Resistance and biomass in Arabidopsis: a new model for Salicylic acid perception.

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Summary.

Salicylic acid (SA) is an essential hormone for plant defence and development. SA perception is usually measured by counting the number of pathogens that grow *in planta* upon an exogenous application of the hormone. We propose a biological SA perception model based on plant fresh weight reduction caused by disease resistance in *Arabidopsis thaliana*. This effect is more noticeable when a chemical analogue of SA is used, such as Benzothiadiazole (BTH). By spraying BTH several times, a substantial difference in plant biomass is observed when compared with the mock treatment. This difference is dose-dependent and does not require pathogen inoculation. The model is robust and allows for the comparison of different Arabidopsis ecotypes, recombinant inbreed lines, and mutants. Our results show that two mutants, *non-expresser of pathogenesis-related genes 1* (*npr1*) and *auxin resistant 3* (*axr3*), fail to lose biomass when BTH is applied. Further experiments showed that *axr3* responds to SA and BTH in terms of defence induction. *NPR1*-related genotypes also confirm the pivotal role of NPR1 in SA perception, and suggest an active program of depletion of resources in the infected tissues.

Introduction.

Plants possess a sophisticated defence system that is triggered depending on the nature of the pathogen. Some plant defences are specialized in necrotrophic pathogens (van Kan 2006) while others are effective against biotrophic pathogens (Bent and Mackey 2007). Salicylic acid (SA) is a key molecule in the triggering of plant defences against biotrophs. SA is also relevant for some developmental events (e.g. Vanacker et al. 2001 and Martinez et al. 2004). Despite its importance in defence, little is still known about this hormone. In Arabidopsis (*Arabidopsis thaliana*) there are transgenic lines have been developed that degrade SA (*NahG*, Friedrich et al. 1995) while others produce more SA (*c-SAS* and *p-SAS*, Mauch et al. 2001). There are also two mutants impaired in SA biosynthesis, such as *eds5/sid1* (Nawrath et al. 2002) and *sid2/ics1* (Wildermuth et al. 2001). Additionally, there are other mutants with less SA, e.g. *eds1* and *pad4* (Wiermer et al. 2005). SA biosynthesis is under a positive feedback loop; SA triggers the expression of *EDS1* and *PAD4*, and these genes are required for the expression of the SA biosynthetic genes. The metabolism of SA is also under control (Shah 2003). Most of the SA present in the plant is conjugated with glucose, forming a pool of temporary inactive SA that can be slowly released in an active form (Nawrath et al. 2005).

The scientific community has made an important effort to find the SA receptor. *NPR1* is the only known gene that, when mutated, renders plants insensitive to SA, and yet it is not clear if it is the receptor itself. It has been found in at least four different screenings (Cao et al. 1994, Delaney et al. 1995, Glazebrook et al.

1996, and Shah et al. 1997), which indicates the essential role played by this gene in SA perception. NPR1 has shown to accumulate in the cytosol and migrate to the nucleus upon SA perception. In the proposed model, SA triggers the expression of a thioredoxin that acts over NPR1 oligomers, rendering monomers that migrate to the nucleus (Tada et al. 2008). NPR1 is degraded by the proteosome in the nucleus, a process that is required for activation of defence when SA is present (Spoel et al. 2009).

In parallel with the search for mutants, other biochemical approaches aimed at searching for proteins with SA binding. While some of the candidates have a strong affinity (Kumar and Klessig 2003) it is likely that none of them is a conventional SA receptor, if such a thing exists.

An intriguing feature of plant defences is the resulting loss of fitness (Heil 2002). It may seem intuitive that, upon a pathogen insult, the plant produces toxic compounds that negatively affect the plant, but other mechanisms can be proposed. For example, the plant may prioritize defence vs. development, redirecting all available resources to stop invasion. A third option is a “scorching earth defence”, i.e. the tissue where the pathogen is perceived is deprived of nutrients. SA negatively regulates the effect of the auxins, and this hormone is a good candidate to be the vehicle used to reduce plant development when a strong defence is triggered (Wang, D. et al. 2007a).

In order to find the genes required for SA perception and its consequences, we had to screen and accurately measure different Arabidopsis genotypes. The exponential growth of the pathogens used (Katagiri et al. 2002) proved to be a handicap. The relationship between plant defence and development is also

affected by the presence of the pathogen in the system. Therefore, we have developed a biological model for the perception of SA in Arabidopsis not based on pathogen inoculation. Instead, we apply benzothiadiazole (BTH). BTH is a biotechnological development of the research in plant defence (Lawton et al. 1996), a chemical analogous of SA that triggers plant defence and biomass loss in a consistent and dose-dependent manner. With this system, we are capable of analyzing the natural and artificial variations of Arabidopsis in response to SA. Small variations were found in both cases. Arabidopsis ecotypes showed no extreme behaviour, and only two mutants were selected, *axr3-1* (A semidominant allele, Ouellet et al. 2001) and *npr1.* Complementary experiments demonstrated that *axr3* can perceive SA, confirming the unique role of *NPR1* and related genes. We also found that the presence of *sni1* in the plant (Li et al. 1999) implies that *NPR1* is relevant for a programmed down-regulation of plant metabolism which could affect the pathogen.

Results.

An experimental model for SA perception.

We are interested in unveiling the signal transduction that starts with salicylic acid (SA) application to Arabidopsis, and results in the triggering of the plant defences. The amounts of SA that trigger plant resistance are close to phytotoxicity, and this is why BTH (Lawton et al. 1996) is commonly used in the laboratory. BTH is a chemical analogous of SA with no phytotoxicity, and it is commercialized under different names (Actigard® and BION® among others, www.syngenta.com). The standard way of measuring SA perception is by a western blot of a defence marker (i.e. PR2, Cao et al. 1997), or by monitoring the growth of a inoculated pathogen, (e.g. *Pseudomonas syringae pv tomato* DC3000 (*Pto*), Katagiri et al. 2002). Figure 1a shows the result of *Pto* inoculation. *Pto* is able to grow several orders of magnitude more in mock-treated plants than in BTH 350 µM treated plants. While the procedure of inoculating and measuring *Pto* is straightforward (Tornero and Dangl 2001), it is subject to important variations; small changes in the initial conditions can lead to a lack of reproducibility. Besides, factors that affect pathogen growth also affect *Pto* measurement. During the experiments, we noticed that BTH treated plants have less biomass than mock-treated plants (Figure 1b). This fact has been described in (Heil 2002) and it is indicated in the label of the commercial product (www.syngenta.com). We repeated the experiments without pathogen inoculation and obtained the same results (data not shown); a single 350 µM application of BTH can reduce Arabidopsis biomass. Since a single treatment lacks reproducibility and there was statistical overlapping between mock vs.

BTH treated plants, we tried different applications and treatments. Briefly, we applied BTH by imbibing, drenching, spraying, and in vitro culture (data not shown). The optimal method consists of spraying BTH four times for two weeks (see Experimental procedures) and recording plant weight when the plants are three weeks old. No special limit was observed after four treatments; up to eight treatments were applied during four weeks with no evident toxicity (data not shown). Increasing the number of treatments improves the difference between mock vs. BTH treated plants when the plants have enough room to grow. When the treatment finished, Col-0 plants outgrew the treatment and were able to set seeds.

The results are more clearly shown when the ratio between BTH and mock treated plants is used (Figure 1c). Different BTH concentrations were used to find the optimal option, starting with 350 µM (used in Figure 1a) and diluting by a factor of 10. BTH concentrations higher than 3.5 µM still produced a measurable effect on Arabidopsis biomass, whereas lower BTH concentrations failed to differentiate mock and BTH treated plants. Therefore, 350 µM is the standard BTH concentration or “High BTH”, and 350 nM the subclinical BTH concentration or “Low BTH”.

Phenotypes of the model

The differences in plant biomass caused by High BTH are numerically significant, corresponding to the strong phenotype of Figure 2a. Figure 2a shows Col-0 treated with mock (left) and High BTH (right); observe the difference in plant size, while the number of leaves is similar. Therefore, a visual

inspection can discern in most cases whether a genotype responds or not to BTH. This is a simple way to evaluate SA perception and characterize the response to BTH in the ecotype Col-0. There were no observable macroscopic plant lesions, so we checked for microscopic lesions. Trypan blue staining pinpoints cell death and membrane damage (and fungi if present, Keogh et al. 1980). Figure 2b, c and d show the Trypan Blue staining of cotyledons from plants treated with mock, Low BTH, and High BTH respectively. While subclinical BTH concentrations produced no measurable effects, standard amounts of BTH triggered program cell death in a small number of cells. Callose depositions are a hallmark of defence induction, and are easily seen with aniline blue under ultraviolet light (Conrath et al. 1989). Therefore, cotyledons of mock, Low BTH, and High BTH treated plants were stained with aniline blue (Figure 2e, f, and g, respectively. Figure 2h, i and j show the same cotyledons exposed to visible light). The result is that mock and subclinical BTH concentrations do not produce callose deposition. Standard BTH concentrations, on the other hand, produce abundant callose depositions, of several sizes and distributions. A 3.3' diaminobenzidine stain showed no difference in Reactive Oxygen Species (ROS) at the time of the sampling (data not shown).

SA is a hormone with a fine-regulated homeostasis. Thus, there is evidence of a positive feedback loop in SA synthesis and of negative regulation upon SA perception (Shah 2003). A plausible explanation of BTH effects on biomass is that it sets in motion a feedback loop that runs unchecked. To verify this hypothesis, the amounts of SA (free and total) in mock, Low BTH, and High

BTH treated plants were measured (Figure 3a). Low BTH induced a small and reproducible increase in the amount of total and free SA (all these values are in agreement with reported concentrations (Defraia et al. 2008)), while High BTH produced a decrease in free SA and a strong decrease in the total amount of SA. The subclinical amounts of BTH do not induce the expression of the marker PR1 (Figure 3b), a standard stress marker (Uknes et al. 1992), nor enough resistance to be detected in *Pto* growth curves (data not shown). Standard BTH concentrations induced a strong PR1 expression, even if the western blot was repeated with only mock and subclinical BTH treatments to avoid a possible signal masking due to the strong High BTH signal (data not shown).

