 2009, 2015), and mutants with different combinations of levels of starch accumulation and expression of QQS (Delvalle et al., 2005; Lu et al., 2008; Wattebled et al., 2008; Zhang et al., 2005). We further determined whether soybean lines that express QQS (Li and Wurtele, 2015b; Li et al., 2015) or overexpress the soybean homolog of NF-YC4 also had altered susceptibility. The resultant data show that expression of QQS or overexpression of its interacting partner NF-YC4 can confer broad-spectrum defense while maintaining normal growth. Further, we show that an interaction between QQS and NF-YCs can extend to a protein as evolutionarily divergent as human NF-YC and explore the segment of QQS associated with this interaction. We used a computational modelling approach and proposed binding sites for QQS peptides at the N-terminus of NF-YC near the histone-binding domain. We confirmed these binding sites experimentally and speculated a model that QQS interacts with NF-YC.

Our results demonstrate that it is possible to simultaneously enhance protein content and defense responses without impairing plant growth; this is true for A. thaliana, the species that naturally contains QQS, and for soybean, an important crop plant that has no QQS homolog. This ability of QQS and NF-YC4 to regulate the allocation of carbon and nitrogen as well as promote plant defense indicates that it may participate in a regulatory hub that bypasses the trade-off between plant growth and defense.

Results

QQS and NF-YC4 mRNA expressions are altered in response to biotic stresses

To examine the expression of QQS and NF-YC4 in response to pathogens, we analysed published transcriptomic data sets in which Arabidopsis plants had been inoculated with a virus, bacterium or oomycete. At 120 h after inoculation (HAI) with TuMV-GFP (Turnip mosaic virus expressing GFP) (Yang et al., 2007), the QQS transcript level was significantly decreased in tissues near the centre of fluorescent GFP foci (zones 0 and 1) (P = 0.01, 0.04) in which Arabidopsis plants had the most accumulation (Figure S1a). No significant changes were detected for the NF-YC4 transcript; however, NF-YC4 expression was near background levels in these samples, making it difficult to determine whether TuMV-GFP affected its expression. Arabidopsis Col-0 plants are susceptible to the bacterial pathogen Pseudomonas syringae pv. tomato (Pto) strain DC3000 (Thimony et al., 2006). QQS transcript level was significantly reduced in plants infected with Pto DC3000 at 7 (P = 0.001) and 24 HAI (P = 0.01), while NF-YC4 transcript level was not significantly affected, although they trend downward at both time points (Figure S1b). In an oomycete (Phytophthora infestans, causing a nonhost resistance response in Arabidopsis) infection experiment (Figure S1c), there were no significant differences in QQS expression between inoculated and mock-inoculated plants, although there appeared to be a trend in which QQS was initially down-regulated at 6 HAI but not at later time points (12 and 24 HAI). NF-YC4 was initially down-regulated at 6 HAI (P < 0.001), but by 24 HAI, its mRNA expression was similar in inoculated and mock-inoculated plants. Taken together, the altered expression of QQS, and to a lesser extent NF-YC4, in response to diverse pathogens is consistent with a model in which QQS plays a role in plant–microbe interactions. Based on these observations, we hypothesized that QQS and NF-YC4 may regulate the expression of defense genes and/or the susceptibility of Arabidopsis to pathogens and possibly pests.

To determine if and how QQS affects expression of defense and other genes under control conditions, RNA-Seq (sequencing) was performed on AtQQS RNAi (RNA interference), AtQQS-OE (overexpressing) and WT Col-0 Arabidopsis plants grown under unstressed conditions (Li et al., 2015). Six hundred and five genes were differentially expressed in AtQQS-OE plants at a false discovery rate (FDR) ≤ 0.05 (Table S1a). In contrast, no gene was
differentially expressed in the AtQQS RNAi plants at this FDR cut-off (Table S1b). Among the differentially expressed genes in AtQQS-OE plants, callose biosynthesis, L-N^2-acetylornithine biosynthesis and monoterpene biosynthesis pathways were over-represented (Li et al., 2015) (Table S1c). These pathways are associated with plant responses to pathogens/herbivores/abiotic stresses (Chen and Kim, 2009; Lewinsohn et al., 1991; Serrano et al., 2014; Shah, 2003).

To further explore the potential relationship between QQS and defense response genes, we selected 13 genes considered as markers for different plant defense responses (Chico et al., 2014; Onkokesung et al., 2014; Seo et al., 2001; Song et al., 2014; Truman et al., 2006; Zhang et al., 2014) and examined the expression of these genes in the RNA-Seq data set using a \( P < 0.05 \) cut-off (Table S2). This analysis identified only four genes that were significantly induced and one that was significantly down-regulated. These modest effects of QQS-OE or QQS RNAi on the expression of canonical plant immune response genes led us to conclude that QQS overexpression does not induce a constitutive large-scale activation of plant defense responses in the absence of biotic stress.

**QQS and NF-YC4 enhance plant antiviral and antibacterial immune responses**

To test the hypothesis that QQS and its interactor NF-YC4 affect Arabidopsis susceptibility to pathogens/pests, we tested responses to infection with representative pathogens (virus and bacteria), and we also sought to determine whether these responses could be extended to pests (aphids and plant-parasitic nematodes). TuMV-GFP was inoculated on Arabidopsis lines with genetically induced differences in QQS or NF-YC4 expression: transgenic AtQQS RNAi, AtQQS-OE and AtNF-YC4-OE in Arabidopsis Col-0* (few trichomes), and T-DNA knockout mutants Atqqs, and Atnfyc4 in Col-0 (trichomes). (a) The numbers of TuMV-GFP infection foci at 120 HAI, (b) the sizes of TuMV-GFP infection foci at 120 HAI and (c) the growth of the Pst DC3000 (DC3000) and CUCPB5115 (Pst DC3000 ΔCEL) bacterial strains were altered in the mutants. CFU, colony forming units. All data in bar charts show mean ± SE (standard error), \( n = 3 \). Statistical significance was determined as described in Appendix S1 ‘Experiment design and statistical methods’: *** \( P < 0.001 \); ** \( P < 0.01 \); * \( P < 0.05 \);  \( P < 0.1 \).
ATQQS RNAi and Atnf-yc4 plants had larger foci by 28%, 53% and 52% (P < 0.001 for all). In contrast, foci on ATQQS-OE and ATNF-YC4-OE plants were 32% and 51% smaller (P < 0.001 for both). Thus, overexpressing either ATQQS or ATNF-YC4 impairs viral infection, while silencing or knocking out these genes enhances viral infection.

