the plant journal

SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

The Plant Journal (2025) **0**, e70174 doi: 10.1111/tpj.70174

Symbiotic plant–bacterial–fungal interaction orchestrates ethylene and auxin signaling for optimized plant growth

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Received 30 October 2024; revised 9 March 2025; accepted 10 April 2025.
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SUMMARY

The complex and mutual interactions between plants and their associated microbiota are key for plant survival and fitness. From the myriad of microbes that exist in the soil, plants dynamically engineer their surrounding microbiome in response to varying environmental and nutrient conditions. The notion that the rhizosphere bacterial and fungal community acts in harmony with plants is widely acknowledged, yet little is known about how these microorganisms interact with each other and their host plants. Here, we explored the interaction of two well-studied plant beneficial endophytes, *Enterobacter* sp. SA187 and the fungus *Serendipita indica*. We show that these microbes show inhibitory growth *in vitro* but act in a mutually positive manner in the presence of Arabidopsis as a plant host. Although both microbes can promote plant salinity tolerance, plant resilience is enhanced in the ternary interaction, revealing that the host plant has the ability to positively orchestrate the interactions between microbes to everyone's benefit. In conclusion, this study advances our understanding of plant–microbiome interaction beyond individual plant–microbe relationships, unveiling a new layer of complexity in how plants manage microbial communities for optimal growth and stress resistance.

Keywords: cross-kingdom signaling, Enterobacter SA187, phytohormones, plant-microbe interaction, salt stress amelioration, Serendiptia indica, sustainable agriculture, symbiosis.

INTRODUCTION

The human population is increasing at an alarming rate and is estimated to reach 10 billion by 2050, meaning that we will need to feed 2 billion more people than today. At the same time, crop production across the globe is going to be severely affected by climate change. Importantly, climate change is projected to have strong negative effects by warming, especially in developing countries with a surging population growth (Rosenzweig et al., 2014). Almost 60% of crop losses are due to abiotic stresses like salinity, drought, and heat. The Green Revolution helped to increase crop yield substantially but also spurred unintended negative consequences, causing detrimental effects on the environment. The increased use of fertilizers, pesticides, and herbicides leads to eutrophication, disbalancing the soil microbial communities and leaching the soil of essential nutrients, and most importantly posing a health risk for farmers and human consumers (Krug et al., 2023; Pingali, 2012). Therefore, it is an urgent need to find alternative farming practices that are more sustainable and less harmful to the environment (Hirt et al., 2023).

The concept of Planetary Health, meaning the interconnection of human health with the health of other components of the global ecosystem, is gaining more and more support in the scientific community. The microbial communities associated with humans, animals, and plants are often referred to as the second genome/extended genotype/eco-holobiont to drive the fitness and performance of almost all living beings on earth (Banerjee & Van Der Heijden, 2023). Soil is the richest reservoir of diverse and complex biological communities where tens of millions of microbes, represented by bacteria, archaea, viruses, fungi, protists, nematodes, etc., coexist and act in a cohort which is key for soil health and the maintenance of biogeochemical cycles (Banerjee & Van Der Heijden, 2023; Hirt et al., 2023; Sokol et al., 2022).

One of the promising approaches to promote sustainable agriculture without causing further degradation of soil health is harnessing the potential of beneficial soil microbes. Plant-associated rhizosphere microbes are often responsible for the adaptation of plants to environmental factors (De Zelicourt et al., 2013). The associated

microbiomes in plants are known to facilitate a range of essential functions such as promoting plant growth and development by altering the root architecture of lateral roots and root hairs to increase nutrient and water uptake and enhancing plant fitness toward various pathogens (Backer et al., 2018; De Zelicourt et al., 2013).

In recent years, desert microbes have emerged as a very powerful tool to tackle several environmental stresses in plants (Alsharif et al., 2020; Blilou & Hirt, 2023; Eida et al., 2018). One of the endophytes, *Enterobacter* sp. SA187, promotes salt tolerance in a wide host range (Synek et al., 2021) by modulating the ethylene signaling pathway, which in turn regulates the sulfur regulon in plants (Andrés-Barrao et al., 2021; De Zélicourt et al., 2018), while another strain of desert microbe, *Pseudomonas argentinensis* SA190, induces drought tolerance in plants that is mediated by ABA-dependent epigenetic priming of aquaporin genes (Alwutayd et al., 2023). The symbiotic associations between plants and beneficial fungi also play an important role in alleviating biotic and abiotic stresses. Fungal hyphae are leaner than roots and hence indirectly facilitate the plants' accessibility to soil pores that are otherwise inaccessible to roots, thereby increasing the surface area for water and nutrient absorption (Evelin et al., 2019). Not only does this symbiotic association increase the efficiency of the uptake and transfer of water and nutrients in plants but also prevents oxidative damage and protects the photosynthetic apparatus under stress conditions (Evelin et al., 2019). *Serendipita* (= *Piriformospora*) *indica* (*S. indica*) is a filamentous root endophytic fungal strain belonging to the family Serendipitaceae, which was isolated from the Thar desert in India (Verma et al., 1998; Weiß et al., 2016) and promotes growth and (a)biotic stress tolerance in plants (Baltruschat et al., 2008; Li et al., 2023; Mosaddeghi et al., 2021; Oelmüller et al., 2009). *S. indica* associates symbiotically with a wide range of hosts and represents an excellent model system to study symbiotic associations of beneficial fungi with host plants.