Natural variation and SA perception.

Once the right conditions were set, we evaluated if Col-0 was the best ecotype to work with. Figure 4 shows the analysis of two sets of ecotypes and Col-0. Figure 4a corresponds to a nuclear core collection of 48 ecotypes (McKhann et al. 2004), while Figures 4b and c shows a set of 96 ecotypes (Nordborg et al. 2005). Col-0 is a valid representative of the ecotypes tested; in the three panels it ranked in the middle of the ecotypes (between 40^{th} and 56^{th} percentiles) when ordered by percentage of plant fresh weight (PFW). Some ecotypes like Col-0, Ws-0, Laer-0 and No-0 were repeated with different stocks (e.g., Col-3, Col-4, Col-5, etc), because they are the background of mutations or are used for mapping. None of them behaved differently (data not shown).

Another option in Natural Variation is to search for quantitative trait locus (QTLs) in mapping populations. This can be done even if the parentals have a

similar behaviour, a phenomena called transgression (Koornneef et al. 2006). We analyzed seven recombinant inbreed lines (RILs) available at the beginning of this research searching for transgression. The RILs were: Col-0 x Nd-1 (Deslandes et al. 1998), Col-gl1 x Kas-1 (Wilson et al. 2001), Cvi-1 x Laer-2 (Alonso-Blanco et al. 1998), Laer-0 x Sha-0 (Clerkx et al. 2004), Bay-0 x Sha-0 (Loudet et al. 2002), Col-4 x Laer-0 (Lister and Dean 1993), and Laer-0 x No-0 (Magliano et al. 2005) (Figure 5 and data not shown). There are three QTLs detected only in the fresh weight of the mock-treated plants (Colgl1 x Kas-1, Laer-0 x Sha-0 and Laer-0 x No-0, Figure 5b, d and e, respectively). There is, however, no significant QTL specific of the response to BTH in terms of fresh weight.

Most signal transductions do not affect SA perception.

The next step was to analyze the wealth of information generated in the form of mutants. SA biosynthesis is regulated by a positive feedback loop, so the first objective was to analyze the mutations related to SA. Thus, we assayed the mutant that failed to perceive SA; *npr1*, mutants of SA biosynthesis; *eds5* and *sid2*, transgenic lines with altered SA content (*NahG* less SA, and *c-SAS* more SA); and mutants with a down regulation of SA biosynthesis, *eds1* and *pad4* (Figure 6a). Only *npr1* failed to respond to SA. This clear result prompted us to keep *npr1* as a negative control, and to extend the list of mutants in defence (Figure 6a and b). Then, we tested mutants in basal resistance (either more resistant or more susceptible), Systemic Acquired Resistance (SAR), specific

resistance, and non-host resistance. None of the tested mutants in defence, except *npr1*, differed from the wild type (wt) in their response to BTH.

SA signal transduction has been reported to crosstalk with several signal transductions (Lopez et al. 2008), Jasmonic Acid, Ethylene, Abscisic Acid, Auxins, Light and ROS being the most commonly cited. Therefore, the response to BTH of a representative set of mutants in each of these pathways was measured. For the Auxin pathway, nineteen mutants were tested (Figure 6c), and only *axr3* did not respond to BTH in a consistent manner. Note that the allele used in this work is *axr3-1*, a semidominant mutation that enhances the stability of the protein (Ouellet et al. 2001). Mutants in other pathways, like Light (Figure 6c), Abscisic Acid (Figure 6d), Ethylene (Figure 6d), ROS (Figure 6d) or Jasmonic Acid and/or response to necrotrophs (Figure 6e), had a similar response to BTH as wt. A complete list of the mutants tested is provided in Table S1.

axr3 and _npr1_ show a distinct response to SA.

The conclusion of Figure 6 and other data not presented is that from a total of 98 mutants tested, only two did not respond to BTH; *npr1* and *axr3*. *NPR1* is a gene clearly involved in SA perception, but the result of *axr3* was unexpected. While it is tempting to discard *axr3* due to the small size of this mutant, other small mutants like *cpr1* (Bowling et al. 1994), show percentages of fresh weight in the same order of magnitude as the wt (Figure 6a). Therefore, a detailed characterization of *axr3* in terms of response to SA and BTH was performed. Figure 7a shows *Pto* growth in Col-0, *npr1* and *axr3* pretreated with mock or

High BTH. Clearly, BTH is able to trigger defence in *axr3*, as opposed to the effect caused in *npr1*. The levels of the PR1 protein were determined by western blot (Figure 7b) in plants either treated with mock or BTH 350 µM and basically showed the same results; while *npr1* fails to induce this defence marker upon High BTH, *axr3* is able to increase the expression of this defence protein. Note that in *axr3* plants there is a small but detectable amount of PR1 even in the mock treatment.

An interesting feature of mutations in *npr1* is that it fails to regulate the levels of SA (Cao et al. 1997). When growing *npr1* in MS plates supplemented with SA 500 µM, the cotyledons are bleached and the plant is unable to grow (Figure 7c). The easiest interpretation is that *npr1* fails to perceive SA, and therefore is unable to trigger SA degradation and SA accumulation has deleterious effects. Col-0 and *axr3* plants, in the other hand, grow in plates containing SA 500 µM (Figure 7c).

npr1-related genes and SA perception

Then we focused on *npr1* and related genes. The previous experiments were repeated with *npr1-1*, but there are eleven alleles of *npr1* (Cao et al. 1994, Delaney et al. 1995, Glazebrook et al. 1996, and Shah et al. 1997). We assayed four of them (Figure 8), and -with some variation- all the alleles tested show no response to BTH in terms of PFW. There are no mutants with an increasing sensitivity to SA; therefore the next best candidates are the transgenics that overexpress *NPR1*. *35S:NPR1* is an overexpression of *NPR1*, and the plants can perceive BTH more strongly, as reported in the literature (Cao et al. 1998).

35S:NPR1:HBD is a version of *NPR1* fused to the glucocorticoid receptor in a *npr1* background (Wang, D. et al. 2005). The result is a protein not subjected to the nuclear vs. cytoplasm traffic, vital to its function in SA perception (Dong 2004). Figure 8 shows that the mere presence of NPR1 in the cytosol is not enough to trigger response to BTH and the nuclear localization is required.

There are five genes in Arabidopsis with a high homology to *NPR1* (Liu et al. 2005). *NPR3* and *NPR4* have been reported to play a key role in plant defence (Zhang et al. 2006) and mutations in *BOP1* and *BOP2* affect the identity of the floral organs and the shape of the leaves (Ha et al. 2007; McKim et al. 2008). Plants from these two double mutants respond to BTH in the same way as in wt (Figure 8).

Regarding its biochemistry, NPR1 has been shown to interact in yeast two-hybrid with two sets of proteins, TGAs (Zhang et al. 1999) and NIMINs (Weigel et al. 2001), and in vivo with some of them. T-DNA insertions in *TGA1* and *TGA7* show small but consistent differences between these mutants and wt in their response to BTH (Figure 8). This small effect is more noticeable when a triple mutant *tga6 tga2 tga5* is used (Zhang et al. 2003), and the plants show an intermediate macroscopic phenotype (data not shown).

The *npr1* phenotypes are quite straightforward, which has lead to a number of suppressor screenings. One of these suppressors is *sni1* (Li et al. 1999), and the double *sni1 npr1* regains the ability to activate defences upon BTH application. Interestingly, the double *sni1 npr1* does not behave as a suppressor in our system (Figure 8). We also tested T3 seeds from insertions in the homolog *NPR2*, the interactors *NIMIN1, NIMIN2, NIMIN3, TGA3* and *TGA4* and

the suppressors *SSI2* (Shah et al. 2001) *SON1* (Kim and Delaney 2002), and *SNI1* but no *npr1*-like phenotype was observed in the segregating families (data not shown).

Discussion

SA perception and plant fitness

Salicylic acid is a necessary hormone in plants for full resistance against biotrophic pathogens such as *Pto*. While the amount of SA can be measured in the laboratory (Defraia et al. 2008), for the quantification of SA perception we usually rely on the growth of the pathogen we are interested to start with. This is a potential circular problem, since we use a tool to answer a question that affects the tool.

Another potential problem is the nature of the pathogen. Pathogens like *Pto* grow exponentially, and small differences in the input lead to considerable differences in the output. There are alternatives, like immunodetection of defence markers (Uknes et al. 1992, Figures 2 and 7), or measurement of phytoalexin accumulation (Glazebrook and Ausubel 1994). These alternatives can produce quantitative data, but are not suitable for high throughput assays.

One side effect of several resistances is their negative effect on plant fitness (Heil 2002). In general, the more resistant an individual is, the less fit it is to compete when the pathogen is not present. There are several hypotheses to explain this fact. The first is that, since the plant produces molecules that eventually stop the growth of the pathogen, it is plausible that the same molecules affect the plant. Other alternative is an economic consideration; the triggering of the defence genes involves the use of resources that have to be obtained from normal plant growth. A somewhat related argument is the “scorching earth” defence, where by turning basal metabolism down, the plant negates the pathogen the nutrients that it requires.

Resistance and fresh weight are inversely correlated

In the case of BTH, a single application can produce measurable effects in terms of plant fresh weight (Figure 1b). This subtle effect measured four days after a single BTH treatment was optimized for measurement and screen. While different ways of applying BTH produce visible differences, the best condition for our goals is to spray the plants with BTH four times on separated dates (see Experimental procedures). This procedure provides us with an accurate quantification of genotypes such as the mutants and ecotypes described above (Figure 1c). But most importantly, it gives us a screen (Figure S1) that can be used to search for new mutants in a high-throughput fashion. In principle, this model is analogous to screen for mutants in auxin perception with plates of 2,4-D (Maher and Martindale 1980). 2,4-D is more stable and have a stronger effect in the plant that the endogenous auxin, like BTH vs. SA. The main differences are that BTH does not work in plates, and it is not lethal. But in both cases we can recover mutations impaired in the perception of the hormone by using an analogue and a set of extreme conditions (Mockaitis and Estelle 2008).