To test the effects of ATQQS and NF-YC4 expression on the growth of a bacterial pathogen, we inoculated the ATQQS and NF-YC4 mutants with Pst DC3000 or the nonvirulent Pst DC3000 ΔCEL mutant (CUCP85115). Pst DC3000 ΔCEL lacks multiple effector genes and grows poorly in planta because it cannot suppress basal defenses (Badel et al., 2006). As expected, Pst DC3000 grew ~1000-fold, whereas ΔCEL grew ~10-fold in WT plants by 4 DPI (Figure 1c). However, the growth of Pst DC3000 was decreased by 63% in ATQQS-OE (P < 0.01) and 88% in ATNF-YC4-OE (P < 0.001), and not significantly altered in the ATQQS RNAi, Atqqs or Atnf-yc4, when compared to WT plants.

The growth of Pst DC3000 ΔCEL was not significantly different in ATQQS-OE, ATNF-YC4-OE and WT plants. However, its growth was significantly increased in ATQQS RNAi, Atqqs or Atnf-yc4 plants (174%, 207% and 102% increase, P < 0.001, <0.01, <0.01) (Figure 1c). Overall, these altered growth patterns indicate that overexpressing either ATQQS or ATNF-YC4 enhances plant immunity to a robust bacterial pathogen. In contrast, silencing or knocking out ATQQS or ATNF-YC4 impairs plant immunity, enabling the nonvirulent ΔCEL to grow better. Combined with the TuMV-GFP results, these data indicate that ATQQS and ATNF-YC4 positively regulate plant immunity to these bacterial and viral strains.

Expression of ATQQS and overexpression of NF-YC4 in transgenic soybeans enhances resistance to viral and bacterial pathogens

To test if ATQQS and NF-YC4 could affect soybean-pathogen interactions, transgenic soybean lines expressing ATQQS (ATQQS-E) or overexpressing GmNF-YC4-1 (GmNF-YC4-1-OE) were inoculated with Bean pod mottle virus (BPMV), and P. syringae pv. glycinea Race 4 (PsgR4) (causing the disease bacterial blight). Systemic infection of BPMV was decreased in transgenic soybean at both 11 (P = 0.084, 0.008 for ATQQS-E and GmNF-YC4-1-OE, respectively) and 13 DPI (P = 0.035, 0.004) (Figure 2a). Growth of PsgR4 was decreased by 55% and 62% in ATQQS-E and GmNF-YC4-1-OE compared to Williams 82 control plants (P = 0.0003, 0.00002) (Figure 2b). These data show that ATQQS and GmNF-YC4-1 can enhance soybean immunity similar to our observations from Arabidopsis.

Decreased susceptibility to aphids in Arabidopsis and soybean plants overexpressing QQS and NF-YC4

To determine whether ATQQS and NF-YC4 might also enhance defense against aphids, the Arabidopsis QQS and NF-YC4 mutants were infested with green peach aphids (Myzus persicae). Green peach aphid population growth was compromised by 27% in the ATQQS-OE compared with the controls (P = 0.04), and 10% (P = 0.34) in the NF-YC4-OE plants. Aphid population growth was increased by 3%, 16% and 28% in the ATQQS RNAi, Atqqs and Atnf-yc4 knockout mutants; however, these differences were not statistically significant (P = 0.86, 0.36, 0.12) (Figure S2). Overall, overexpression of ATQQS seems to increase resistance to pests such as aphids in Arabidopsis.

To test whether soybean lines expressing QQS or overexpressing NF-YC4 have a similar anti-aphid phenotype, the ATQQS-E, GmNF-YC4-1-OE and control soybean lines were infested with soybean aphids (Aphis glycines). Soybean aphid population growth was compromised in ATQQS-E by 31%–34% (P = 0.09, 0.06) and in GmNF-YC4-1-OE lines by 37%–45% (P = 0.02, 0.05) (Figure 3a), demonstrating that ATQQS and GmNF-YC4 mediate reduced susceptibility to aphids.

Decreased susceptibility to nematodes in soybean plants expressing QQS or overexpressing NF-YC4

The soybean cyst nematode (SCN, Heterodera glycines) infects soybean and causes significant yield losses (Niblack et al., 2002). To investigate whether ATQQS or GmNF-YC4 could enhance nematode defense in soybean, the roots of ATQQS-E and GmNF-YC4-1-OE, and control soybean lines were grown in soil infested with SCN. The number of SCN females on the roots after a single generation was decreased in the GmNF-YC4-1-OE lines by 41%–47% (P = 0.04, 0.05), with a similar, although not significant,
trend observed for the AtQQS-E soybean lines (reductions of 18%–19%; \( P = 0.85, 0.28 \)) (Figures 3b and S3).

Decreased sudden death syndrome symptoms in soybean plants expressing QQS or overexpressing NF-YC4 in the field

AtQQS-E, GmNF-YC4-1-OE and WT soybean plants were grown in the field, inoculated with Fusarium virguliforme, the fungus that causes sudden death syndrome (SDS). Foliar SDS symptoms started to appear at growth stage R5 (beginning of pod filling). There was a significant difference in foliar disease index (FDX = disease incidence \times disease severity/9) in mutants compared with the WT. The AtQQS-E (\( P = 0.008, 0.009 \)) and GmNF-YC4-1-OE (\( P = 0.00005, 0.00004 \)) mutants showed 70% and 90% less disease, than the WT (Figure 3c).