There has been an increasing interest to study microbial communities, searching for synergism between two or more beneficial microbes, especially between bacterial and mycorrhizal fungal strains (Berrios et al., 2023; Bonfante & Anca, 2009). It was suggested that the "DefenseBiome" of a plant could play a vital role in plant fitness under stress conditions (Liu et al., 2020). A number of studies have shown the potential of *S. indica* to form close associations with rhizobacteria and that this interaction plays an important role in plant growth promotion. Different endophytic bacterial isolates were shown to differ in their ability to interact with *S. indica*, thereby influencing the beneficial effect of *S. indica* on plants (Del Barrio-Duque et al., 2019). Several of the economically important rhizobacteria showed beneficial to inhibitory effects on *S. indica*, thus proving the concept of a delicate balance between rhizospheric organisms in nature (Del Barrio-Duque et al., 2019; Kumar Bhuyan et al., 2015; Varma et al., 2013; Vyshakhi & Anith, 2021).

Salt stress, a major abiotic stress, negatively affects morphological, biochemical, and molecular processes in plants, diminishing agricultural productivity. The excess of sodium ions causes changes in metabolic activity, induces oxidative damage affecting overall growth and development in plants (Munns & Tester, 2008). High salt concentrations in the soil alter the soil porosity, causing low soil water potential leading to water stress, eventually destabilizing the cell membranes and triggering protein degradation due to toxic cellular sodium ion levels (Acharya et al., 2024). The membrane depolarization due to high ionic levels can influence the uptake of essential nutrients from soil, reducing yield quality and quantity (Isayenkov & Maathuis, 2019). Furthermore, salt stress reduces the photosynthetic activity of plants, curbing biomass accumulation and altering source-sink dynamics. Apart from the genetic engineering of salt-tolerant plants, which is a long-term and expensive effort, rhizosphere microbes present an affordable and readily available solution to tackle the harmful effects of high salinity in plants (Alsharif et al., 2020; Backer et al., 2018; De Zelicourt et al., 2013).

The soil microbiome and plants are interconnected via the rhizosphere where communication occurs by chemical signals that enable the establishment of symbiotic relationships between plant and rhizosphere microbes. Many microbes are known to stimulate plant hormonal pathways directly or indirectly by producing hormones or their precursors which are either taken up by roots directly or can manipulate the hormonal balance of the host plant to promote plant growth and stress resilience (De Zélicourt et al., 2018; González Ortega-Villaizán et al., 2024; Khatabi et al., 2012; Tzipilevich et al., 2021). In some cases, the microbe establishes a feedback loop where the activation of the immune system in the plant leads to reactive oxygen species (ROS) production which will lead to auxin production in the bacteria. Thereby, auxins such as indole-3-acetic acid (IAA) stimulate root colonization by bacteria which in turn induces plant fitness by changing root architecture as well as by providing protection against pathogens (Tzipilevich et al., 2021). Auxins are hormones that affect a plethora of growth and developmental processes in plants (Semeradova et al., 2020). The intricate balance and crosstalk between phytohormones are key for plant growth, development and response to various (a)biotic stresses. In particular, the sophisticated cooperation of auxin and ethylene is known to regulate many developmental and stress response pathways in plants (Lewis et al., 2011; Méndez-Bravo et al., 2019; Muday et al., 2012; Růžička et al., 2007). While on the one hand auxin can induce ethylene biosynthesis and signaling, on the other hand the growth responses to ethylene are mainly auxin-dependent

(Zemlyanskaya et al., 2018). Changes in ethylene levels result in an imbalance of the auxin concentration gradient in plants. Various auxin mutants show root-specific ethylene insensitive phenotypes suggesting the intricate cooperation between these two phytohormones (Qin et al., 2019).

In this study, we assessed the interaction of two well-studied root endophytic beneficial microbes, the bacterium *Enterobacter* sp. SA187 and the fungus *S. indica* when applied individually or as a binary consortium (*Bicom.*) to the model plant *Arabidopsis thaliana*. Our findings highlight that the two microbes negatively interact on synthetic media in the presence of a carbon source. However, in the absence of a carbon source, Arabidopsis engages the two microbes in a tripartite positive interaction thereby promoting plant resilience to salinity.

RESULTS

Enterobacter sp. SA187 and *S. indica* act in a synchronized manner to promote plant growth and salinity tolerance

To comprehend whether SA187 and *S. indica* cooperate synergistically to alleviate salinity stress in plants, plant growth parameters were measured after 16 days of growth in the absence and presence of SA187, *S. indica* alone, or in the combination of both microbes (*Bicom.*) on ½ MS and ½ MS supplemented with 100 mM NaCl (Figure 1A). While plants grown on ½ MS control plates did not show any major changes in the plant growth phenotype, on salt plates, the plants inoculated with SA187 or *S. indica* displayed enhanced growth compared to mock-treated plants (Figure 1B; Figure S1). Interestingly, the plants co-cultivated with both microbes (*Bicom.*) performed better than the plants that were individually colonized by the microbes (Figure 1B). The effect of enhanced growth was further evaluated by measuring the fresh weight of plants. Again, the control plants had slightly increased but no significant increase in the average shoot weight, but under salt conditions, the shoot weight was significantly increased with much pronounced growth in *Bicom.*-colonized plants (Figure 1C). The inoculation with SA187 or *S. indica* resulted in 49% and 42%, respectively, while upon *Bicom.* colonization, the beneficial index in shoots reached 76% (Figure 1D). Just like in shoots, the roots of salt-treated colonized plants had greater fresh weight – 68% in SA187-colonized and 46% in *S. indica*-colonized plants, which was more pronounced again (101%) when plants were colonized with the consortium (Figure 1E,F). Although microbial colonization with either of the microbial strains or the Bicom. did not change primary root length significantly (Figure 1G), the density of lateral roots was increased significantly (Figure 1H) suggesting that colonization with SA187 and *S. indica* as a *Bicom.* induces more lateral root formation in Arabidopsis than when colonized individually. These results explain the synergistic effect of plant growth and salt tolerance of Arabidopsis by SA187 and *S. indica*.

