Balancing trade-offs between biotic and abiotic stress responses through leaf age-dependent variation in stress hormone cross-talk

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In nature, plants must respond to multiple stresses simultaneously, which likely demands cross-talk between stress-response pathways to minimize fitness costs. Here we provide genetic evidence that biotic and abiotic stress responses are differentially prioritized in Arabidopsis thaliana leaves of different ages to maintain growth and reproduction under combined biotic and abiotic stresses. Abiotic stresses, such as high salinity and drought, blunted immune responses in older rosette leaves through the phytohormone abscisic acid signaling, whereas this antagonistic effect was blocked in younger rosette leaves by PBS3, a signaling component of the defense phytohormone salicylic acid. Plants lacking PBS3 exhibited enhanced abiotic stress tolerance at the cost of decreased fitness under combined biotic and abiotic stresses. Together with this role, PBS3 is also indispensable for the establishment of salt- and leaf age-dependent phyllosphere bacterial communities. Collectively, our work reveals a mechanism that balances trade-offs upon combined stress-related phytohormone salicylic acid (5). Molecular cross-talk between ABA and SA provides control stress-response cross-talk. At the organism level, this mechanism balances stress-response trade-offs to maintain plant growth and reproduction during combined stresses. We also show that this leaf age-dependent stress-response prioritization influences the establishment of plant-associated leaf bacterial communities. This study illustrates the importance of active balancing of stress-response trade-offs for plant fitness maintenance and for interaction with the plant microbiota.

Significance

Plants are exposed to conflicting stresses simultaneously in nature. As stress responses are costly, plants likely coordinate these responses to minimize fitness costs. The nature and extent to which plants employ inducible mechanisms to cope with combined physical and biological stresses remains unknown. We identify a genetic mechanism by which leaves of distinct ages differentially control stress-response cross-talk. At the organism level, this mechanism balances stress-response trade-offs to maintain plant growth and reproduction during combined stresses. We also show that this leaf age-dependent stress-response prioritization influences the establishment of plant-associated leaf bacterial communities. This study illustrates the importance of active balancing of stress-response trade-offs for plant fitness maintenance and for interaction with the plant microbiota.

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Age and developmental stage are important factors influencing stress responses in animals and plants. For instance, as _A. thaliana_ plants age, SA-mediated immunity is enhanced (18). Plants also display age-dependent responses at the organ level. Young _A. thaliana_ rosette leaves exhibit greater SA accumulation and SA-mediated immunity in comparison with older rosette leaves (19). Evidence for leaf age-dependent variation also exists for abiotic stress (20, 21). Based on the optimal defense theory (ODT), in defending themselves against herbivores, plants prioritize tissues, such as young leaves, that are more valuable for the whole plant (22). However, whether leaf age-dependent variation in stress responses is a simple prioritization analogous to the ODT or an active strategy to increase plant fitness is not understood.

Here we show that biotic and abiotic stress responses are differentially prioritized in a leaf age-dependent manner in _A. thaliana_ to maintain fitness under combined stresses. Abiotic stresses dampen immunity in old rosette leaves, whereas the SA signaling components PBS3 and NPR1 protected young rosette leaves from ABA-mediated immune suppression. _pbs3_ mutant plants exhibited enhanced abiotic stress tolerance but showed compromised fitness maintenance under combined biotic and abiotic stresses. Defining a hitherto uncharacterized link between stress signaling cross-talk and microbiota structure, _PBS3_ is indispensable for the proper establishment of salt stress- and leaf age-dependent bacterial communities that balance SA-dependent cross-talk between SA and ABA signaling is a critical determinant of plant performance during combined stresses.

**Results**

**Leaf Age Controls ABA–SA Cross-Talk Independently of Vegetative Phase Change.** To gain insights into mechanisms underlying the cross-talk between ABA and SA signaling, we investigated the impact of ABA on the SA response in _A. thaliana_. We found that pretreatment with ABA unexpectedly blocked SA-mediated induction of the SA-response marker gene _PATHOGENESIS RELATED PROTEIN 1 (PR1)_ in young rosette leaves (L06 to L08; numbers refer to the positions of rosette leaves (L06 to L08; numbers refer to the positions of rosette leaves) (5) only in a subset of rosette leaves (L12; Fig. 1A and B), indicative of a leaf age-specific effect of ABA on the SA response. In contrast, expression of the ABA-response marker gene _RESPONSE TO ABA 18 (RAB18)_ (23, 24) was independent of leaf age (Fig. 1C and SI Appendix, Fig. S1A).