In order to use the biological model, several steps have to be taken. First we need to characterize plant response in terms of macroscopic, microscopic and molecular phenotypes, to be sure that the observed effects on fresh weight correspond to the activation of plant defences. Second is the ecotype to be used, because Col-0 may not be the best background. And thirdly, there is the question of genetic specificity; the biological model proposed should not mislabel mutants that affect the growth of the pathogen as a mutant in SA

perception (e.g. *cpr1,* Figure 6a), and it should correctly label *npr1* as defective in SA perception.

The response to BTH in terms of PFW is dose-dependent (Figure 1c). The highest BTH concentration tested is 350 µM, a concentration frequently used in Arabidopsis (Lawton et al. 1996). To put it in context, this corresponds to approximately nine times the dose recommended in tomato for *Pto* infection (www.epa.gov), but it is six times lower than SA concentrations used in Arabidopsis (2 mM, e.g. Aviv et al. 2002). The loss of fresh weight can be detected as low as 3.5 µM, but not at 350 nM. Low BTH is unable to trigger cell death or callose deposition (Figure 2c and f). High BTH, on the other hand, is able to cause cell death in a small number of cells (Figure 2d), as also reported in the literature for SAR (Alvarez et al. 1998) and labelled as micro-HRs. While a plausible hypothesis was that these micro-HR sites are similar, we did not observe any oxidative burst (data not shown). It is therefore possible that the micro-HRs are different, and while in SAR they are caused by oxidative burst, the cell death shown in Figure 2d is caused by other effector. Another alternative is that in our model a transient oxidative burst occurs immediately after the treatments, but it disappears when the tissue is stained (5 days after the last treatment). In any case, the small number of cell deaths observed does not account for the difference in PFW, and it seems an effect rather than a cause of resistance. While it has been reported that BTH by itself does not strongly trigger callose depositions (Kohler et al. 2002), a second mock treatment after BTH had the ability to do so. Consistently with this result, (we

sprayed the plant several times), there is a strong callose staining with High BTH.

The next step was to analyze the molecular events that occur in this system. The amount of SA is under the control of feedback loops, positive in SA biosynthesis and negative in SA accumulation (Shah 2003). Therefore, it was relevant to measure the amount of SA in this system. High BTH produced a strong reduction in the amount of total SA (Figure 3a). There is a small reduction in the amount of free SA, but it is clear that the plant responded to High BTH with a reduction of the conjugated form of SA (mainly glucoside) (Nawrath et al. 2005). Therefore, this constitutes additional evidence in favour of a negative feedback loop that regulates the accumulation of SA. The other piece of evidence is the amount of SA in *npr1* (Cao et al. 1997). This mutant has more SA than the wt, both in mock and pathogen-inoculated plants. Another form of this phenotype is the low tolerance of *npr1* plants to SA in vitro (Figure 7c). It can not detect SA, and therefore it can not avoid SA accumulation and toxicity.

The detection of the defence marker PR1 (Figure 3b) and *Pto* growth (Figure 1a and data not shown) confirm that fresh weight loss and disease defence are closely correlated, as low concentrations that do not produce fresh weight loss, do not trigger defence. Correspondingly, high concentrations are able to produce both phenotypes.

SA perception in natural variation

Before starting the search for new mutants, the best genotype has to be chosen. Col-0 is the ecotype most widely used for mutant screening (www.arabidopsis.org), but it could be an extreme ecotype in response to BTH. Figure 4 shows that Col-0 is a representative Arabidopsis ecotype, because it ranks between the 40th and 56th percentile among the collections tested. Another reason for these experiments was to search for natural variation, but there is no extreme ecotype in the response to BTH.

We also searched for transgression in seven RILs (Figure 5 and data not shown), but found none. The three QTLs found are only relevant to the differences in growth when a mock treatment is applied, but there is no difference in the response to BTH. This does not mean that there are not variations in the SA response (van Leeuwen et al. 2007), but with the populations and system under study, none was both significant and specific to SA perception.

SA perception in defence and signalling mutants

From the comprehensive list of mutants tested, there is no evidence of desensitization. That is, mutants that have more SA than in wt (*e.g. c-SAS* and *cpr1*) are still able to respond to exogenous BTH applications (Figure 6). A direct consequence is that we can assay genotypes that are more resistant to bacteria and unequivocally discriminate if it is due to an enhanced SA perception. So far we have found no evidence for such genotype, with the exception of *35S:NPR1* (see below). Regarding the different kind of defences,

mutants in SAR, basal, specific (or gene-for-gene) and non-host resistance were tested and found not to be different from the wt, with the exception of *npr1*, as discussed below.

The more we study plant biology, the clearer it becomes that everything is interconnected. If two decades ago plant defence and development could be seen as two separate programs, evidence in the last years reveals a much more intricate signal network with complex interactions. Thus, there are reports on the interactions between SA and Auxins, Light perception, Ethylene, Jasmonic Acid, Abscisic Acid and ROS, among others (reviewed by Lopez et al. 2008). Mutations in pathways different than Auxin do not have a measurable impact on SA perception when measured as described. Regarding Auxins, only *axr3* does not respond to BTH in weight, and there is no visible difference between mock and BTH treated plants (data not shown). *AXR3* belongs to the family of IAAs, genes that are rapidly induced with auxins, and behave as activators or repressors of the auxin response (Reed 2001). The allele of *axr3* used is a dominant mutant that stabilizes the protein, causing an increase in auxin perception and phenocopying the overexpression of the wt protein (Ouellet et al. 2001). It is tempting to speculate that AXR3 is the link between defence and development.

In favour of this hypothesis, there are solid evidences of the interaction between SA and Auxins (Wang, D. et al. 2007a) and the overexpression of *AXR3* reproduces the *axr3* phenotype (Reed 2001). Thus, the phenotype that responds to BTH could be explained by an increase in the amount of the AXR3 protein. However, this hypothesis has serious drawbacks. *AXR3* is slightly

repressed under pathogenic conditions (www.genevestigator.com), which does not fit with a prominent role in the response to BTH. Mechanistically, exogenous Auxin applications reduces SA perception (Wang, D. et al. 2007a). But *axr3* has Auxin hypersensitivity, so instead of sensing more SA, it should perceive less SA, which contradicts the model. A closer examination proves that *axr3* is indeed able to perceive SA and BTH, as measured by *Pto* growth, western blot of PR1, and tolerance to SA in plates (Figure 7). This perception is slightly attenuated (Figure 7a and 7b), as expected by the interaction between Auxins and SA.

The second hypothesis is that the small size of the plant does not allow it to lose weight, as it is already at minimal levels. The average weight of *axr3* in mock is minor than Col-0 with BTH in Figure 6c, while in other replications both weights were similar (data not shown). The difference with the first hypothesis is that the small fresh weight is not related to the interaction between resistance and development, and other mutants of small size could present no differences between mock and BTH. The results of our experiments support this second hypothesis.

NPR1- related genotypes mark the relationship between plant defence and development.

NPR1 is a gene necessary for SA perception (Figure 6), among other roles in plant defence (Pieterse and Van Loon 2004) and development (Vanacker et al. 2001). The extreme *npr1* phenotype in response to BTH (Figure 6) is not allele specific, because the available alleles behave in the same way. It is worth

mentioning that the *npr1-3* allele is still functional for the so-called Induced Systemic Resistance (Pieterse and Van Loon 2004). In fact, the overexpression of *NPR1* fused to the glucocorticoid receptor (*35S:NPR1:HBD* in Figure 8) reproduces the same phenomenon, i.e. a functional NPR1 protein that is unable to migrate to the nucleus. Therefore, the response to BTH is dependent on the NPR1 protein acting in the nucleus. The overexpression of *NPR1* produces an increasing sensitivity to SA and its analogues in terms of pathogen growth and defence markers (Cao et al. 1998; Friedrich et al. 2001), and we can reproducibly detect this enhanced SA perception (Figure 8).

In the Arabidopsis genome there are five genes with high homology to *NPR1* (Liu et al. 2005). Certain functional redundancy could exist in the genes of this family; therefore we assayed loss of function mutations in these genes. Fortunately, there are two double mutants available, *npr3 npr4* and *bop1 bop2*, and none of them has a consistent difference with wt. In the case of *NPR2*, T3 seeds from a T-DNA insertion (Table S1) were found to be like wt (data not shown). Therefore, there is no measurable functional redundancy, at least in a *NPR1* wt background.

NPR1 interacts with proteins from two families of genes, *TGAs* (Zhang et al. 1999) and *NIMINs* (Weigel et al. 2001). *TGAs* are a subclass of the family of *bZIP* transcription factors (Jakoby et al. 2002) that physically interact with promoters of PR genes (Johnson et al. 2003). The transcriptional activation of these PR genes is dependent on the interaction of the TGAs with NPR1 (Després et al. 2000). There are reports about functional redundancy in this family (Jakoby et al. 2002), and yet T-DNA insertions in *tga1* and *tga7* have a

small but measurable phenotype in our model (Figure 8). The best indication of the significant role of this gene family in SA perception is the phenotype of the triple mutant *tga6 tga2 tga5* (Zhang et al. 2003, Figure 8). In this case the phenotype is visible to the naked eye (data not shown). T3 seeds from T-DNA insertions in *TGA3* and *TGA4* (Table S1) were phenotypically similar to wt (data not shown). *NIMINs* are a family of three small genes, and their proteins interact in vitro with NPR1. Mechanistically, *NIMIN* genes would act as repressors of SA signalling (Weigel et al. 2001). T3 seeds from T-DNA insertions in *NIMIN1, NIMIN2* and *NIMIN3* (Table S1) were found to behave like wt (data not shown).