Interaction between SA187 and *S. indica*

The enhanced plant growth and salt resilience in plants colonized with SA187 and *S. indica* together (*Bicom.*) led us to reason whether both these microbes interact with and influence the growth of their counterparts. To answer this question, we inoculated SA187 and *S. indica* next to each other on the medium suitable for bacterial (LB), fungal (PDA) or plant growth (½ MS). On ½ MS, only *S. indica* showed scanty growth of very fine hyphal filaments, while SA187 did not grow at all, suggesting that the root exudates/sugars from plants are important for the proper growth of both microbes in the nutrient deficient conditions on basal ½ MS w/o a carbohydrate source (Figure 2A). Surprisingly, although the bacterial and fungal growth was supported on their respective media for bacteria or fungi, respectively, SA187 and *S. indica* on the same media showed a negative interaction with a clear inhibition zone between the microbial colonies (Figure 2A).

To compare this negative interaction between SA187 and *S. indica in vitro* with the situation *in planta*, we determined the level of fungal and bacterial colonization in colonized Arabidopsis. For this, we performed qRT-PCR with *S. indica* specific marker gene *EF-H*, relative to the expression of the plant *GAPDH* gene, as described (Bakshi et al., 2015). The expression level of *PiEF-H* was significantly increased in both non-salt (40%) and salt (185%) conditions when the fungus was co-cultivated with SA187 (Figure 2B). Trypan Blue staining of Arabidopsis roots demonstrated enhanced fungal hyphae and sporulation in *Bicom.* colonized plants compared with plants colonized only with *S. indica* (Figure 2C). Although the expression levels of the SA187 marker gene (*infB*) were similar in SA187 or *Bicom.* colonized plants (Figure 2D), the colony forming units (CFU/plant) were considerably higher due to the enhanced plant growth (Figure 2E). As ethylene is essential for establishing the biotropic symbiosis between *S. indica* and plant at the contact site (Khatabi et al., 2012) we decided to investigate the colonization associated expression of the ethylene biosynthetic gene (*ACS4*) and also the spatiotemporal induction of ethylene response using ethylene reporter lines (EBF2:GUS) after 3 and 7 days post inoculation (dpi). After 3 days of inoculation, the expression of *ACS4* in microbe-colonized plants was not very different than in mock colonized plants (Figure 2G); however, the microbes inoculated plants showed slightly increased GUS staining which was not only localized to root tip – like in mock colonized plants, but migrated higher up in the root tissues, especially in SA187 and *Bicom.* colonized plants (Figure 2G).

Intriguingly, after 7 days of colonization, both microbes singly, that is, with SA187 and *S. indica*, significantly increased the expression of *ACS4* (Figure 2F) which was corroborating the increased EBF2:GUS intensity (Figure 2G). Interestingly, the *Bicom.* displayed a massive increase in both *ACS4* expression and GUS staining showing that the additive effect of SA187 and *S. indica* in ethylene levels of *Bicom.* plants could be the plausible reason behind improved *S. indica* colonization and increased lateral root density (Figure 1) in these plants.