Effects of QQS expression on Arabidopsis defense are not associated with starch and protein content

The importance of carbohydrates, such as sucrose and trehalose, to signalling in response to several biotic stresses (Singh et al., 2011; Tauzin and Giardina, 2014) led us to investigate the possible interactions between QQS expression level, starch content and pathogen defense. Specifically, because QQS overexpression reduces susceptibility to pathogens (Figure 1) and decreases starch content (Li and Wurtele, 2015b; Li et al., 2015), we tested whether the decreased susceptibility to TuMV might be coupled to starch content, independent of QQS expression level. This investigation was enabled by Arabidopsis starch mutants that represent the four possible combinations of the altered QQS transcript level and starch content, as previously determined in plants grown under long-day (LD) conditions (Table S3): (1) Atss1 (starch synthase I knockout (Delvalle et al., 2005), QQS ↑, starch ↓); (2) Atss3 (starch synthase III knockout (Zhang et al., 2005), QQS ↑, starch ↑); (3) Atsex4-5 (glucan phosphatase knockout (Lu et al., 2008), QQS ↓, starch ↑) and (4) Atisa1/isa3/pu1 (a triple knockout of starch debranching enzymes (Wattebled et al., 2008), QQS ↓, starch ↓). Because our pathogen bioassays were conducted under short-day (SD) conditions, we tested the composition and level of QQS expression for each of these mutants and their corresponding WT controls under SD conditions. Starch accumulation and QQS transcript levels in lines under SD conditions (Figure 4a,b) were similar in trend to the same lines under LD conditions (Li et al., 2015). When compared with the corresponding WT controls under SD conditions, the protein content was similar in low-starch mutant Atss1 (\( P = 0.003 \)) and high-starch mutant Atss3 (\( P = 0.029 \)), but lower in high-starch mutant Atsex4-5 (\( P < 0.001 \)) (Figure 4c). These QQS and starch-perturbed lines were inoculated with TuMV-GFP. At 5 DPI, the TuMV-GFP infection foci sizes in the high-QQS-transcript-level mutants (Atss1 and Atss3) were 27% and 36% smaller than the
WT control ($P < 0.001$ for both) (Figure 4d). In contrast, the TuMV-GFP foci in the low-QQS-transcript-level mutants (Atsex4-5 and At Isis1/isa3/pu1) were 16% and 11% larger than the WT control ($P = 0.002$, $<0.05$). The starch accumulation, AtQQS and AtNF-YC4 transcript levels were also tested for the QQS and NF-YC4 mutants under SD conditions (Figure S4a,b). The resistance to TuMV-GFP increases in plants with higher QQS transcript levels and appears to be independent of starch content (Table S3).

The QQS interaction with Arabidopsis NF-YC4 and human NF-YC

Our findings that QQS and NF-YC4 may play an important role in plant defense led us to investigate the interaction between QQS and NF-YC in more detail. The heterotrimeric NF-Y transcription factor complex is conserved across eukaryotic species (Laloum et al., 2013; Nardini et al., 2013) and modulates gene expression in part via binding to the CCAAT box promoter motif (Nardini et al., 2013; Ripodas et al., 2014). Our previous study demonstrated that AtNF-YC4 binds to QQS between aa 73 and 162 (Li et al., 2015). Here, we used a computational model to analyse potential QQS/NF-YC4 interaction sites (Figure S5a). We dissected this potential interaction by screening five fragments of QQS (primers used are in Table S4), selected based on the secondary structure model prediction, for their ability to interact with NF-YC4 in pull-down assays (Figure 5a). Pull-down assays using maltose-binding protein (MBP)-NF-YC fragments as bait indicate that the 12-N-terminal aa of QQS (QQS-1-12), the 49-C-terminal-aa (QQS-11-59) and the 19-C-terminal-aa (QQS-41-59) peptides interact with AtNF-YC4, while the middle-35-aa (QQS-13-47) and the 12-C-terminal-aa (QQS-48-59) peptides do not bind to AtNF-YC4 (Figure 5b).

To explore the cross-kingdom universality of the QQS/NF-YC interactions and to extend the biological significance of the interactions between QQS and NF-YC, we investigated whether the QQS fragments also interact with human NF-YC (HsNF-YC). Protein sequence alignments indicated that the HsNF-YC shares a conserved H2A domain with AtNF-YC4; thus, the entire HsNF-YC as well as the N-terminal fragment of HsNF-YC (aa 1-145, HsNF-YC-1-145) was used (Figure S5b). MBP pull-down assays indicate there is a physical interaction between all tested QQS fragment peptides (QQS-1-12, QQS-11-59 and QQS-41-59) and the HsNF-YC-1-145 peptide (Figure 5c). Reciprocal Glutathione Sepharose Tag (GST) pull-down assays using bound QQS fragments as bait show that the QQS-11-59 and QQS-41-59 peptides pulled down AtNF-YC4, HsNF-YC and HsNF-YC-1-145 (Figure 5d). The QQS-1-12 fragment failed to pull down any of the NF-YC proteins.
Potential QQS interactions with NF-Y