NPR1 is the only gene necessary for SA perception, and several suppressor screenings have been made to identify other players (Li et al. 1999). T3 seeds from T-DNA insertions in *SSI2*, and *SNI1* behave like wt (data not shown). Interestingly, the double *sni1 npr1* does not behave as a suppressor in our system (Figure 8). *SNI1* encodes a nuclear protein rich in leucine and it is assumed to be a negative SAR regulator (Li et al. 1999). We were able to confirm the suppression of the *npr1* phenotype by *sni1* in *Pto* growth curves (data not shown), but not in weight. An obvious hypothesis is that the signal that goes from SA to NPR1 somehow splits into two; one is repressed by *SNI1* and activates defence genes (e.g. *PR1*), eventually causing the measurable reduction of the infection. The other is *SNI1* independent, and reduces the growth of the plant. This branching could be achieved through different signal thresholds, since *sni1* induces defences at lower concentrations of SA analogues (Li et al. 1999) both in wt and in *npr1*. In any case, the evidence that a genotype produces defence (PR gene expression included) with no loss of

fresh weight, contradicts the first two hypotheses presented to explain the interaction between plant defence and development (“defence is toxic”, and “defence is expensive”). Thus, the third hypothesis (“scorching earth defence”) is favoured by the results presented here. In other words, the plant has two programs: active synthesis of defences and active depletion of nutrients.

Experimental procedures.

Inoculation and plant treatment.

For all the experiments, *Arabidopsis thaliana* was sown in small pots, kept at 4 °C for 3 days and then transferred to growing conditions under a short-day regime (8 hours of light (150 µmol m-2 s-1) at 21 °C, 16 hours of dark at 19 °C). The treatments, inoculations, and sampling started 30 minutes after the initiation of the artificial day to ensure reproducibility. *Pseudomonas syringae pv. tomato* DC3000 (*Pto*) containing pVSP61 (empty vector) were maintained as described (Ritter and Dangl, 1996). The bacteria were grown, inoculated and measured as described (Tornero and Dangl 2001) with minor changes. Trypan Blue and Aniline Blue staining were performed as described (Tornero et al. 2002; Conrath et al. 1989, respectively). For all the experiments, three independent treatments were performed (three independent sets of plants sown and treated on different dates), only two in the case of the large collection of ecotypes.

BTH and fresh weight.

Benzothiadiazole (BTH, CGA 245704), in the form of commercial product (Bion® 50 WG, a gift from Syngenta Agro S.A. Spain) was prepared in water for each treatment and applied with a household sprayer. When indicated, a mock inoculation of distilled water was performed. The treatments were conducted on the 8^{th}, 11^{th}, 15^{th}, and 18^{th} day (day 0 is when plants are transferred to growing conditions), and the weight of the plants recorded on the 21^{st} day. For each genotype and treatment, 15 plants were weighed in 3 groups of 5. The mock treatment was considered to have a value of 100, and the average and

standard deviation of the percentage of the fresh weight resulting from the BTH treatment are represented.

Western blot.

Immunodetection of PR1 protein was carried out as described (Wang, D. et al. 2005), using an Amersham ECL Plus Western Blotting Detection Reagents (GE HealthCare, Little Chalfont, UK). The second antibody was a 1:25000 dilution of Anti-Rabbit IgG HRP Conjugate (Promega, Madison, USA). Chemiluminescent signals were detected using a LA-3000 Luminescent Image Analyzer (Fujifilm Life Science, Stamford, CT, USA).

SA in plates and *in planta*.

Arabidopsis seeds were surface-sterilized for 10 min in 70% ethanol and for 10 min in commercial bleach. Then, five washes were done with distilled water and the seeds were distributed on agar plates. The medium contains 0.5x Murashige and Skoog salts (Duchefa BV, Haarlem, the Netherlands), 0.6% (w/v) Phyto Agar (Duchefa), 2% (w/v) sucrose, with or without SA 500 µM (final concentration). The result was evaluated 10 days after transferring to growing conditions. For the measurement of SA *in planta*, three samples of 250 mg were frozen in liquid nitrogen. SA extraction was performed as described by (Mayda et al. 2000).

QTL mapping.

Plants of Arabidopsis were treated with either mock or benzothiadiazole (BTH) 350 µM as described above. The weight of five plants per line and the genotype of each line were used as input for the program WinQTLCart (Wang, S. et al. 2007b), that calculates the probability that a QTL is link to a particular region of the genome. The populations analyzed were: Col-0 x Nd-1, 98 lines (Deslandes et al. 1998); Col-gl1 x Kas-1, 115 lines (Wilson et al. 2001); Cvi-1 x Laer-2, 50 lines (Alonso-Blanco et al. 1998); Laer-0 x Sha-0, 114 lines (Clerkx et al. 2004); Bay-0 x Sha-0, 162 lines (data not shown, Loudet et al. 2002); Col-4 x Laer-0, 85 lines (Lister and Dean 1993); and Laer-0 x No-0, 135 lines (Magliano et al. 2005).

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Supporting information

Table S1. List of ecotypes, populations and mutants tested.

Figure S1. Picture of Col-0 and *npr1.1* after mock and BTH 350 µM treatments

Figure legends:

1.-BTH increases disease resistance and decreases biomass accumulation. (a), Arabidopsis plants were pretreated with either mock or benzothiadiazole (BTH) 350 µM and then inoculated with *Pseudomonas syringae pv tomato* isolate DC3000 (*Pto*) one day later. Three days later, the bacteria (measured as Logarithm of colony forming units per plant) were measured. (b), Plant weight of (a) before bacterial extraction, in mg of fresh weight. (c), The same effect after considerable optimization that includes four treatments (see Experimental procedures). All panels show the average and standard deviations, and at least three independent experiments were performed with similar results.

2.-The continuous triggering of plant resistance produces a distinctive macroscopic and microscopic phenotype. (a), Macroscopic phenotype of plants either treated with mock (left) or with BTH 350 µM (right) at the same time as Figure 1c. BTH-treated plants have the same number of leaves as mock-treated plants, and are able to survive and set seeds. (b), (c) and (d) correspond to Trypan blue stains, unveiling cell death and membrane damage. (e), (f) and (g) show Aniline blue stains under ultraviolet light, which detects callose depositions. (h), (i), and (j) are the same micrographs under visible light. (b), (e) and (h) are from representative plants treated with mock, (c), (f) and (i) are from BTH 350 nM treated plants, and (d), (g), and (j) are from BTH 350 µM treated plants. Only BTH 350 µM produces microscopic cell death in few and

isolated cells (dark blue staining outside the veins in (d)), and triggers plant defence, as observed in the callose depositions (fluorescent in (g)).

3.-SA accumulation and defence induction upon BTH application. (a) Quantification of SA upon mock, BTH 350 nM, and BTH 350 µM treatments as described in Figure 1c. Both free and total SA (i.e. glucosylated derivates released after hydrolysis plus the free SA) were measured, showing the average and standard deviations of three samples. (b) Western blot for PR1. This defence marker was immunodetected in samples from the same experiments as in (a). The arrow points to the expected size of PR1 (14 kDa).

4.- Arabidopsis ecotypes tested show a similar phenotype. Two collections of ecotypes were tested as described in Figure 1c. (a) Col-0 and the McKhann collection (McKhann et al. 2004) ranked for its percentage of fresh weight. (b) and (c) Col-0 and the Nordborg collection (Nordborg et al. 2005) were measured in two separate lots. The full names of the ecotypes shown, as well as other ecotypes tested, are listed in Table S1. None of the ecotypes tested shows an extreme behaviour under these conditions, and Col-0 ranked between 40^{th} and 56^{th} percentile in the three panels.

5.- There are no significant QTLs in the tested populations specific to SA perception. Plants were treated with either mock or BTH 350 µM as described in Figure 1c. The output showed is the logarithm of odds (LOD, in the Y axis) that a QTL is link to a particular region of the genome (X axis). The horizontal

line shows the threshold of significance. The continuous lines show the QTLs for mock, and the dotted line the QTLs for BTH treatment. The populations analyzed were (a) Col-0 x Nd-1, (b) Col-gl1 x Kas-1, (c) Cvi-1 x Laer-2, (d) Laer-0 x Sha-0, (e) Laer-0 x No-0, (f) Col-4 x Laer-0.

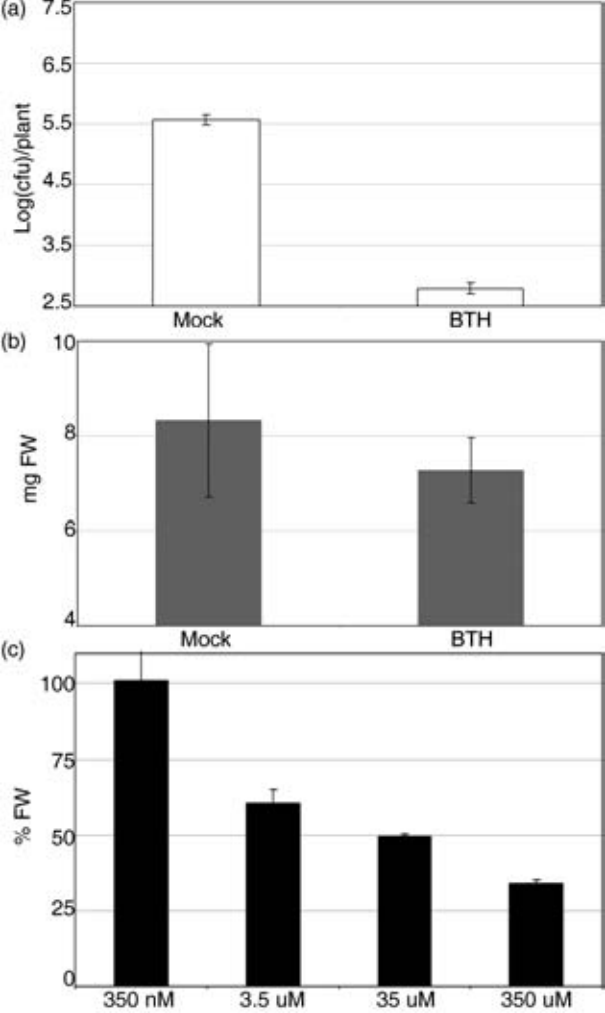
6.-A collection of mutants points to only two candidates for SA perception. All the mutants were tested as described in Figure 1c. The complete list is shown in Table S1. (a) and (b) show defence mutants, (c) corresponds to mutations in Auxin and Light signalling, (d) mutations in Abscisic Acid, Ethylene and Reactive Oxygen Species, and (e) mutations in Jasmonic Acid and/or response to necrotrophs. *35FM* stands for *35S:FMO1, 35DIR* is *35S:DIR1, phyA/B* is the double *phyA phyB, 35HAB* is *35S:HAB1, abi/hab* is *abi1-2 hab1-1, 35ERF* is *35S:ERF1, atrb-D* is *atrboh-D,* and *atrb-F* is *atrboh-F*.