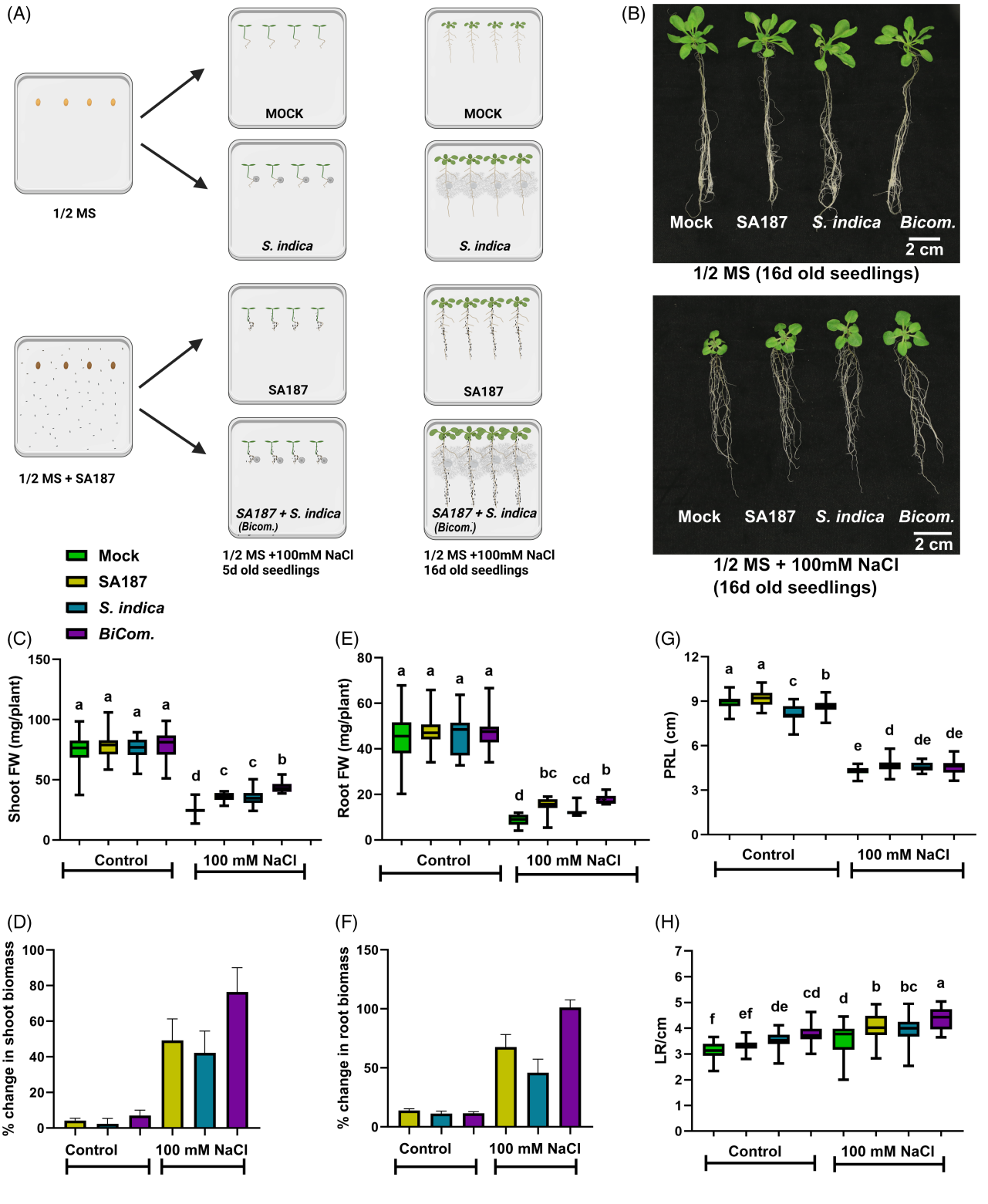

Figure 1. SA187 and *Serendipita indica* act in a coordinated manner to promote plant growth and salinity tolerance.
(A) Workflow for plan screening assay under 100 mM NaCl.
(B) Growth of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized plants grown under ½ MS (control) or treated with ½ MS + 100 mM NaCl conditions for 16 days.
(C, D) Shoot fresh weight (C) and percentage change in shoot biomass (D) of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized plants grown under control (½ MS) or treated with ½ MS + 100 mM NaCl conditions for 16 days. Box plot represents data from six individual biological replicates ($n \geq 36$). The letters on the top (C) denote significance difference (One-way ANOVA).
(E, F) Root fresh weight (E) and percentage change in root biomass (F) of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized plants grown under control (½ MS) or treated with ½ MS + 100 mM NaCl conditions for 16 days. Box plot represents data from six individual biological replicates ($n \geq 36$). The letters on the top (E) denote significance difference (One-way ANOVA).
(G, H) Primary root length (PRL) (G) and lateral root density (LR/cm) (H) of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized plants grown under control (½ MS) or treated with ½ MS + 100 mM NaCl conditions for 9 days. Box plot represents data from six individual biological replicates ($n \geq 36$). The letters on the top denote significance difference (One-way ANOVA, Tukey test for multiple comparison).

SA187 and *S. indica* regulate the Na^+ and K^+ ion contents in plants to support plant growth under saline conditions

The concentration of Na^+ and K^+ ions and the Na^+/K^+ ratio is regarded as an important determinant of salinity stress tolerance in plants (Shabala & Pottosin, 2014). Therefore, we measured these ion concentrations in shoots and roots of control and salt-treated plants. The Na^+ content in shoots of non-salt-treated, as well as salt-treated, plants colonized with SA187, *S. indica*, and *Bicom.* was slightly decreased, while, on the contrary, in roots the Na^+ levels were higher than in non-colonized plants (Figure 3A). The K^+ concentration was increased in shoot and root tissues by all microbial combinations (Figure 3B). To get a better understanding, the ratio between sodium and potassium ions was calculated. The Na^+/K^+ ratios in shoot samples were lower in both control and salt-treated plants, especially for *Bicom.*-colonized plants under salt conditions, suggesting that under stress conditions the *Bicom.*-colonized plants have improved their resilience to regulate the harmful Na^+ (Figure 3C). These results suggest that Na^+ ions are kept out of the shoot system, thereby protecting the leaves from the detrimental effect of salt stress. In contrast, in roots, colonized plants showed even higher Na^+ ion levels than non-colonized plants. However, these increases were largely compensated by the higher K^+ ion contents in roots such that the overall Na^+/K^+ ratios were lower than in non-colonized roots. In summary, the more strongly reduced Na^+/K^+ ratios in *Bicom.*-colonized plants explain the improved synergistic salt stress tolerance of Arabidopsis compared to the SA187 or *S. indica* singly colonized or the non-colonized plants.

S. indica promotes nutrient uptake (like calcium or phosphate) in plants. We therefore also measured the P and Ca contents in shoot and root samples under control and salt-treated conditions. The P levels (Figure 3D) were found to be increased only when *S. indica* was present, while the Ca^+ concentration (Figure 3E) was increased with both SA187 and *S. indica*, suggesting an increased uptake of these nutrients.