The QQS-1-12 and QQS-41-59 fragments each bind to AtNF-YC4 and to human NF-YC. Furthermore, a sequence alignment of QQS-1-12 and QQS-41-59 with NF-YBs identified a 7-aa consensus motif REQEIVY (QQS-5-11) and a 10-aa motif VARLKMRLVI (QQS-41-49) that are similar to motifs within NF-YB in the N-terminal region near the histone-binding domain (Figure S5c). The consensus sequences are R[E/D]Q[E/D]-[Y/F/W] and [V/I]-R[L/I]-[K/R]-[I/V/L]. QQS-5-11 aligns to the disordered region and the coil near the N-terminus (NF-YB-51-57), whereas QQS-41-49 aligns to the α1 helix and loop-1 region (NF-YB-62-70) (Figure 6a,b). Based on computational analyses of the contact maps of NF-YB-51-57 and NF-YB-62-70 in the NF-YB and NF-YA dimer, and of the NF-YANF-YB/NF-YC trimer and DNA complex (Figure 6a,b), we propose that the QQS N-terminus and C-terminus bind to NF-YC at the same binding sites as NF-YB-51-57 and NF-YB-62-70, and we further propose that the middle region QQS (QQS-12-40) may form a loop to bring the two QQS binding sites close to each other (Figure 6c). The structure of the NF-YANF-YB/NF-YC and DNA complex in the region of NF-YB-51-57 and NF-YB-62-70 shows that the disordered region located at NF-YB-51-57 and the structured region located at NF-YB-62-70 are buried in the cavity formed by the hydrophobic interface of NF-YC-4 and hydrophilic interfaces of DNA and NF-YA (Figure S6). The sequence diversity and structural flexibility in this region of plant NF-YBs are consistent with this domain providing specific recognition for the NF-YA/NF-YB/NF-YC association and modulating DNA transcription via an interaction with DNA. Taken together, these data lead us to speculate, proposing a model in which QQS binds to NF-YC, potentially dissociating NF-YB or preventing NF-YB binding...
In vivo experimentation will be important to validate this model.

Searches of the patented protein sequence database (http://www.ebi.ac.uk/patentdata/proteins) identified nine plant-related peptides with the motif R[E/D]Q[D/E]-[Y/F/W][L/V] (QQS-1-12 region) and three candidates with the motif [V/I]-R[L/I]M[K/R]-[I/V/L]QQS-41-49 region), but without histone-like motifs (Figure S7). (No polypeptides in the database contained both the

(Figure 6d). In vivo experimentation will be important to validate this model.

Searches of the patented protein sequence database (http://www.ebi.ac.uk/patentdata/proteins) identified nine plant-related peptides with the motif R[E/D]Q[D/E]-[Y/F/W][L/V] (QQS-1-12 region) and three candidates with the motif [V/I]-R[L/I]M[K/R]-[I/V/L]QQS-41-49 region), but without histone-like motifs (Figure S7). (No polypeptides in the database contained both the
Transgenic plants expressing these sequences were tolerant to an herbicide (Guo et al., 2015; Wu et al., 2015), but to our knowledge have not been tested for pathogen susceptibility (Table 55a,b). We propose that polypeptides with these motifs may bind to NF-YC, as QQS does, and modify plant NF-Y associations and regulate transcription.

Discussion

QQS may be the first orphan gene identified as having a biochemically characterized molecular function (Li et al., 2009). It interacts with a conserved transcription factor, NF-YC4 (Li et al., 2015). Both QQS and NF-YC4 regulate carbon and nitrogen allocation, affecting starch and protein accumulation in leaves and seeds (Li and Wurtele, 2015b; Li et al., 2009, 2015). When QQS or NF-YC4 is up-regulated, starch is decreased and protein is increased. The tight linkage of QQS expression to environmental stresses and genetic perturbations has led us to the hypothesis that QQS provides a homeostatic function and optimizes tolerance to biotic/abiotic perturbations by mediating crosstalk between primary metabolism and environmental changes (Arendsee et al., 2014; Li and Wurtele, 2015b; Li et al., 2009, 2015).

In Arabidopsis plants, the expression of QQS, and to a lesser extent of NF-YC4, is responsive to biotic stimuli and is differentially regulated depending on the pathogen (Figure S1, Arendsee et al., 2014). For example, Pst DC3000 and TuMV are pathogenic to Arabidopsis; QQS and NF-YC4 transcripts are down-regulated following exposure to each of these pathogens. In contrast, P. infestans is not pathogenic to Arabidopsis; following P. infestans inoculation QQS and NF-YC4 expression is initially repressed, but by 24 HAI it is not. These kinetics fit well with previous observations that P. infestans completes its early stages of infection within 6 HAI as it would in a susceptible host, but by 24 HAI, a resistance reaction in the form of a hypersensitive response is activated in Arabidopsis (Huitema et al., 2003). Overall, the data indicate that QQS expression is down-regulated by successful pathogens during disease. This is consistent with our observations in transgenic plants that QQS overexpression leads to decreased susceptibility. The most likely explanation of why the RNAsi and knockout plants are not more susceptible to Pst DC3000 than the WT plants is because Pst DC3000 has one or more effectors that can suppress the basal defense mechanisms mediated by QQS and NF-YC4. Therefore, knocking out the QQS or NF-YC4 plant genes does not benefit this bacterium. However, the antibacterial effects of defense mediated by QQS and NF-YC4 are revealed when we use the Pst DC3000 ΔCEL mutant, which lacks the ability to transfer key effectors into the plant. Pst DC3000 ΔCEL grows more rapidly in mutants with QQS or NF-YC4 gene expression reduced or eliminated, relative to its growth in WT plants. This indicates that basal defenses may not be as effective in mutant plants underexpressing QQS or NF-YC4, allowing a debilitated bacterial pathogen to better colonize the plant.

In GmNF-YC4-1-OE soybeans, there is no QQS, but soybean immunity is enhanced. Taken together, these data indicate that although expression of QQS clearly promotes enhancement of plant immunity across several species, the QQS-NF-YC4 interaction is not required to enhance plant immunity.