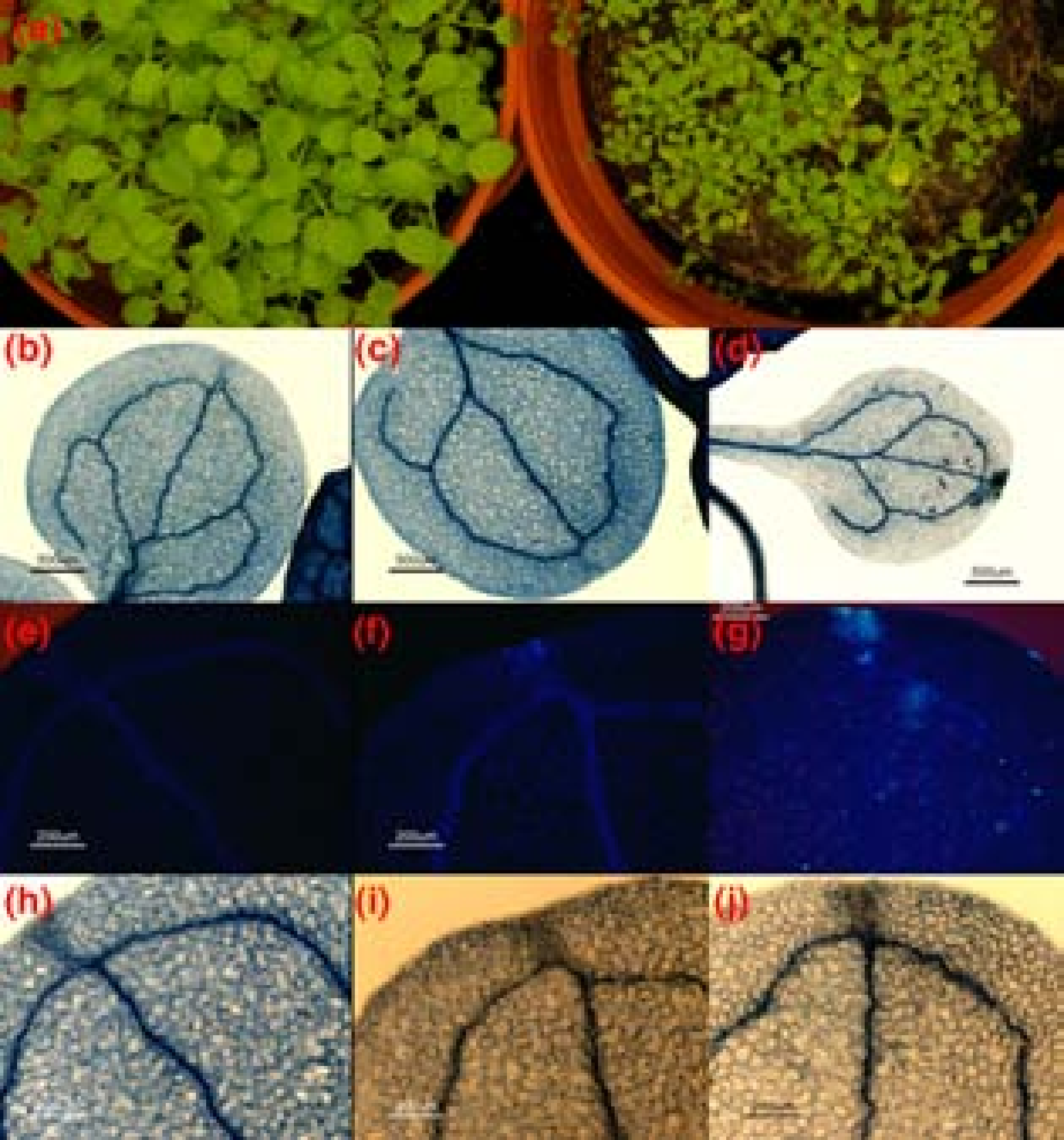
7.-*axr3* can sense SA and BTH. (a) *Pto* growth in Col-0, *npr1* and *axr3* treated either with mock or BTH 350 µM, as described in Figure 1a. (b) Western blot for PR1 of the same experiment as (a), but prior to bacterial inoculation. The arrow points to the expected size of PR1 (14 kDa). (c) Phenotype of the same genotypes in MS plates supplemented with SA 500 µM. *npr1* plants do not perceive SA and therefore can not avoid accumulation to toxic levels. Col-0 and *axr3* can perceive SA and are able to grow in this medium.

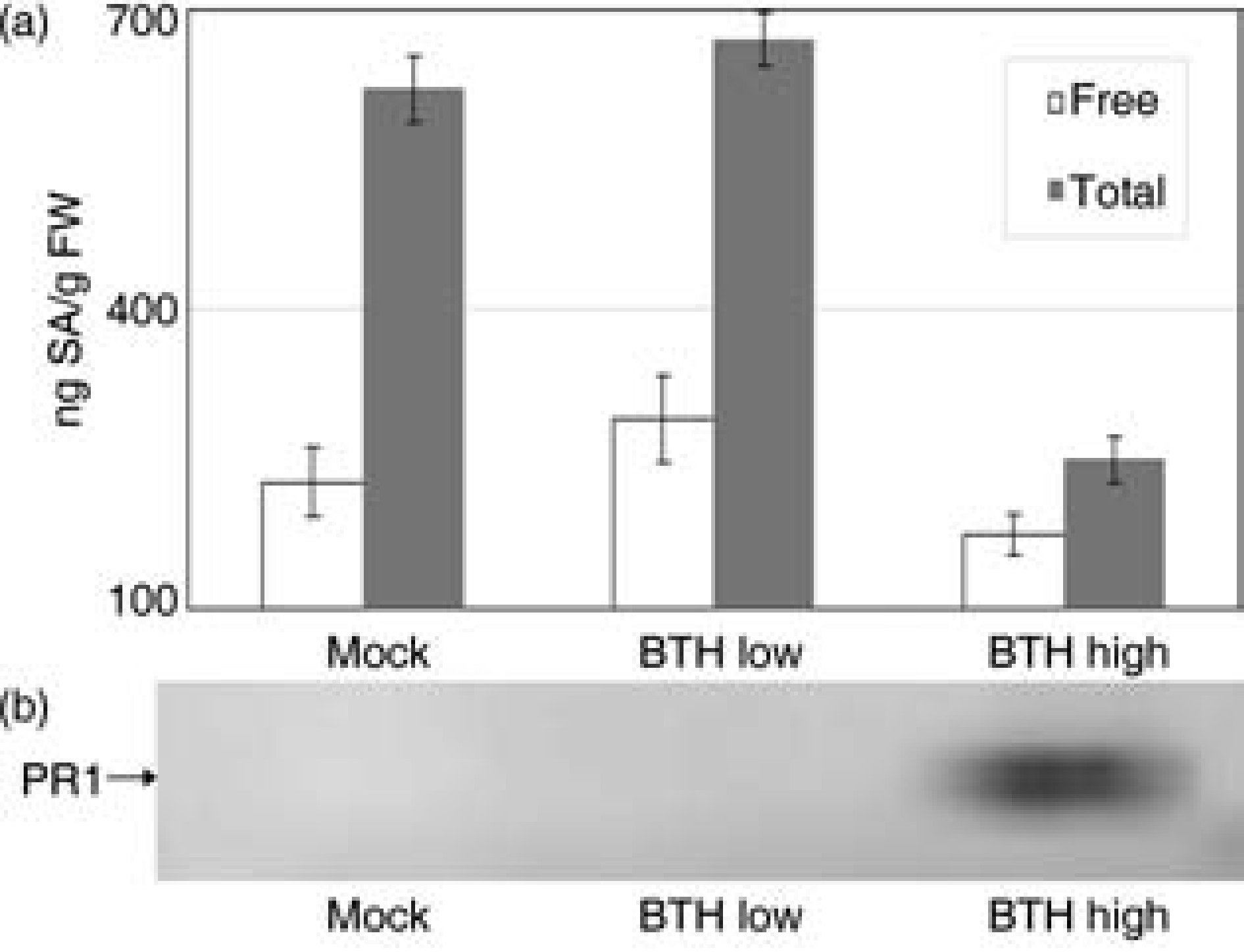
8.-Behaviour of genotypes related to NPR1 in SA perception. Alleles of *npr1*, loss of function of related genes and transgenic plants that overexpress