Transcriptional analysis of *Bicom.* colonization induces auxin and immunity pathways in Arabidopsis

To analyze the transcriptional changes and thus to gain insight into the molecular mechanism behind the enhanced growth by *Bicom.*, we carried out the RNA-seq analysis on plants non-colonized or colonized with SA187, *S. indica*, and *Bicom.*, and grown for 16 days on either ½ MS (data not shown) or ½ MS + 100 mM NaCl. After filtering for the *P* value (0.05) and Log_2 fold change (>1 for upregulated and <1 for downregulated genes), a total of 1002 and 1280 genes were found to be differentially expressed in shoots and roots, respectively. Hierarchical clustering of the DEGs from the root transcriptome was resolved into 12 clusters (Figure S3) but was not informative for gene ontology (GO) enrichment analysis using AgriGO (TAIR10) (Tian et al., 2017). The differentially expressed genes (DEG) from the shoot transcriptome were resolved into eight clusters (Figure 4A; Figure S2), with GO term enrichment of cluster 3 and cluster 7. Cluster 3 gene functions were strongly related to auxin transport, response, and signaling, while cluster 7 GO terms were related to auxin response, oxidative stress, and immune response (Figure 4B). As auxin-related GOs were dominating both clusters 3 and 7, we selected the genes from both clusters and compared their expression levels with those of non-colonized plants. The enrichment analysis based on Log_2 values showed that the majority of the genes which were highly upregulated in *Bicom.*-colonized plants were SAURs and IAAs – *IAA1*, *IAA5*, *IAA6*, *IAA19*, *IAA29*, *IAA34*, and genes responding to auxin, for example, *ACS4*, *BG1*, *HAT2*, and *XTH19* (Figure 4C).

Taken together, the changes in the transcriptome profiles showed that *Bicom.* controls auxin signaling and auxin-responsive genes, suggesting that an auxin-dependent mechanism might be controlling the enhanced plant growth and tolerance in *Bicom.*-colonized plants.

Dynamic regulation of immunity-related genes during SA187 and *S. indica* colonization of Arabidopsis

To address the enhanced colonization of *S. indica* in roots inoculated with *Bicom.*, we carried out gene expression