The levels of resistance to pathogens and herbivores observed in Arabidopsis and soybean plants overexpressing QQS or NF-YC4 are consistent with quantitative resistance as opposed to qualitative resistance. Quantitative resistance is typically defined as incomplete resistance that can be broad spectrum, whereas qualitative resistance is generally considered to be complete resistance and is conferred by resistance genes with relatively narrow specificities (Poland et al., 2009). The plants overexpressing QQS and NF-YC4 had significant and reproducible increases in resistance to the pathogens and herbivores tested, and although it was not complete, this resistance may be useful for augmenting qualitative and quantitative resistance traits already present in soybean. Quantitative resistance takes on many forms, and there are several potential mechanisms that include: variants of nucleotide-binding site leucine-rich repeat proteins, pattern recognition receptors, loss-of-function alleles of susceptibility genes and variation in host metabolism (French et al., 2016). Given that plants overexpressing QQS and NF-YC4 also have increased partitioning of resources into protein versus carbohydrate, we hypothesize that the mechanism underlying quantitative resistance in these plants is related to metabolism.

Disaccharide carbohydrates are important regulators of plant defenses against pathogens (sucrose) and aphids (trehalose) (Singh et al., 2011; Tauzin and Giardina, 2014). Furthermore, sugar and starch contents are often modified during plant–microbe interactions (Tauzin and Giardina, 2014). For example, aphid feeding increases trehalose metabolism, sucrose content and starch content of infected Arabidopsis and tomato plants (Singh and Shah, 2012; Singh et al., 2011); it has been proposed that trehalose provides a signalling mechanism that enhances the conversion of sucrose to starch and consequently reduces the sucrose available to phloem-feeding aphids (Singh and Shah, 2012). Thus, a potential explanation of the decreased susceptibility to pathogens/pests in Arabidopsis and soybean plants overexpressing QQS and NF-YC4 is that defenses are induced as a result of altered plant composition. However, comparisons of Arabidopsis starch mutants with altered QQS expression show that decreased susceptibility to TuMV is not associated with the starch content and thus is more likely regulated by QQS expression level independent of the changes in starch content. The soybean aphid is a specialist feeder that can only colonize soybean and its winter hosts, whereas the green peach aphid is a generalist, adapted to overcome diverse plant defense strategies and colonizes hundreds of different species (Blackman and Eastop, 2000; Tilmont et al., 2011); this difference in specificity might explain the difference in efficacy of the QQS pathway against these insects in our assays.

Constitutively activated defenses that are effective against pathogens or herbivores can be costly to plants, resulting in decreased biomass and seed yield (Benedetti et al., 2015; Heidel et al., 2004; Heil et al., 2000; Redman et al., 2001). In contrast, induced defenses are much less costly, because they are only deployed when the plant is under attack (Conrath et al., 2015). We have previously shown in glasshouse and field studies that overexpression of QQS in Arabidopsis, soybean, rice and maize does not affect the growth nor reduce the yield of these plants compared with control plants (Li and Wurtele, 2015b; Li et al., 2009, 2015), and overexpression of NF-YC4 does not appear to impact the growth. Therefore, the enhanced-defense phenotype associated with QQS and its interacting factor is not concomitant with a growth cost, as might be expected if QQS overexpression activated constitutive defenses. Another line of evidence supporting induced versus constitutive defense in plants with up-regulated QQS is that constitutive defenses to pathogens and
herbivores are associated with strong expression of many defense-related genes (Tian et al., 2014; Ward et al., 1991). However, in the QQS-OE plants, only a handful of defense-related genes are minimally induced. This indicates that entire pathogen- or herbivore-induced defense pathways are not constitutively activated by QQS. Thus, we propose that the plant immune system is more rapidly induced when QQS is overexpressed, leading to decreased susceptibility to pathogens/pests. The broad-spectrum resistance we observed could indicate that QQS or NF-YC4 overexpression activates a form of priming, which would render plant defenses more responsive to biotic stresses (Conrath et al., 2015).

This research has exciting implications with respect to the concept of the so-called defense-versus-yield trade-off, which is thought to be due in part to the competition among different metabolic pathways for limited plant resources (Huot et al., 2014; Mitra and Baldwin, 2014; Robert-Seilaniantz et al., 2011). Inducible defenses benefit a plant, because when it is not under attack, resources are not allocated to defense and instead support growth and development. Overexpression of QQS or NF-YC4 has the unusual result that the plant is protected against a range of biotic stresses; however, there is no detected trade-off between this increased plant defensive ability and growth of the plants. QQS, in part through its interaction with NF-YC4, appears to function at a nexus that controls the allocation of resources to protein, starch and primes the plant response to biotic stimuli (Arendsee et al., 2014; Li et al., 2009). We note that in soybean, GmNF-YC4 overexpression tended to have a greater effect on improved defense to pathogens/pests than the AtQQS expression pointing to a need for additional research to understand the role of GmNF-YC4 in soybean defenses.

The increased susceptibility to pathogens in Atqq and AtNF-ycd lines indicates that QQS and NF-YC4 may be important in plant defense. The small protein QQS, although encoded by an orphan gene unique to the model plant A. thaliana, interacts with NF-YC from as divergent a species as humans. Previous studies indicated that NF-YB and NF-YC interact through histone fold motifs (Romier et al., 2003). Our data on interaction of QQS and NF-YC led us to propose two additional interaction motifs (QQS-5-11 and QQS-41-49), which mediate QQS binding to the N-terminus of NF-YC, near NF-YC histone-binding domain. These motifs may have been neglected in previous studies because they are unstructured and therefore not manifested in the crystal structures of NF-Y (Nardini et al., 2013). Sometimes two proteins with a completely unrelated sequence can fold into the same structure and complete the same biological function. QQS contains these motifs, which we speculate may alter the ability of NF-YB to bind to NF-YC, thus modulating its function.

In conclusion, QQS expression is differentially regulated in Arabidopsis after exposure to pathogens, implicating QQS as having a role in plant defense. Experiments with transgenic Arabidopsis and soybean lines altered in expression of QQS or its interacting partner NF-YC4 revealed the ability of each of these genes to confer quantitative protection against pathogens/pests. QQS and NF-YC4 may control allocation of primary resources to defense, thus linking two highly prized agronomic traits, pathogen resistance and yield. The broad resistance or reduced susceptibility conferred by QQS and NF-YC4 provides a new model to explore how plant defense mechanisms interconnect with primary metabolism, and how a recently evolved gene can play a role in this process. From a broader vantage point, the study reveals the promise of orphan genes as untapped resources for crop improvement.