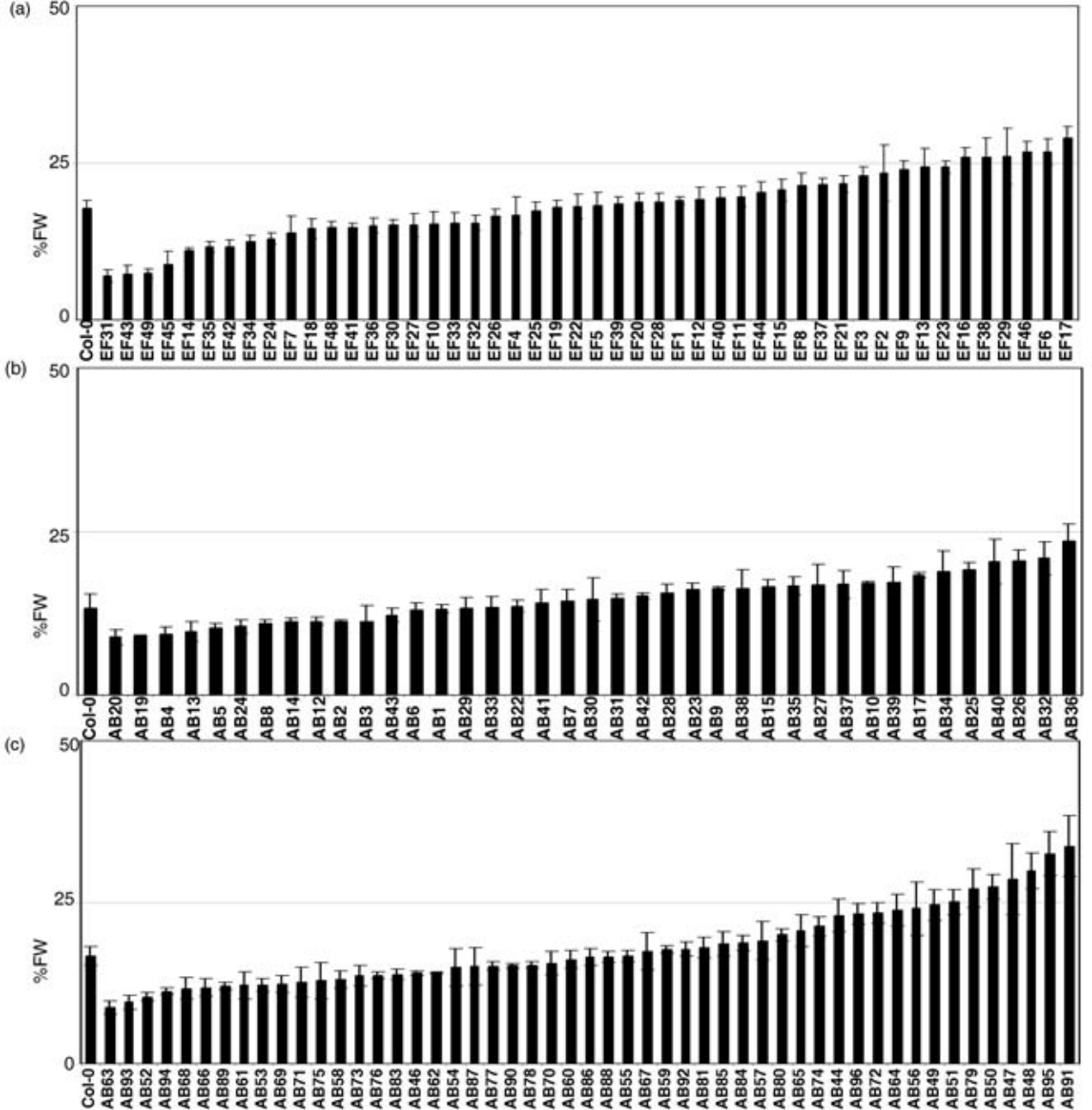
the protein were assayed as described in Figure 1c. *3xtga* stands for the triple mutant *tga6 tga2 tga5* and *NPR1HBD* for the transgenic *35S:NPR1:HBD*.

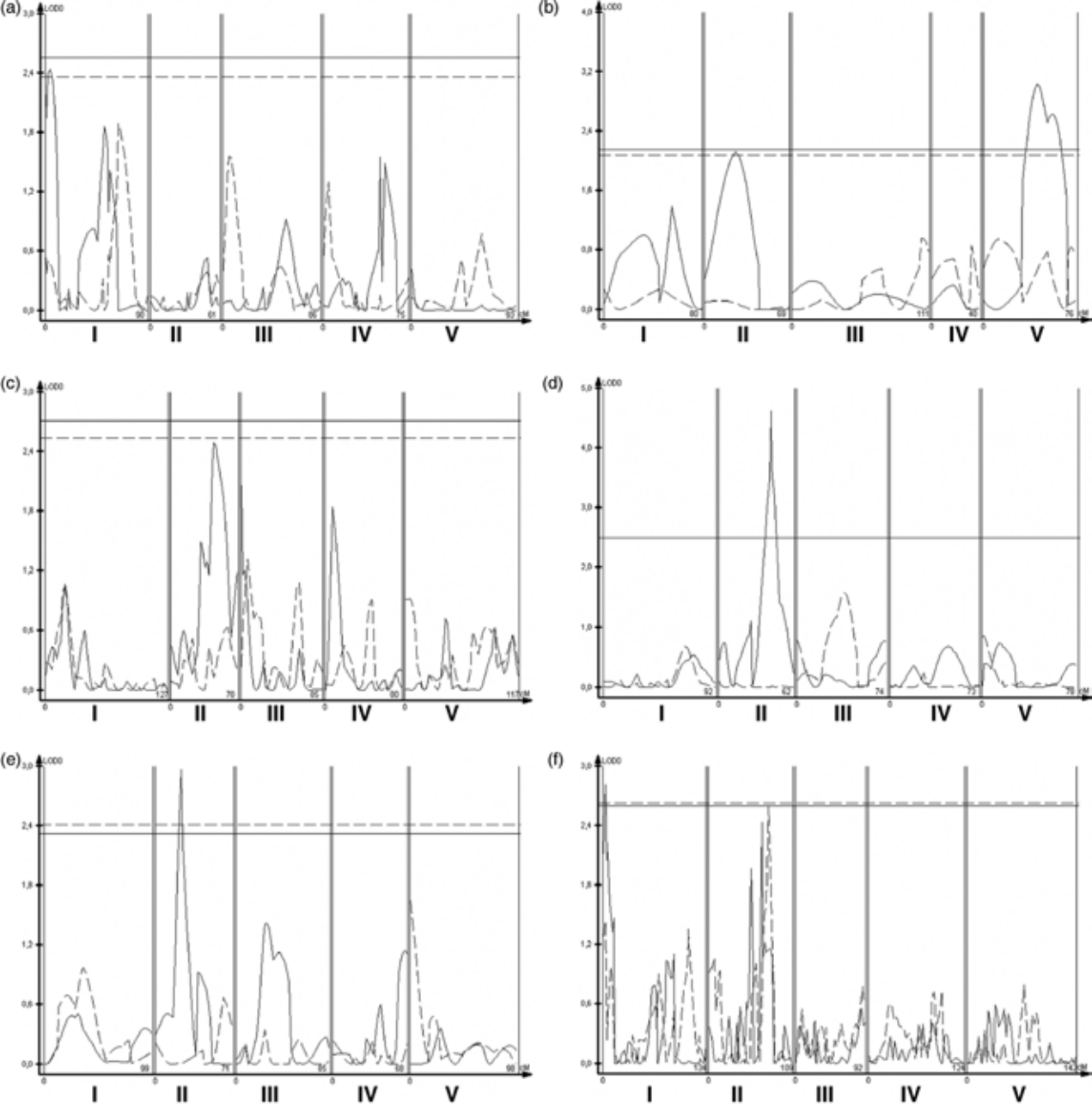
Figures.

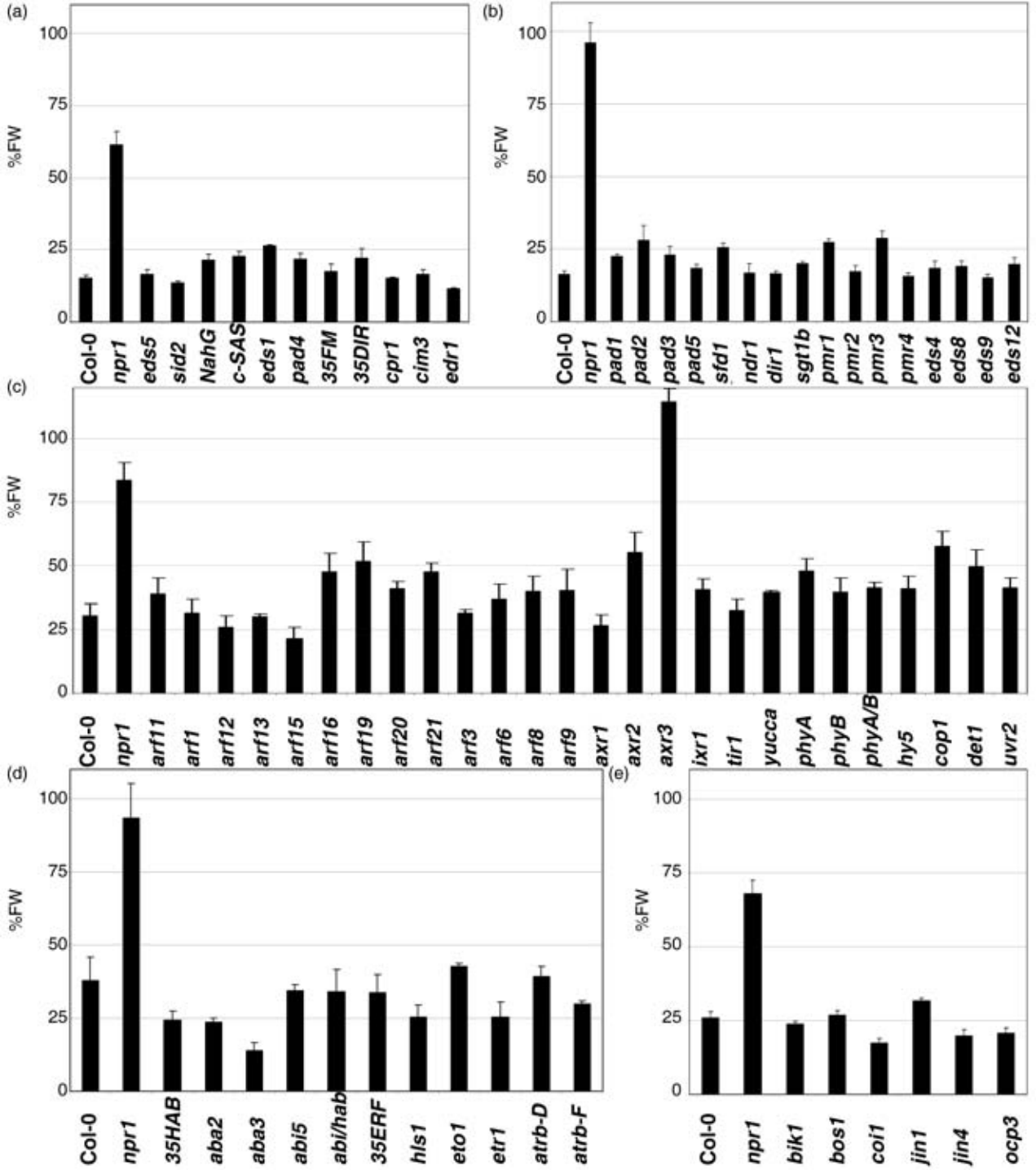

(a)
7.5
6.5
5.5
4.5
3.5
2.5
Log(cfu)/plant
Mock
BTH
(b)
10
8
6
4
mg FW
Mock
BTH
(c)
100
75
50
25
0
% FW
350 nM
3.5 uM
35 uM
350 uM