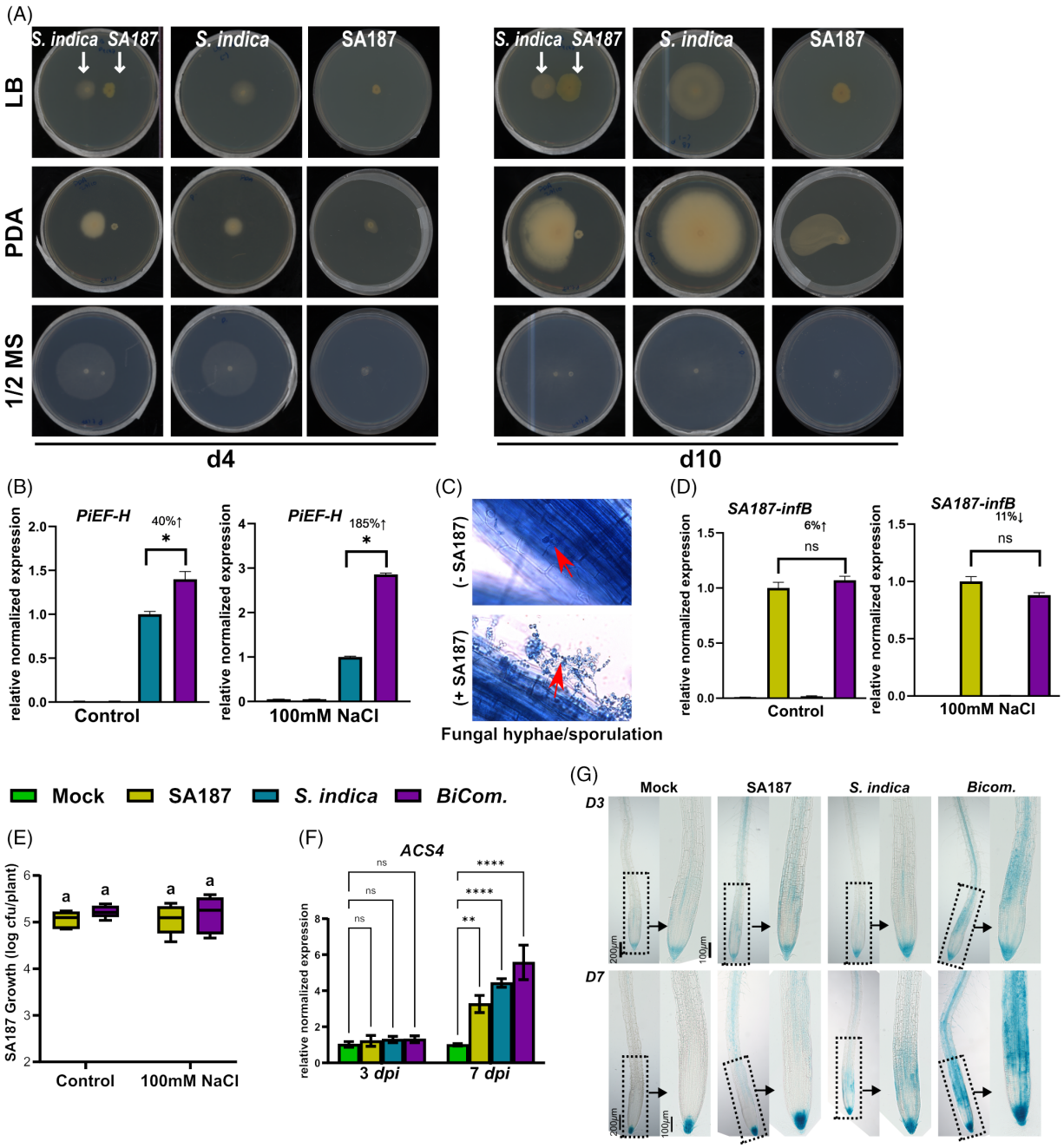

Figure 2. SA187 promotes *Serendipita indica* growth in plants.
(A) Plug inoculation assay showing the interaction of SA187 and *S. indica* on LB, PDA, and ½ MS plates at two different time points, day 4 and 10.
(B) qRT-PCR of fungal *PiEF-H* transcript, normalized to GADPH, in plants non-inoculated (mock) or colonized with SA187, *S. indica* or *Bicom.* grown on ½ MS or ½ MS + 100 mM NaCl plates. Data in the plot represents average ± SEM of three biological replicates. "*" Indicates the significant difference between the two dataset (*t*-test) and the percentage change in level of expression of *Pi.EF-H* between *S. indica* and *Bicom.*-colonized plants is denoted on the top.
(C) Microscopy image showing the Arabidopsis root colonization by *S. indica* in absence (– SA187) or presence (+ SA187) of SA187. Arrow shows increase in hyphae and sporulation of *S. indica* by SA187.
(D) qRT-PCR of SA187 *infB* transcript, normalized to UBI, in plants non-inoculated (mock) or colonized with SA187, *S. indica* or *Bicom.* grown on ½ MS or ½ MS + 100 mM NaCl plates. Data in the plot represents average ± SEM of three biological replicates. Significant difference between the two dataset (*t*-test) and the percentage change in level of expression of *infB* between *SA187* and *Bicom.*-colonized plants is denoted on the top. ns = non significant.
(E) CFU count of SA187 in plants colonized with SA187 or *Bicom.* under ½ MS or ½ MS + 100 mM NaCl conditions. Box plot represents data from five individual biological replicates. The significant difference is denoted by alphabet (Two-way ANOVA).
(F) *ACS4* expression in mock- and *SA187*, *S. indica*, or *Bicom.* inoculated seedlings after 3 and 7 days of colonization. * indicates significant difference from WT at indicated time point. **, $P < 0.01$, ****, $P < 0.0001$ as determined by Two way ANOVA with Dunnett's multiple comparisions test.
(G) Ethylene reporter, pEBF2:GUS, visualizing the relative auxin response in primary root tips of mock- and *SA187*, *S. indica*, or Bicom.-inoculated seedlings.

analysis of immune response genes at early and late time points of colonization in Arabidopsis. The genes involved in plant defense response towards microbial infection, like *AZI1* and *EARL1*, were suppressed after 7 dpi (Figure 4D). NPR1 is a transcriptional co-activator of defense response genes in plants, while NIMIN1 inhibits the activation of NPR1-dependent SAR genes at the onset of SAR by binding the C-terminus of NPR1, thus repressing its activity (Hermann et al., 2013). We found that SA187 and *Bicom.*-inoculated plants increased the expression of NIMIN1 genes right from early stages of colonization, while *S. indica* induced NIMIN1 expression in the roots of 7 dpi plants (Figure 4D). In line with this, the expression of NPR1 was found to be reduced from day 1 of colonization with microbes, with *Bicom*. showing the highest level of NPR1 suppression (Figure 4D).

Role of auxin signaling during SA187 and *S. indica* colonization of Arabidopsis

The significant changes in expression of auxin-related genes prompted us to check for the auxin response in plants upon colonization by SA187 and *S. indica*. To this end, we observed the auxin response reporter *DR5:GUS*, whose expression correlates with auxin levels in plants. After 3 days of colonization, the microbial colonization did not show any major changes in GUS intensity in primary roots, but lateral roots and lateral root primordia showed enhanced GUS levels (Figure 4E). On day 7, the DR5:GUS staining was slightly enhanced in primary roots of plants colonized with *Bicom*. however, like at day 3, the lateral roots and lateral root primordia again showed enhanced GUS signal (Figure 4E). Although SA187 and *S. indica* displayed enhanced auxin response, this response was significantly increased in Bicom.-colonized plants. These results support the notion that increased auxin levels could be the reason for the enhanced lateral root density and plant biomass in Bicom.-treated plants.

To confirm the role of auxin in the symbiotic interaction of Arabidopsis with SA187 and *S. indica*, we tested several auxin mutants. Under saline conditions, auxin transport mutants (*pin2* and *aux/laxQ*) were strongly compromised, while the *arf7arf19* mutants showed a marginal increase in the beneficial effect of *S. indica* (Figure 5A; Figure S5). In contrast, *Bicom*. maintained the same growth enhancement as SA187 in these mutants (Figure 5A). These results suggest that auxin transport, which is key in maintaining the auxin concentration gradient across plant tissues, and plant auxin responses may be important for the interaction of *S. indica* with Arabidopsis.

The role of the ethylene signaling pathway in SA187 and *S. indica* promoted salinity tolerance

Since SA187 is well known to act via ethylene signaling (De Zélicourt et al., 2018; González Ortega-Villaizán et al., 2024; Li et al., 2023), we also tested ethylene pathway mutants for their involvement during the *Bicom*. interaction. Under non-salt conditions, we did not see any differences in the plant biomass in ethylene mutants (Figure S4). Interestingly, both SA187 and *S. indica* require the ethylene signaling pathway, as the beneficial growth phenotype of these microbes was either lost for SA187 or partially compromised for *S. indica* in the ethylene signaling mutants *ein2* and *ein3*, respectively (Figure 5B). However, the impaired beneficial effect of *S. indica* in the ethylene biosynthesis mutant *acs-hept* could be rescued by SA187 (Figure 5B; Figure S6). Therefore, these results show that ethylene signaling is at the core of SA187 and *S. indica* interaction with plants, and that SA187-induced ethylene signaling promotes the *S. indica* plant growth phenotype.