To systematically explore the leaf age-dependency of ABA-mediated transcriptional changes, we conducted RNA sequencing (RNA-seq) experiments. Although ABA-triggered transcriptome changes in old (L7) and young (L12) rosette leaves were similar overall, some gene clusters exhibited leaf age-dependent expression patterns (Fig. 1D, SI Appendix, Fig. S1 C and D, and Dataset S1). These results support our finding that expression of a subset of ABA-regulated genes is leaf age-dependent.

We next examined the physiological significance of leaf age-dependent effects of ABA on immunity. The bacterial pathogen _Pseudomonas syringae pv. tomato_ DC3000 ( _Pto_ ) is known to trigger ABA accumulation through the action of type III effectors (25, 26). Therefore, we reasoned that the disarmled bacterial effector _hrcC_ triggers ABA accumulation through the action of type III effectors, which bind to the promoters of SNAC-A TFs in vitro (29), and a subset of _PBS3_, _RAB18_ expression in young rosette leaves (L12; Fig. 1E and SI Appendix, Fig. S1B). This is consistent with an ABA-mediated suppression of the SA response in old but not in young leaves (Fig. L4) and with SA-mediated immunity restricting _Pto hrcC−_ growth (27).

We speculated that the observed leaf age-dependent ABA effects can be linked to leaf developmental stage because the onset of detectable suppression of the SA response by ABA correlates approximately with the onset of a vegetative phase change from juvenile to adult leaves in Col-0 plants (28). To explore this possibility, we employed transgenic _A. thaliana_ overexpressing the miRNA miR156a in which the expression of juvenile traits is markedly prolonged (28). However, similar to WT, we observed that ABA effects on _Pto hrcC−_ growth were dependent on leaf age despite the juvenile trait being manifest in younger and older leaves (Fig. 1 F and G). Thus, leaf age but not vegetative phase change likely controls ABA–SA cross-talk, with marked consequences for the SA response and bacterial resistance.

**ABA Suppresses Immunity via the ABA RESPONSIVE ELEMENT BINDING PROTEIN–SNAC-A Transcription Factor Cascade.** ABA RESPONSIVE ELEMENT (ABRE) BINDING PROTEIN (AREB) transcription factors (TFs) redundantly regulate a major part of ABA-mediated transcriptional changes (24) (SI Appendix, Fig. S2A). We found that ABA-triggered suppression of immunity against _Pto hrcC−_ in old leaves was compromised in _areb_ triple mutant plants (areb1 areb2 abf3) (24), indicating that ABA-triggered immune suppression requires AREB-mediated transcription. Next, we further dissected this transcriptional cascade. AREB TFs have been shown to bind to the promoters of SNAC-A TFs in vitro (29), and a subset of SNAC-A TFs, _ANAC019, 055, and 072_, whose gene expression is induced by ABA, are involved in the suppression of SA accumulation (30). These SNAC-A TFs redundantly control a subset of ABA responses such as _SA26_ expression and senescence, but not _RAB18_ expression (23). Therefore, we tested whether SNAC-A TFs collectively regulate ABA-mediated immune suppression. Indeed, we found that ABA-mediated immune suppression in old leaves was compromised in _snac-a_ sept mutant ( _snac-a_ sept anac019 anac055 anac072) and _anac002_ ( _anac019_ atf1 anac081 _atf2 anac102 anac032) (23) plants (Fig. 2A).