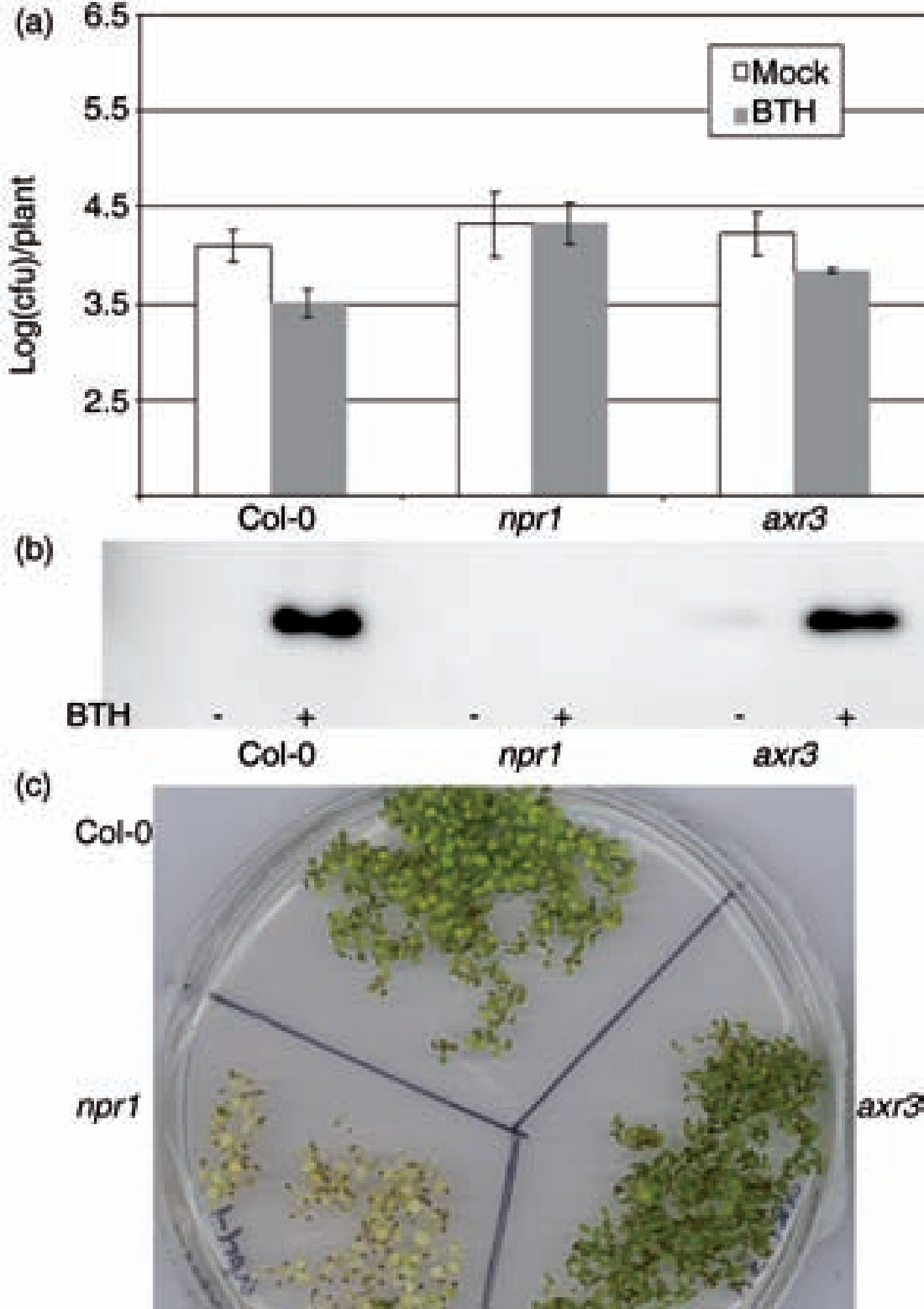

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)

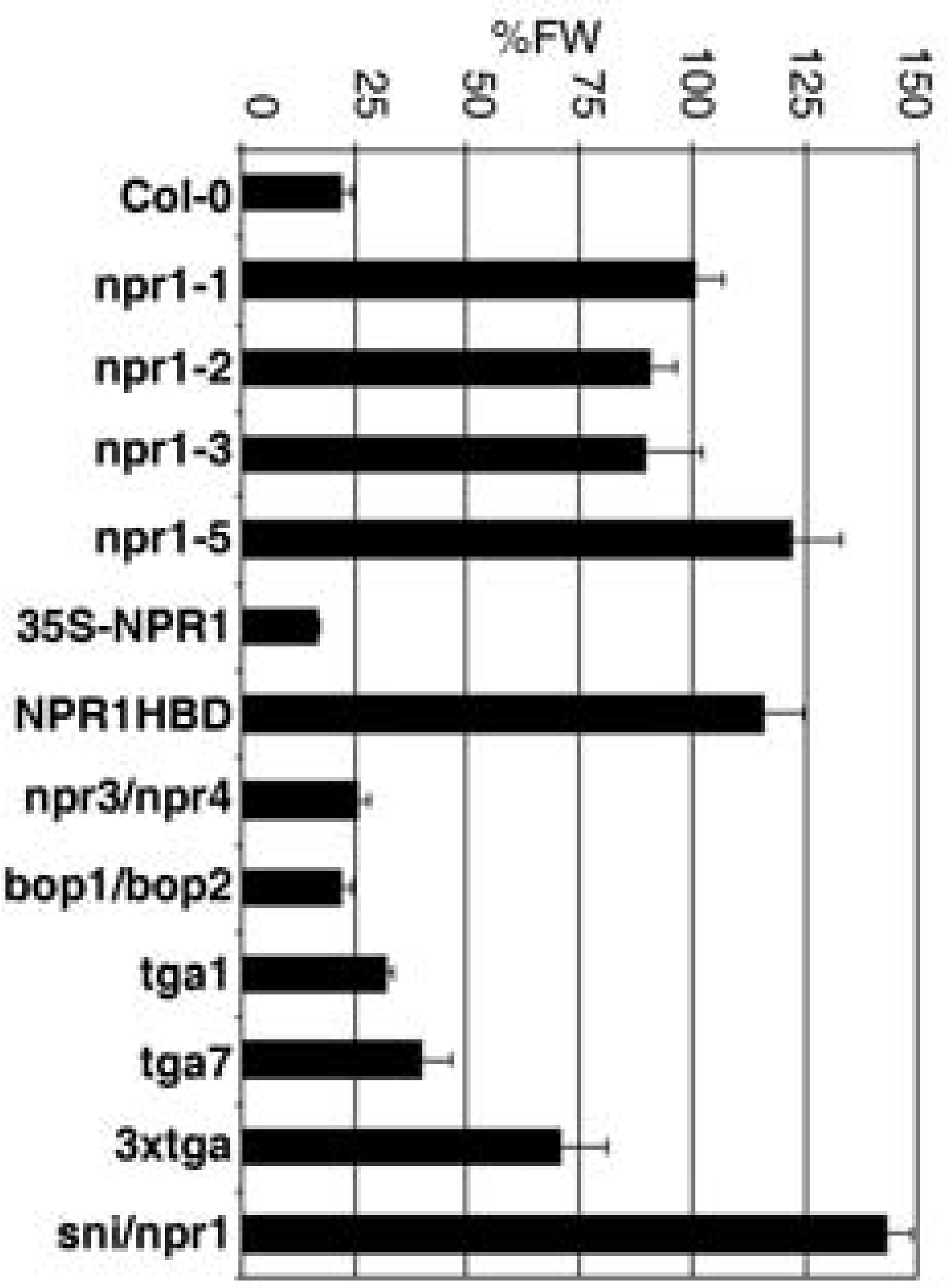

(a)
700
400
100
ng SA/g FW
Free
Total
Mock
BTH low
BTH high
(b)
PR1
Mock
BTH low
BTH high


(a)
50
25
0
%FW
Col-0
EF31
EF43
EF49
EF45
EF14
EF35
EF42
EF34
EF24
EF7
EF18
EF48
EF41
EF36
EF30
EF27
EF10
EF33
EF32
EF26
EF4
EF25
EF19
EF22
EF5
EF39
EF20
EF28
EF1
EF12
EF40
EF11
EF44
EF15
EF8
EF37
EF21
EF3
EF2
EF9
EF13
EF23
EF16
EF38
EF29
EF46
EF6
EF17
(b)
50
25
0
%FW
Col-0
AB20
AB19
AB4
AB13
AB5
AB24
AB8
AB14
AB12
AB2
AB3
AB43
AB6
AB1
AB29
AB33
AB22
AB41
AB7
AB30
AB31
AB42
AB28
AB23
AB9
AB38
AB15
AB35
AB27
AB37
AB10
AB39
AB17
AB34
AB25
AB40
AB26
AB32
AB36
(c)
50
25
0
%FW
Col-0
AB63
AB93
AB52
AB94
AB68
AB66
AB89
AB61
AB53
AB69
AB71
AB75
AB58
AB73
AB76
AB83
AB46
AB62
AB54
AB87
AB77
AB90
AB78
AB70
AB60
AB86
AB88
AB55
AB67
AB59
AB92
AB81
AB85
AB84
AB57
AB80
AB65
AB74
AB44
AB96
AB72
AB64
AB56
AB49
AB51
AB79
AB50
AB47
AB48
AB95
AB91