DISCUSSION

Plants are meta-organisms that possess distinct microbiomes and tight symbiotic associations with the microorganisms in their surroundings. Rhizosphere microorganisms influence the composition and productivity of plants, and therefore the richness of the belowground microbial species is considered to be a predictor of aboveground plant diversity and productivity (Mendes et al., 2013). Salinity is a major abiotic stress factor limiting agricultural yield around the globe. There are a number of reports showing that PGPRs alleviate salt stress by modulating the physiological, biochemical, and molecular responses to salinity in plants (Giannelli et al., 2023). SA187 is an *Enterobacter* species that colonizes a wide range of plants (Synek et al., 2021) and promotes plant tolerance to salt stress in an ethylene-mediated manner by attenuating the salt stress-induced sulfur starvation response in plants (Andrés-Barrao et al., 2021; De Zélicourt et al., 2018). *S. indica* is a beneficial fungus exhibiting a wide host range of plant species and promoting plant growth and development by inducing early flowering, higher biomass, and altering plant metabolism (Li et al., 2023). *S. indica* modulates plant hormone levels and enzymatic activities at the same time as it enhances plant nutrient uptake (Li et al., 2023). Recently, the concept of microbe–microbe interactions in shaping plant host–microbe interactions has gained widespread interest. *S. indica* is a model beneficial fungal species that is also known to display inter-kingdom synergy with several other growth-promoting bacteria (Bandyopadhyay et al., 2022; Dabral et al., 2020; Del Barrio-Duque et al., 2019; Jahandideh Mahjen Abadi et al., 2021; Kumar et al., 2012; Singh et al., 2022). In this study, we combined the multi-stress tolerance-inducing microbes *Enterobacter* sp. SA187 and *S. indica* to test the inter-kingdom interaction between these two beneficial microbes with Arabidopsis under ambient and salt stress conditions.

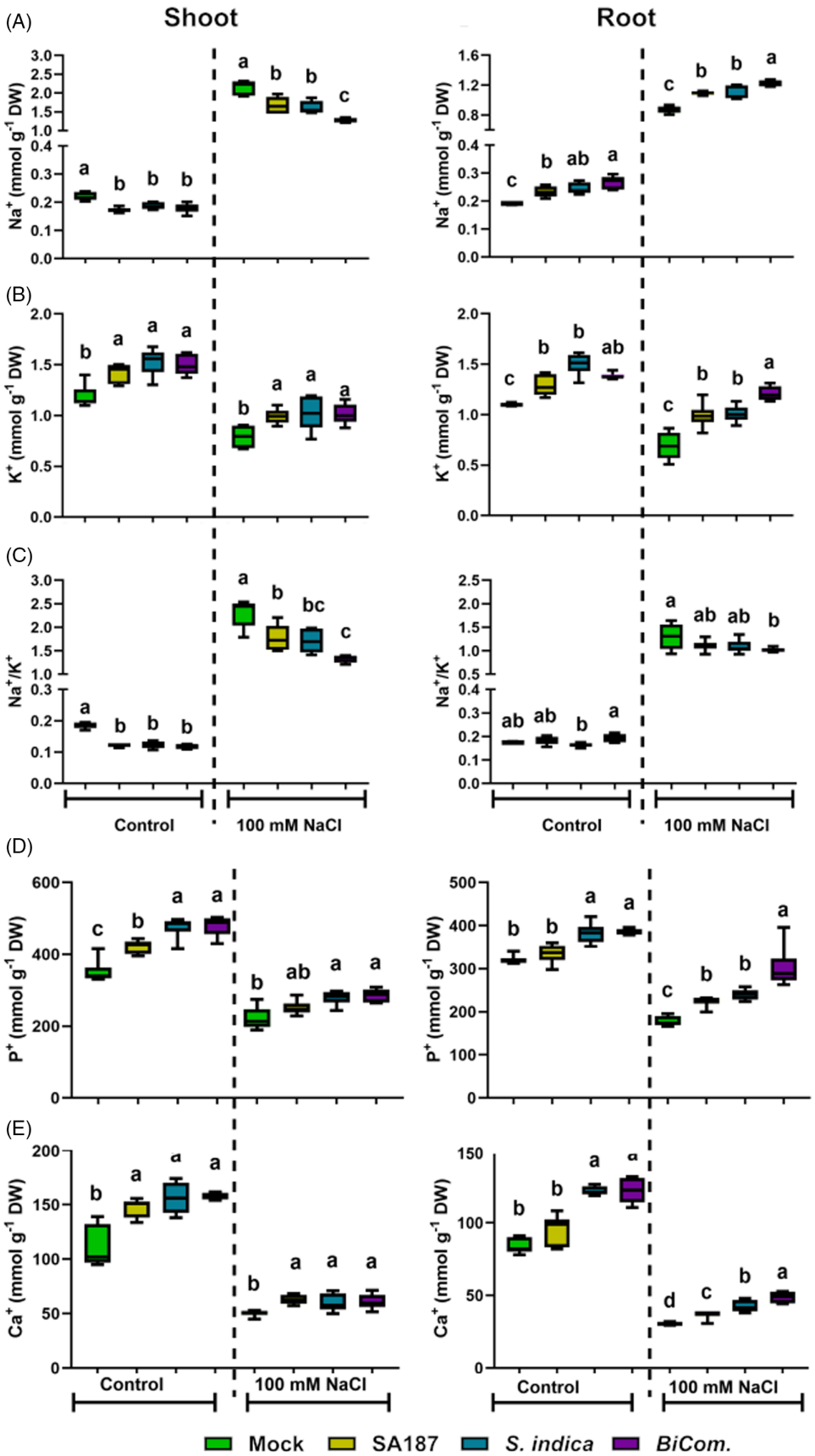

Figure 3. Ion content in Arabidopsis seedlings.
Shoot and root Na^+ content (A) K^+ content (B) and $^+Na/ K^+$ ratio (C) P content (D) and Ca^+ content (E) of 21-day-old mock or SA187, *Serendipita indica*, *Bicom*. inoculated Arabidopsis seedlings on control ½ MS and ½ MS + 100 mM NaCl. Box plot represents data from three individual biological replicates (n = 6). The significant difference from the mock in each treatment is denoted by alphabet (One-way ANOVA, tukey test).