Plants overexpressing _ANAC002, 019, 055, or 072_ show enhanced abiotic stress tolerance (3, 31). Conversely, we found that salt tolerance was impaired in _snac-a_ sept plants (Fig. 2B and SI Appendix, Fig. S2B). Expression of _ANAC019, ANAC032_, and _ANAC072_ after ABA treatment was higher in old than in young rosette leaves in an _AREB-dependent_ manner (Fig. 2C), suggesting that this _ANAC_ induction is under control of the AREBs. It has been shown that _SA_ induction requires _ETHYLENE INSENSITIVE2 (EIN2)_ (23), a key component of ethylene signaling (32). Accordingly, we found that ABA-triggered leaf age-dependent expression of _SNAC-A_ s and suppression of immunity against _Pto hrcC−_ were impaired in ein2 plants (Fig. 2A and C), whereas _RAB18_ induction remained intact (SI Appendix, Fig. S2A). This corroborates the conclusion that these SNAC-A TFs play an important role in ABA-mediated suppression of immunity in old leaves. Collectively, these results suggest that _snac-a_ sept plants exhibit an altered balance between biotic and abiotic stress responses.

To explore the evolutionary conservation of the leaf age-dependent effect of ABA on immunity, we investigated _Arabidopsis lyrata_, a close relative of _A. thaliana_. We found that ABA suppresses immunity against _Pto hrcC−_ and induces SNAC-A expression in a leaf age-dependent manner as in _A. thaliana_ Col-0 plants (Fig. 2 D–F) (33).
treatment in young leaves of Col-0, was retained in sid2 but abolished in pbs3 plants (Fig. 3B). Given that PBS3 has been proposed to protect SA from degradation (36), we conclude that the increase in total SA elicited by ABA in young leaves may be caused by reduced SA degradation rather than increased SA biosynthesis via SID2.
Fig. 2. ABA triggers immune suppression in old leaves through AREB and ANAC TFs. (A) Old leaves (OL) and young leaves (YL) of 4–5-wk-old Col-0, areb1 areb2 abf3 (areb), ein2, and anac septuple mutant (snac-a sept) plants were infiltrated with Pto hrcC− (OD$_{600}$ = 0.0002) 24 h after 500 μM ABA spray or mock. Bacterial growth was measured at 2 days postinoculation (dpi). Data represent means ± SEM calculated from three independent experiments, each with at least five biological replicates, by using a mixed linear model. Different letters indicate significant differences (adjusted P < 0.005). (B) Shoot fresh weight of Col-0 and snac-a sept seedlings grown on MS plates containing 100 mM NaCl or mock for 10 d. The box plots show combined data from three independent experiments, each with at least eight biological replicates. Different letters indicate significant differences (adjusted P < 0.05). (C) AtANAC019, AtANAC032, and AtANAC072 expression levels in old and young leaves of 4–5-wk-old Col-0 and areb1 areb2 abf3 (areb) plants 24 h after 500 μM ABA spray or mock were determined by quantitative RT-PCR. Data represent means ± SEM calculated from at least three biological replicates by using a mixed linear model. (D) Pto hrcC− (OD$_{600}$ = 0.0002) was infiltrated into old and young leaves of 5–6-wk-old A. lyrata plants 24 h after 500 μM ABA spray or mock. Bacterial growth was measured at 2 dpi. Data represent means ± SEM calculated from three independent experiments, each with at least five biological replicates, by using a mixed linear model. Different letters indicate significant differences (adjusted P < 0.05). (E) AlANAC019, AlANAC032, and AlANAC072 expression levels in old and young leaves of 5–6-wk-old A. lyrata plants 24 h after 500 μM ABA spray or mock were determined by quantitative RT-PCR. Data represent means ± SEM calculated from at least three biological replicates by using a mixed linear model. Different letters indicate significant differences (adjusted P < 0.05). (F) Leaf numbers in 5–6-wk-old A. lyrata showing old and young leaves. (A–D) n.s., not significant (*P < 0.05 and **P < 0.01, two-tailed Student’s t-tests).
Notably, we found that young leaves of \textit{pbs3} plants are vulnerable to ABA-mediated suppression of immunity against \textit{Pto hrcC}, whereas \textit{sid2} plants exhibited a WT-like phenotype (Fig. 3C). In addition, we found that the enhanced SA-induced \textit{PR1} expression by ABA in young leaves is compromised in \textit{pbs3} plants whereas ABA-induced \textit{RAB18} expression remains intact (\textit{SI Appendix, Fig. S3A and B}). Finally, increased free and total SA accumulation was impaired in \textit{pbs3} leaves upon treatment with \textit{flg22}, a peptide epitope derived from bacterial flagellin that stimulates SA accumulation (27) (\textit{SI Appendix, Fig. S3C and D}). Thus, \textit{PBS3} is required for immunity-triggered SA accumulation. In young leaves, however, increased total SA accumulation and SA response upon ABA treatment might be important to protect young leaves from ABA-mediated immune suppression, which is impaired in \textit{pbs3} plants.