Experimental procedures

Plant materials

Arabidopsis thaliana mutants with perturbed QQS or NF-YC4 expression have been previously generated and characterized: AtQQS RNAi (Li et al., 2009), AtQQS-OE (Li and Wurtele, 2015b) and AtNF-YC4-OE (Li et al., 2015) transformants in an ecotype Columbia (Col-0) background with few trichomes; and T-DNA knockout mutants Atqq (Li et al., 2015) and Atnf-yc4 (Kumimoto et al., 2010; Li et al., 2015) in a Col-0 background with trichomes. For genotype with more than two independent lines that were verified in our previous studies, one representative line was used in current experiments due to growth chamber space. Arabidopsis mutants in starch metabolism with different levels of QQS expression and starch content, including Atts1 [ecotype Wassilewskija (Ws)], Atts3, Atses4-5 and Atsi3/4a3/pu1 (ecotype Col-0 for the latter three mutants), are knockout mutants of starch synthase I, III, a plant-specific glucan phosphatase and triple knockout mutant of starch debranching enzymes (Delvalle et al., 2005; Lu et al., 2008; Wattebled et al., 2008; Zhang et al., 2005).

Soybean plants: the GmNF-YC4-1 ( Glyma06g17780) overexpressing (GmNF-YC4-1-OE) and Arabidopsis QQS-expressing (AtQQS-E) soybean lines in the Williams 82 background, expressed under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter, were generated previously, and the plant composition and expression level of QQS or GmNF-YC4-1 have been quantified (Li and Wurtele, 2015b; O’Conner et al., 2018).

Plant selection and growth, RNA-Seq, TuMV-GFP inoculation assay, BPMV-GFP inoculation assay, Pseudomonas inoculation assay, Aphid infestation, SCN bioassay, Field SDS experiment, RNA isolation and real-time PCR, Composition analysis, Mapping the QQS and NF-YC interaction, Protein expression and purification, Pull-down assay, and Experiment design and statistical methods are provided in Appendix S1 in Supporting Information.

Accession numbers: Sequence data from this article can be found under the following accession numbers in The Arabidopsis Genome Information Resource: QQS (At3g30720), NF-YC4 (At5g63470), and in LegumeIP: GmNF-YC4-1 (Glyma06g17780).

Acknowledgements

We thank Diane Luth and Kan Wang at ISU PTF for soybean transformation; Kent Berns for field management; David Soh for testing in SCN-infested soil; and ISU Soil and Plant Analysis Laboratory for protein combustion. We thank BGI for transcriptome sequencing and processing sequencing data. This material is based in part upon work supported by: NSF award numbers MCB-0951170 (to E.S.W. and L.L.) and IOS 1546858 (to E.S.W.), Iowa Soybean Association (to L.L., and to S.A.W.), ISU Research Foundation (to L.L.), ISU Center for Metabolic Biology, and USDA NIFA Hatch project 3808 (to S.A.W.).
Author contributions
L.L. designed the research. M.Q., W.Z., J.D.H., U.K., S.O. and L.L. performed research. X.Z., Y.W., C.D., D. M., D.N., G.C.M., G.L.T., S.A.W. and L.L. analysed data. L.L., E.S.W. and S.A.W. wrote the manuscript with inputs from M.Q., W.Z., X.Z., D.N., G.C.M. and G.L.T.