(a)
LODD
I
II
III
IV
V
(b)
LODD
I
II
III
IV
V
(c)
LODD
I
II
III
IV
V
(d)
LODD
I
II
III
IV
V
(e)
LODD
I
II
III
IV
V
(f)
LODD
I
II
III
IV
V


(a)
%FW
100
75
50
25
0
Col-0
npr1
eds5
sid2
NahG
c-SAS
eds1
pad4
35FM
35DIR
cpr1
cim3
edr1
(b)
%FW
100
75
50
25
0
Col-0
npr1
pad1
pad2
pad3
pad5
sfd1
ndr1
dir1
sgt1b
pmr1
pmr2
pmr3
pmr4
eds4
eds8
eds9
eds12
(c)
%FW
100
75
50
25
0
Col-0
npr1
arf11
arf1
arf12
arf13
arf15
arf16
arf19
arf20
arf21
arf3
arf6
arf8
arf9
axr1
axr2
axr3
ixr1
tir1
yucca
phyA
phyB
phyA/B
hy5
cop1
det1
uvr2
(d)
%FW
100
75
50
25
0
Col-0
npr1
35HAB
aba2
aba3
abi5
abi/hab
35ERF
hls1
eto1
etr1
atrb-D
atrb-F
(e)
%FW
100
75
50
25
0
Col-0
npr1
bik1
bos1
coi1
jin1
jin4
ocp3


(a)
6.5
5.5
4.5
3.5
2.5
Log(cfu)/plant
Mock
BTH
Col-0
npr1
axr3
(b)
BTH
-
+
-
+
-
+
Col-0
npr1
axr3
(c)
Col-0
npr1
axr3


%FW
0
25
50
75
100
125
150
Col-0
npr1-1
npr1-2
npr1-3
npr1-5
35S-NPR1
NPR1HBD
npr3/npr4
bop1/bop2
tga1
tga7
3xtga
sni/npr1

Mutant	Ecotipe if not Col-0	Other names	Figure 5	Keyword	Keyword
35S:HAB1			d	ABA	
aba2-1	Laer		d	ABA	
aba3-2	Laer		d	ABA	
abi1-2/hab1-2			d	ABA	
abi5-3	Laer		d	ABA	
arf11.1			c	Auxins	
arf1-2			c	Auxins	
arf12.1			c	Auxins	
arf13.1			c	Auxins	
arf15.1			c	Auxins	
arf16.1			c	Auxins	
arf19.1			c	Auxins	
arf20.1			c	Auxins	
arf21.1			c	Auxins	
arf3.1			c	Auxins	
arf6.1			c	Auxins	
arf8.2			c	Auxins	
arf9.1			c	Auxins	
axr1-3			c	Auxins	
axr2-1			c	Auxins	
axr3-1			c	Auxins	
ixr1-1			c	Auxins	
tir1-1			c	Auxins	
yucca			c	Auxins	
35S-FMO1			a	Defence	
fmo1			Data not shown	Defence	
ald1			Data not shown	Defence	NPR1-independent
dth9			Data not shown	Defence	NPR1-independent
eds1-1	Ws-0		Data not shown	Defence	R genes

eds12			b	Defence	
eds1-2	Laer-0		Data not shown	Defence	R genes
eds1-2	Col-0		a	Defence	R genes
eds4			b	Defence	
eds5.1		sid1	a	Defence	Salicylic acid
eds5.3		sid1	Data not shown	Defence	Salicylic acid
eds8-1			b	Defence	
eds9-1			b	Defence	
ocp11			Data not shown	Defence	Non-host
pad1-1			b	Defence	
pad2-1			b	Defence	
pad3-1			b	Defence	
pad4-1			a	Defence	R genes
pad5-1			b	Defence	
pbs3			Data not shown	Defence	Auxins
sgt1a	Ws-0		Data not shown	Defence	
why			Data not shown	Defence	NPR1-independent
cim3			a	Defence	
cpr1			a	Defence	Constitutive defence
cpr5			Data not shown	Defence	Constitutive defence
dnd1			Data not shown	Defence	Constitutive defence
edr1			a	Defence	Constitutive defence
nho1			Data not shown	Defence	Non-host
pen1-1			Data not shown	Defence	Non-host
pen2-1			Data not shown	Defence	Non-host
pmr1-1			b	Defence	Non-host
pmr2-1			b	Defence	Non-host
pmr3-1			b	Defence	Non-host
pmr4-1			b	Defence	Non-host
hsp90-2			Data not shown	Defence	R genes

rar1/ndr1			Data not shown	Defence	R genes
rar1-21			Data not shown	Defence	R genes
rpm1-1			Data not shown	Defence	R genes
rps5-2			Data not shown	Defence	R genes
sgt1b			b	Defence	R genes
c-SAS-10			a	Defence	Salicylic acid
NahG	Laer-0		Data not shown	Defence	Salicylic acid
NahG			a	Defence	Salicylic acid
NahG	Ws-0		Data not shown	Defence	Salicylic acid
sid2		eds16	a	Defence	Salicylic acid
35S-DIR1			a	Defence	
dir1			b	Defence	
ndr1-1			b	Defence	R genes
sfd1-1			b	Defence	
sfd1-2			Data not shown	Defence	
35S-ERF1			d	Ethylene	
ein2-1			Data not shown	Ethylene	
ein2-5			Data not shown	Ethylene	
eto1-1			d	Ethylene	
eto2			Data not shown	Ethylene	
eto3			Data not shown	Ethylene	
etr1-3			d	Ethylene	
hls1-1			d	Ethylene	
bik1			e	JA-Necrotrophs	
bos1			e	JA-Necrotrophs	
coi1			e	JA-Necrotrophs	
jin1			e	JA-Necrotrophs	
jin4		jar1	e	JA-Necrotrophs	
ocp3			e	JA-Necrotrophs	
cop1-4			c	Light	

det1-1			c	Light	
hy5-215			c	Light	
phyA/phyB	Laer		c	Light	
phyA-201	Laer		c	Light	
phyB-5	Laer		c	Light	
uvr2-1			c	Light	
atrboh-D			d	ROS	
atrboh-F			d	ROS	
rcd1			Data not shown	ROS	

Keyword	Keyword	Ref
		Saez et al. 2004
		Leon-Kloosterziel et al. 1996
		Leon-Kloosterziel et al. 1996
		Saez et al. 2006
		Finkelstein and Lynch 2000
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Okushima et al. 2005
		Leyser et al. 1993
		Timpte et al. 1995
		Ouellet et al. 2001
		Scheible et al. 2001
		Dharmasiri et al. 2005
		Zhao et al. 2001
SAR		Bartsch et al. 2006
SAR		Bartsch et al. 2006
		Song et al. 2004
SAR	Basal defence	Mayda et al. 2000
	Basal defence	Parker et al. 1996

	Basal defence	Glazebrook et al. 1996
	Basal defence	Parker et al. 1996
	Basal defence	Parker et al. 1996
	Basal defence	Glazebrook et al. 1996
	Basal defence	Nawrath et al. 2002
	Basal defence	Nawrath et al. 2002
	Basal defence	Glazebrook et al. 1996
	Basal defence	Glazebrook et al. 1996
	Basal defence	Agorio and Vera 2007
		Glazebrook and Ausubel 1994
		Glazebrook and Ausubel 1994
		Glazebrook and Ausubel 1994
	Basal defence	Glazebrook and Ausubel 1994
		Glazebrook and Ausubel 1994
	Basal defence	Warren et al. 1999
		Azevedo et al. 2002
	Basal defence	Desveaux et al. 2004
		Maleck et al. 2002
		Bowling et al. 1994
		Bowling et al. 1997
		Yu et al. 1998
		Frye and Innes 1998
		Lu et al. 2001
		Collins et al. 2003
		Lipka et al. 2005
		Vogel, J. and Somerville 2000
		Vogel, J. and Somerville 2000
		Vogel, J. and Somerville 2000
		Vogel, J. and Somerville 2000
		Hubert et al. 2003

		Tornero et al. 2002
		Tornero et al. 2002
		Grant et al. 1995
		Warren et al. 1998
JA-Necrotrophs		Tör et al. 2002
		Mauch et al. 2001
SAR	Basal defence	Lawton et al. 1995
SAR	Basal defence	Lawton et al. 1995
SAR	Basal defence	Lawton et al. 1995
SAR	Basal defence	Wildermuth et al. 2001
SAR		Maldonado et al. 2002
SAR		Maldonado et al. 2002
SAR		Century et al. 1995
SAR		Nandi et al. 2004
SAR		Nandi et al. 2004
		Berrocal-Lobo et al. 2002
		Alonso et al. 1999
		Alonso et al. 1999
		Guzmán and Ecker 1990
		Vogel, J.P. et al. 1998
		Woeste et al. 1999
		Guzmán and Ecker 1990
		Lehman et al. 1996
		Veronese et al. 2006
		Mengiste et al. 2003
		Xie et al. 1998
		Berger et al. 1996
		Berger et al. 1996
		Coego et al. 2005
		McNellis et al. 1994

		Chory et al. 1989
		Oyama et al. 1997
		Reed et al. 1993
		Nagatani et al. 1993
		Reed et al. 1993
		Jiang et al. 1997
		Torres et al. 2002
		Torres et al. 2002
		Ahlfors et al. 2004

S1. Picture of Col-0 and *npr1.1* after mock and BTH 350 µM treatments. Plants were mock treated (left) or BTH 350 µM treated (right) as described in Experimental procedures. The genotypes are Col-0 (up) and *npr1.1* (down). Part of this picture is shown in Figure 2A.