Given that \textit{NPR1}, encoding the SA receptor (37, 38), is required for the majority of the SA response (37) (\textit{SI Appendix, Fig. S3B}), we also included \textit{npr1} plants in our analysis. We found that, similar to \textit{pbs3}, young leaves of \textit{npr1} plants are not protected against ABA-mediated immune suppression (Fig. 3C). These and previous results might suggest that \textit{NPR1} is required for \textit{PBS3} function or vice versa (39). ABA promotes \textit{NPR1} degradation, which correlated with reduced \textit{PR1} expression, whereas SA antagonizes this (6). Together, \textit{NPR1} might be protected from ABA-mediated degradation by higher SA levels in young leaves. Our finding that expression of \textit{SNRK2.8}, an important resistance interactor of \textit{NPR1} (40), is strongly suppressed by ABA in only old leaves supports this hypothesis (\textit{SI Appendix, Fig. S1 C and D}).

\textbf{PBS3 Regulates the Trade-Off Between Biotic and Abiotic Stress Responses.} Abiobic stresses such as salinity and drought activate ABA biosynthesis (41). To test whether endogenous ABA accumulation induced by abiotic stresses triggers leaf age-dependent immune suppression, we measured \textit{Pto hrcC} growth in \textit{A. thaliana} plants after salt or drought treatment. To avoid the known pleiotropic effects of SA hyperaccumulation in \textit{npr1} on plant performance (42, 43), we focused on \textit{pbs3} plants. Similar to the ABA treatment described here earlier, young leaves of \textit{Col-0} plants were protected from salt and drought stress-triggered immune suppression (Fig. 4A and B). Abiotic stress-triggered suppression of immunity against \textit{Pto hrcC} in old leaves was not seen in \textit{aba2}, which is impaired in ABA biosynthesis (44) (Fig. 4A and B), indicating that abiotic stress-induced immune suppression is dependent on endogenous ABA and/or derived metabolites from ABA (45). In contrast to \textit{WT Col-0}, young leaves of \textit{pbs3} plants were not protected from the abiotic stress-triggered immune suppression (Fig. 4A and B). Thus, the protective role of \textit{PBS3} in young leaves is physiologically relevant.

Considering the extensive cross-talk between biotic and abiotic stress responses in both directions (2), we hypothesized that young leaves of \textit{pbs3} are hyperresponsive to abiotic stress compared with those of \textit{WT}. To measure a leaf-specific abiotic stress response output, we determined the accumulation of proline, which serves as a cellular osmoprotectant (46). Upon salt stress, proline levels and expression of \textit{P5CS1}, which encodes an enzyme that catalyzes the rate-limiting step in proline biosynthesis (46), were significantly higher only in young leaves of \textit{pbs3} compared with \textit{Col-0} (Fig. 4C and D). Together with the compromised immune phenotype, 4–5-wk-old \textit{Col-0}, \textit{sid2}, and \textit{pbs3} plants 48 h after spray with 500 \textmu M ABA or mock. Data represent means ± SEM calculated from three biological replicates by using a mixed linear model. Different letters indicate significant differences (adjusted \( P < 0.05 \)). (C) Old and young leaves of 4–5-wk-old \textit{Col-0}, \textit{sid2}, \textit{pbs3}, and \textit{npr1} plants were infiltrated with \textit{Pto hrcC} (\textit{OD}600 = 0.0002) 24 h after 500 \textmu M ABA spray or mock. Bacterial growth was measured at 2 days postinoculation. Data represent means ± SEM calculated from three independent experiments, each with at least five biological replicates, by using a mixed linear model. Different letters indicate significant differences (adjusted \( P < 0.005 \); \( * P < 0.05 \) and \( ** P < 0.01 \), two-tailed Student’s \( t \) tests). n.s., not significant.
In agreement with this, under severe salt stress (300 mM NaCl), abiotic stress responses is shifted toward abiotic stress tolerance.