References
Arendsee, Z.W., Li, L. and Wurtele, E.S. (2014) Coming of age: orphan genes in plants. Trends Plant Sci. 19, 698–708.
Badel, L.L., Shimizu, R., Oh, H.S. and Collmer, A. (2006) A Pseudomonas syringae pv. tomato avrE1 HopM1 mutant is severely reduced in growth and lesion formation in tomato. Mol. Plant Microbe Interact. 19, 99–111.
Benedetti, M., Pontiggia, D., Raggi, S., Cheng, Z., Scaloni, F., Ferrari, S., Ausubel, F.M. et al. (2015) Plant immunity triggered by engineered in vivo release of oligogalacturonides, damage-associated molecular patterns. Proc. Natl Acad. Sci. USA, 112, 5533–5538.
Blackman, R.L. and Eastop, V.F. (2000) Aphids on the World’s Crops: An Identification and Information Guide. Chichester and New York, NY: Wiley.
Bolton, M.D. (2009) Primary metabolism and plant defense–fuel for the fire. Mol. Plant Microbe Interact. 22, 487–497.
Bowling, S.A., Clarke, J.D., Liu, Y., Klessig, D.F. and Dong, X. (1997) The ctr5 mutant of Arabidopsis expresses both NPR1-dependent and NPR1-independent resistance. Plant Cell, 9, 1573–1584.
Carvunis, A.R., Rolland, T., Wapinski, I., Calderwood, M.A., Yildirim, M.A., Simonis, N., Charleoteaux, B. et al. (2012) Proto-genes and de novo gene birth. Nature, 487, 370–374.
Chen, X.Y. and Kim, J.Y. (2009) Callose synthesis in higher plants. Plant Signal Behav. 4, 489–492.
Chico, J.-M., Fernández-Barbero, G., Chini, A., Fernández-Calvo, P., Díez-Díaz, M. and Solano, R. (2014) Repression of jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their IAZ repressors in Arabidopsis. Plant Cell, 26, 1967–1980.
Chisholm, S.T., Coaker, G., Day, B. and Staskawicz, B.J. (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell, 124, 803–814.
Clarke, J.D. (1998) Uncoupling PR gene expression from NPR1 and bacterial resistance: characterization of the dominant Arabidopsis cpr 6-1 mutant. Plant Cell, 10, 551–570.
Clough, S.J., Fengler, K.A., Yu, J.C., Lippok, B., Smith, R.K. and Bent, A.F. (2000) The Arabidopsis dfn1 “defense, no death” gene encodes a mutated cyclic nucleotide-gated ion channel. Proc. Natl Acad. Sci. USA, 97, 9323–9328.
Conrath, U., Bevers, G.J., Langenbach, C.J. and Jaskiewicz, M.R. (2015) Linking genes of unknown function with abiotic stress responses by high-throughput phenotype screening. Mol. Plant Microbe Interact. 28, 365–369.
Lalamr, T., De Mita, S., Gamas, P., Baudin, M. and Nebel, A. (2013) CCAAT-box binding transcription factors in plants: Y so many? Trends Plant Sci. 18, 157–166.
Lewinsohn, E., Gijzen, M. and Croteau, R. (1991) Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species. Plant Physiol. 96, 44–49.
Li, L. and Wurtele, E.S., Iowa State University (2012) Materials and methods for modifying a biochemical component in a plant, US 20120221167 A1, US patent and trademark office.
Li, L. and Wurtele, E.S., Iowa State University (2015a) Increasing protein content by modifying transcriptional repressor binding site in NF-YC4 promoter, US Provisional Pat. App. No. 62/117,924, U.S. patent and trademark office.
Li, L. and Wurtele, E.S. (2015b) The QQS orphan gene of Arabidopsis modulates carbon and nitrogen allocation in soybean. Plant Biotechnol. J. 13, 177–187.
Li, L., Foster, C.M., Gan, Q., Nettleton, D., James, M.G., Myers, A.M. and Wurtele, E.S. (2009) Identification of the novel protein QQS as a component of the starch metabolic network in Arabidopsis leaves. Plant J. 58, 485–498.
Li, L., Zheng, W., Zhu, Y., Ye, H., Tang, B., Arendsee, Z.W., Jones, D. et al. (2015) QQS orphan gene regulates carbon and nitrogen partitioning across species via NF-YC interactions. Proc. Natl Acad. Sci. USA, 112, 14734–14739.
Liu, J.-Z., Horstem, H.D., Braun, E., Graham, M.A., Zhang, C., Xavarez, D., Qi, W.-L. et al. (2011) Soybean homologs of MPK4 negatively regulate defense responses and positively regulate growth and development. Plant Physiol. 157, 1363–1378.
Lu, Y., Savage, L.J., Ajaiwa, I., Imre, K.M., Yoder, D.W., Benning, C., Dellapenna, D. et al. (2008) New connections across pathways and cellular processes: industrialized mutant screening reveals novel associations between diverse phenotypes in Arabidopsis. Plant Physiol. 146, 1482–1500.
Luhua, S., Hegie, A., Suzuki, N., Shulaev, E., Luo, X., Cenariu, D., Ma, V. et al. (2013) Linking genes of unknown function with abiotic stress responses by high-throughput phenotype screening. Physiol. Plant. 148, 322–333.
Mitra, S. and Baldwin, I.T. (2014) RubPCase activase (RCA) mediates growth-defense trade-offs: silencing RCA redirects jasmonic acid (JA) flux from JA-isoleucine to methyl jasmonate (MeJA) to attenuate induced defense responses in Nicotiana attenuata. New Phytol. 201, 1385–1395.
Nardini, M., Gnesutta, N., Donati, G., Gatta, R., Forni, C., Fossati, A., Vonrenen, C. et al. (2013) Sequence-specific transcription factor NYF- displays histone-like DNA binding and H2B-like ubiquitination. Cell, 152, 132–143.
Nisback, T., Arelli, P., Noel, G., Opperman, C., Orf, J., Schmitt, D., Shannon, J. et al. (2002) A revised classification scheme for genetically diverse populations of Heterodera glycines. J. Nematol. 34, 279.
O’Connor, S., Neudorf, A., Zheng, W., Qi, M., Zhao, X., Du, C., Nettleton, D. et al. (2018) From Arabidopsis to crops: the Arabidopsis QQS orphan gene modulates nitrogen allocation across species. In: Engineering Nitrogen Utilization in Crop Plants (Shrawat, A., Zayed, A., and Lightfoot, D.A., eds), Springer.
Onokoosung, N., Reichelt, M., van Doorn, A., Schuurink, R.C., van Loon, J.J.A. and Dicke, M. (2014) Modulation of flavonoid metabolites in Arabidopsis thaliana through overexpression of the MYB75 transcription factor: role of
kaempferol-3,7-dihannoside in resistance to the specialist insect herbivore *Pliric brassicae*. *J. Exp. Bot.* **65**, 2203–2217.

Petroni, K., Kumimoto, R.W., Gnesutta, N., Calvezani, V., Fornari, M., Tonelli, C., Holt, B.F. 3rd et al. (2012) The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. *Plant Cell* **24**, 4777–4792.

Poland, J.A., Balint-Kurti, P.J., Wisser, R.J., Pratt, R.C. and Nelson, R.J. (2009) Shades of gray: the world of quantitative disease resistance. *Trends Plant Sci.* **14**, 21–29.

Redman, A.M., Cipollini, D.F. Jr and Schultz, J.C. (2001) Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. *Oecologia* **126**, 380–385.

Ripodas, C., Cua, J., Battaglia, M., Baudin, M., Niebel, A., Zanetti, M.E. and Blanco, F. (2014) Transcriptional regulators of legume-rhizobia symbiosis: nuclear factors Ys and GRAS are two for tango. *Plant Signal Behav.* **9**, e28847.

Robert-Seilaniantz, A., Grant, M. and Jones, J.D. (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-saliclylate antagonism. *Annu. Rev. Phytopathol.* **49**, 317–343.

Romier, C., Cocchiarella, F., Mantovani, R. and Moras, D. (2003) The NF-YB/NF-RIPodas, C., Clua, J., Battaglia, M., Baudin, M., Niebel, A., Zanetti, M.E. and Blanco, F. (2014) Transcriptional regulators of legume-rhizobia symbiosis: nuclear factors Ys and GRAS are two for tango. *Plant Signal Behav.* **9**, e28847.