By genotype or salt stress, levels of IAA and ACC were dependent

Interestingly, Col-0 and pbs3 plants accumulated comparable levels of basal and salt stress-induced ABA in leaves of different ages (Fig. 4F). We also determined the levels of other phytohormones, namely auxin (indole-3-acetic acid; IAA), jasmonic acid and its precursor 12-oxo-phytodienoic acid, SA, and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (47) in Col-0 and pbs3 leaves of different ages upon salt stress or mock treatment. Although these hormone levels were mostly unaffected by genotype or salt stress, levels of IAA and ACC were dependent on leaf age (SI Appendix, Fig. S4), which may influence the leaf age-dependent stress cross-talk. In summary, these results suggest that, during combined stress, PBS3 lowers the abiotic stress response and enhances immunity in young leaves.

**PBS3 Is Required for the Maintenance of Plant Growth and Reproduction Under Combined Stress.** We employed two experimental systems to test whether the altered balance of leaf age-dependent stress cross-talk in pbs3 plants affects plant fitness-related traits during combined stresses. In the first system, we combined salt stress with infection with the obligate biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis (Hpa)*, whose growth is sensitive to SA-mediated immunity (48). Considering that many plant pathogens, including *Hpa*, require high humidity for successful infection, co-occurrence of drought stress with pathogen infection is unlikely to be common. Therefore, we used mild salt stress as an abiotic stress factor, which can co-occur with pathogen infection (49). Salt pretreatment reduced oomycete biomass in the young leaves of Col-0 WT, whereas, in pbs3 plants, salt stress promoted *Hpa* growth in old and young leaves (Fig. 5A). We included snac-a sept plants in the plant performance assay because, in this genotype, the balanced trade-offs of abiotic and biotic stress responses were shifted in the opposite direction compared with pbs3 plants (Figs. 2A and B, 3C, and 4). Furthermore, we included sid2 plants because of their deficiency in SA biosynthesis but indistinguishable leaf age-dependent stress cross-talk vs. Col-0 plants (Fig. 3C). We found that single salt and *Hpa* stress reduced shoot fresh weight of all tested genotypes, with *Hpa* infection having greater negative consequences on growth of Col-0, pbs3, and sid2, except snac-a sept plants, whose growth suffered more severely from salt stress (Fig. 5B). This *snac-a* sept plant phenotype is consistent with our observation that *snac-a* sept plants exhibited lowered tolerance to mild salt stress in a germfree environment (Fig. 2B).

Interestingly, the effects of combined stress on plant growth were similar to those of *Hpa* single stress in WT and sid2 plants,
Leaf age-dependent variation in biotic and abiotic stress cross-talk contributes to plant fitness-related traits under combined stresses. (A) Hpa growth 8 d after inoculation in old leaves (OL) and young leaves (YL) of 4–5-wk-old Col-0 and pbs3 plants following 75 mM NaCl or water (Mock) soil drench treatment for 2 d. Data are means ± SEM calculated from at least three biological replicates by using a mixed linear model. (B) Shoot fresh weight of Col-0, snac-a sept, pbs3, and sid2 plants challenged with mock, NaCl, Hpa, or both NaCl and Hpa (Methods). The box plots show combined data from at least three independent experiments for Col-0, pbs3, and sid2 and two independent experiments for snac-a sept mutant plants, each with at least eight biological replicates. (A and B) *P < 0.05, **P < 0.01, and ***P < 0.001, two-tailed Student’s t tests; n.s., non-significant. (C) Old and young leaves of 4–5-wk-old Col-0 and pbs3 plants were infiltrated with Pto cor− (OD400 = 0.0002) 1 d after water, 500 μM ABA, or 100 mM NaCl treatment. Bacterial growth was measured at 2 days postinoculation. Data represent means ± SEM calculated from three independent experiments, each with at least five biological replicates, by using a mixed linear model. Different letters indicate significant differences (adjusted P < 0.005). (D) The number of siliques in Col-0 and pbs3 plants after water (Mock), 50 mM NaCl (NaCl), Pto cor− (Pto), or both NaCl and Pto. The box plots show combined data from three independent experiments, each with at least 10 biological replicates. Statistical analysis was performed by using log-transformed siliques numbers. Different letters indicate significant differences (adjusted P < 0.005).

which exhibited leaf age-dependent stress cross-talk (Figs. 3C and 5B). In contrast, combined stress had more severe consequences on plant growth than the single Hpa and salt stress in pbs3 and snac-a sept plants, respectively (Fig. 5B). Thus, these results suggest that a loss in leaf age-dependent stress-response cross-talk is associated with a plant growth penalty during combined stress conditions.

In the second assay, we used salt stress and infection with Pto cor− as an additional biotic and abiotic stress combination. The Pto cor− strain lacks the phytotoxin coronatine that can suppress immune responses such as SA accumulation and MAPK activation (30, 50) but is more virulent compared with Pto hrcC− (Figs. 1E and 5C). Similar to Pto hrcC− infection, immune suppression by ABA treatment and salt stress was leaf age-dependent in Col-0 but independent of leaf age in pbs3 plants (Fig. 5C). To evaluate whether A. thaliana reproduction was affected by abiotic and biotic response cross-talk, we measured the number of siliques under single salt or Pto cor− stress or in combination in Col-0 and pbs3 plants. Under our experimental conditions, Pto cor− infection alone did not affect silique numbers of Col-0 and pbs3 plants, but moderate salt stress resulted in a reproductive penalty (Fig. 5D). In Col-0 plants, the effect of combined stress on silique numbers was similar to that of salt stress alone, whereas the negative effect of combined stress exceeded the single salt stress in pbs3 plants (Fig. 5D). Together, these findings indicate that PBS3, which is necessary for balancing leaf age-dependent biotic and abiotic stress responses based on leaf age, is required for the maintenance of growth and reproduction under combined biotic and abiotic stress.
PBS3 Coordinates Leaf Age- and Salt Stress-Dependent Phyllosphere Microbiota Assembly. To test the impact of leaf age-dependent variation in cross-talk between ABA and SA signaling on biotic components beyond pathogens, we compared the composition of leaf-associated bacterial communities under abiotic stress or components beyond pathogens, we compared the composition of leaf-associated bacterial communities under abiotic stress or components beyond pathogens, we compared the composition of leaf-associated bacterial communities under abiotic stress or components beyond pathogens, we compared the composition of leaf-associated bacterial communities under abiotic stress.

**A** Leaf age x Treatment effect, 13.5% of variance; p < 0.001

**B** Genotype x Leaf age effect (Mock) 16.7% of variance; p < 0.001

**C** Genotype x Leaf age effect (Salt) 12.2% of variance; p < 0.001

**D** Genotype x Treatment effect (OL) 17.3% of variance; p < 0.001

**E** Genotype x Treatment effect (YL) 13.6% of variance; p < 0.001

*Fig. 6. PBS3 shapes the leaf age- and salt stress-dependent assembly of leaf bacterial communities. (A–E) Canonical analysis of principle coordinates of bacterial β-diversity Bray-Curtis distances based on bacterial 16S rRNA profiling of leaf bacterial communities in WT Col-0, pbs3, and aba2 (A) or Col-0 and pbs3 plants (B–E). Plants were grown in natural Cologne soil treated with water (mock) or 75 mM NaCl (salt) for 6 wk. Constrained analysis was performed for leaf age x treatment effect (A), genotype x leaf age effect under mock (B) or salt stress (C), and genotype x treatment effect in old leaves (OL; D) or young leaves (YL; E).*
Discussion

We have unveiled a genetically controlled mechanism by which *A. thaliana* plants balance trade-offs between conflicting biotic and abiotic stress responses by integrating these differently in young and old leaves. Moreover, we find that leaf age-dependent preference of stress responses balances trade-offs to increase plant growth and reproduction during combined stress. Thus, our findings define a plant strategy to maintain fitness in nature, where plants are often exposed to multiple stresses simultaneously (51), and demonstrate the physiological significance of stress-hormone cross-talk at the organismal level.