Song, S., Huang, H., Gao, H., Wang, J., Wu, D., Liu, X., Yang, S. et al. (2014) The cuticle and plant defense to pathogens. *Front. Plant Sci.* **5**, 274.

Shah, J. (2003) The salicylic acid loop in plant defense. *Curr. Opin. Plant Biol.* **6**, 365–371.

Singh, V. and Shah, J. (2012) Tomato responds to green peach aphid infestation with the activation of trehalose metabolism and starch accumulation. *Plant Signal Behav.* **7**, 605–607.

Singh, V., Louis, J., Ayre, B.G., Reese, J.C., Pegadaraju, V. and Shah, J. (2011) TREHALOSE PHOSPHATE SYNTHASE1-dependent trehalose metabolism promotes *Arabidopsis thaliana* defense against the phloem-feeding insect *Myzus persicae*. *Plant J.* **67**, 94–104.

Smedegaard-Petersen, V. and Stolen, O. (1981) Effect of energy-demanding defense reactions on yield and grain quality in a powdery mildew-resistant barley cultivar. *Phytopathology* **71**, 396.

Song, S., Huang, H., Gao, H., Wang, J., Wu, D., Liu, X., Yang, S. et al. (2014) Interaction between MYC2 and ETHYLENE INSENSITIVE3 mediates antagonism between jasmonate and ethylene signaling in Arabidopsis. *Plant Cell* **26**, 263–279.

Tazin, A.S. and Giardina, T. (2014) Sucrose and invertases, a part of the plant defense response to the biotic stresses. *Front. Plant Sci.* **5**, 293.

Thilmony, R., Underwood, W. and He, S.Y. (2006) Genome-wide transcriptional analysis of the Arabidopsis thaliana interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli* O157:H7. *Plant J.* **46**, 34–53.

Tian, D., Peiffer, M., De Moraes, C.M. and Felton, G.W. (2014) Roles of ethylene and jasmonic acid in systemic induced defense in tomato (*Solanum lycopersicum*) against *Helicoverpa zeae*. *Planta* **239**, 577–589.

Tilmon, K.J., Hodgson, E.W., O’Neal, M.E. and Ragsdale, D.W. (2011) Biology of the Soybean Aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. *J. Integr. Pest Manag.* **2**, A1–A7.

Truman, W., de Zabala, M.T. and Grant, M. (2006) Type III effectors orchestrate a complex interplay between transcriptional networks to modify basal defense responses during pathogenesis and resistance. *Plant J.* **46**, 14–33.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ah-Goy, P. et al. (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* **3**, 1085–1094.

Wattebled, F., Planchot, V., Dong, Y., Szydłowski, N., Pontoire, B., Devin, A., Ball, S. et al. (2008) Further evidence for the mandatory nature of polysaccharide debranching for the aggregation of semicrystalline starch and for overlapping functions of debranching enzymes in Arabidopsis leaves. *Plant Physiol.* **148**, 1309–1323.

Wissler, L., Gadau, J., Simola, D.F., Helmkampf, M. and Bornberg-Bauer, E. (2013) Mechanisms and dynamics of orphan gene emergence in insect genomes. *Genome Biol. Evol.* **5**, 439–455.

Wu, W., Tabaska, J., Kovalic, D., Qiu, B.X. and Guo, L. (2015) Isolated novel nucleic acid and protein molecules from soy and methods of using those molecules to generate transgenic plants with enhanced agronomic traits. *Google Patents.*

Wuff, B.B., Horvath, D.M. and Ward, E.R. (2011) Improving immunity in crops: new tactics in an old game. *Curr. Opin. Plant Biol.* **14**, 468–476.

Yang, C., Guo, R., Jie, F., Nettleton, D., Peng, J., Carr, T., Yeakley, J.M. et al. (2007) Spatial analysis of *Arabidopsis thaliana* gene expression in response to tumip mosaic virus infection. *Mol. Plant Microbe Interact.* **20**, 358–370.

Zhang, X., Myers, A.M. and James, M.G. (2005) Mutations affecting starch synthase III in Arabidopsis alter leaf starch structure and increase the rate of starch synthesis. *Plant Physiol.* **138**, 663–674.

Zhang, X., Zhu, Z., An, F., Hao, D., Li, P., Song, J., Yi, C. et al. (2014) Jasmonate-activated MYC2 represses ETHYLENE INSENSITIVE3 activity to antagonize ethylene-promoted apical hook formation in Arabidopsis. *Plant Cell* **26**, 1105–1117.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** AtQQS and AtNF-YC4 transcript levels are altered in response to plant pathogens.

**Figure S2** Aphid performance on Arabidopsis QQS and NF-YC4 mutants.

**Figure S3** SCN female counts were decreased in soybean AtQQS- and GmNF-YC4-1-OE mutants after a single 30-d nematode generation.

**Figure S4** Starch accumulation, and AtQQS and AtNF-YC4 transcript levels in Arabidopsis QQS and NF-YC4 mutants under short-day conditions.

**Figure S5** QQS and NF-YC interaction.

**Figure S6** Interaction of the N-terminal region in structure 4 AWL.

**Figure S7** Searching the QQS-like protein in patented protein sequence database.

**Table S1** Genes with significant changes in the QQS-OE and QQS RNAi mutants. (See separate Excel file.)

**Table S2** Expression of five genes involved in plant defense have altered expression in plant lines that overexpress or underexpress QQS.

**Table S3** Mutants in starch metabolism with altered QQS or NF-YC4 transcript level, altered starch/protein level and their resistance to pathogens.

**Table S4** Sequences of primers and DNA oligonucleotides used for Figure 5.

**Table S5** Sequence information for selected sequences from Figure S7.

**Appendix S1** Supplementary experimental procedures.