Young leaves serve as a better energy source compared with old leaves because cellular components such as the photosynthesis apparatus are more intact (52–55). The ODT explains why plants prioritize young leaves over old leaves for defense against insect herbivores by postulating that young leaves constitute a higher value for the whole plant, where value is correlated with the cost of having that tissue removed (22). Our study shows that young leaves exhibit higher biotic stress responses but lower abiotic stress responses compared with old leaves. Thus, our work suggests that young leaves are not simply protected from stresses because of their higher value in a manner similar to the ODT, but rather that plants actively balance the trade-off between biotic and abiotic stress responses through leaf age-dependent variation in stress hormone cross-talk. *pbs3* and *snac-a sept* plants, in which this balancing trade-off mechanism was absent, exhibit fitness penalties during combined biotic and abiotic stress conditions. In nature, actively maintaining fitness during combined stresses might be crucial for plant reproduction, and the balancing trade-off mechanism adds another dimension to our understanding of how plants cope with complex and fluctuating environments.

Our genetic analysis revealed that leaf age-dependent stress response prioritization during combined stress is controlled by *PBS3* and NPR1, components of SA signaling. However, SA biosynthesis via *SID2* in the isochorismate pathway was not required. Hence, this mechanism is distinct from plant age-dependent control of plant immunity as described during age-related resistance, which is fully dependent on *SID2* in *A. thaliana* (18). Another study reported that expression of ENHANCED DISEASE SUSCEPTIBILITY5 (*EDS5*), required for SA accumulation, shows leaf age-dependency in *A. thaliana* (56). However, *EDS5* expression is higher in older compared with younger leaves. Therefore, this *EDS5* expression pattern does not explain why young leaves are protected from ABA-triggered immune suppression. Thus, the leaf age-dependent trade-off between biotic and abiotic stress responses during combined stress is regulated by a mechanism distinct from the previously described age-dependent variation in stress responses. Our findings also indicate that the function of SA is not limited to plant–microbe interactions, but has wider implications for plant fitness maintenance under combined stress. Consistent with this, *A. thaliana* *npr1* mutants exhibited reduced fitness in the field but not under controlled standard conditions (57).

Our data show that salt stress, leaf age, plant ABA biosynthesis, and *PBS3* influence the structure of leaf-associated bacterial communities. Factors determining microbiota structure and contributions of plant commensals to plant health and fitness are beginning to be defined (13). Our findings demonstrate that leaf age-dependent variation in biotic and abiotic stress cross-talk is not limited to interactions with microbial pathogens, but also influences associations with resident leaf commensals. In the context of the observed differential biotic and abiotic stress response prioritization in younger and older leaves, this raises the intriguing possibility that the corresponding distinctive leaf-resident bacterial communities are adapted to contribute preferentially to biotic and abiotic stress tolerance, respectively. Irrespective of this, our work identifies a leaf age-dependent genetic interaction among immunity, the leaf-associated bacterial microbiota, and abiotic stress tolerance, which might determine plant fitness in natural environments.

Materials and Methods

Plants were grown in a chamber at 22 °C with 60% relative humidity and a 12-h light period for 4 wk before transfer to another chamber at 22 °C with 60% relative humidity and a 12-h light period before treatments. All *A. thaliana* plants used were in the Col-0 accession background. The details and procedures of plant materials and growth conditions, bacterial infection, *Hpa* infection, performance assay, quantitative PCR, RNA-seq, SA measurements, probe quantification, quantification of multiple phytohormones, bacterial 16S rRNA gene profiling, and statistical analysis, as well as the gene accession numbers used in this study, are provided in SI Appendix, SI Materials and Methods.

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