The combined effect of SA187 and *S. indica* was found to be more effective than the individual microbes. The shoot biomass increased from 49% and 42% in SA187 and *S. indica*, but to 76% in *Bicom*. The effects of *Bicom*. on the root biomass were even more drastic, reaching up to 101% of increase when compared to singly colonized SA187, *S. indica*, or non-colonized plants (Figure 1). Both microbes control root architecture via plant hormone signaling. SA187 induces root hair formation and lateral roots in Arabidopsis in an ethylene-dependent manner (De Zélicourt et al., 2018). *S. indica* can synthesize auxin and gibberellins and thereby stimulate lateral root formation and increase root surface area, which helps in the uptake of water and minerals under stress conditions (Ansari et al., 2013). *S. indica* can also induce ethylene formation and thus stimulate lateral root formation (Bakshi et al., 2015). When treated as a consortium, SA187 and *S. indica Bicom*. resulted in a massive increment in both shoot and root tissues, as well as denser and bushier roots (Figure 1) indicating a positive interaction between these microbes in promoting salt tolerance in plants.

The concept of 'cry for help' explains that the rhizodeposits from plants (e.g., exudates, border cells, and mucilage) are key regulators of diversity and activity of microorganisms on plant roots. Plants control their surrounding microbial diversity for their own benefit by selectively stimulating beneficial microbes with traits that benefit plant growth and health (Mendes et al., 2013). Our *in vitro* interaction assays with SA187 and *S. indica* clearly showed a negative interaction between these microbes on all tested media (Figure 2A). Interestingly, upon bringing Arabidopsis as a third partner into the system, this interaction was no more negative. This contrasting behavior can be explained by the inability of the two microbes to grow on media lacking a carbon source. Whereas LB and PDA support the growth of the two microbes, nutrient competition becomes evident. However, on the plant growth medium ½ MS which is a purely synthetic medium, containing no carbon source, both strains undergo carbon starvation. In the presence of Arabidopsis, however, the plant offers both microbes sugars, organic acids and other carbohydrates as nutrient source. Surprisingly, both microbes engage in a symbiotic interaction and fungal colonization is even stimulated in the presence of the bacterium SA187 (Figure 2B,C). Since ethylene is essential for effective root colonization by *S. indica* (Khatabi et al., 2012), and we observed a pronounced accumulation of GUS signal in pEBF2:GUS roots indicating increased ethylene signaling in *Bicom*. plants (Figure 2G), we hypothesize that the enhanced ethylene production by SA187 boosts *S. indica* colonization in *Bicom*. roots. To date, many bacterial strains – known as Mycorrhiza helper bacteria (MHB), have been reported to enhance mycorrhizal growth and symbioses with plants by simulating mycelial extension, increasing root-fungus contacts and colonization, and protecting the harmful environmental effects on mycorrhizal hyphae (Frey-Klett et al., 2007). The notion that SA187 promotes growth of *S. indica* in the presence of a plant provides evidence that SA187 might be acting as a helper bacteria for *S. indica* colonization and the symbiotic association with the plant host. Fungal exudates, such as trehalose, serve as nutrients/chemoattractants for MHB and bacteria using fungal hyphae as a highway to migrate along the plant rhizosphere (Shi et al., 2023). The switch from antagonistic to a symbiotic behavior of SA187 and *S. indica* in the presence of a plant clearly shows the complex regulation of inter-kingdom interactions between plant-bacterial–fungal interactions in the rhizosphere.

The rhizosphere microbiome significantly influences nutrient uptake in plants to maintain growth under stress conditions. One of the ways to achieve salinity tolerance in plants is by maintaining low Na^+ levels while increasing K^+ concentrations under stress conditions. Plants that maintain low Na^+/K^+ ratios are able to perform better under salt stress conditions (Rawat et al., 2023; Sun et al., 2015). To maintain growth under saline conditions, plants either have to actively exclude sodium ions either by secreting them via salt glands, sequestering them in the vacuole, or expulsing them back into the soil. The reduction of Na^+ in the shoots while their increase in the roots (Figure 3A) in singly or dually SA187 and *S. indica*-colonized plants could suggest that Na^+ in shoots is actively pumped back via the xylem to arrive in the roots again, as suggested by our previous studies with several beneficial bacteria (Eida et al., 2019). Alternatively, Na^+ might become sequestered in root cells so that it is not getting transported to shoots, thereby enabling shoots to maintain photosynthetic activity. In contrast to the Na^+ levels, increased K^+ levels were observed in shoots as well as in roots by SA187 or *S. indica*-colonized plants, whereas the highest increases in K^+ were observed in *Bicom*-colonized plants (Figure 3B). In this way, *Bicom*-colonized plants had the lowest Na^+/K^+ ratios in shoots and roots of salt-treated plants (Figure 3C). In summary, whereas both SA187 and *S. indica* modulate the salt stress response in plants by reducing the plant Na^+/K^+ ratios (De Zélicourt et al., 2018; Gill et al., 2016), *Bicom*. further enhances the reduction of the Na^+/K^+ ratios, especially in the shoots of salt-treated plants (Figure 3C).

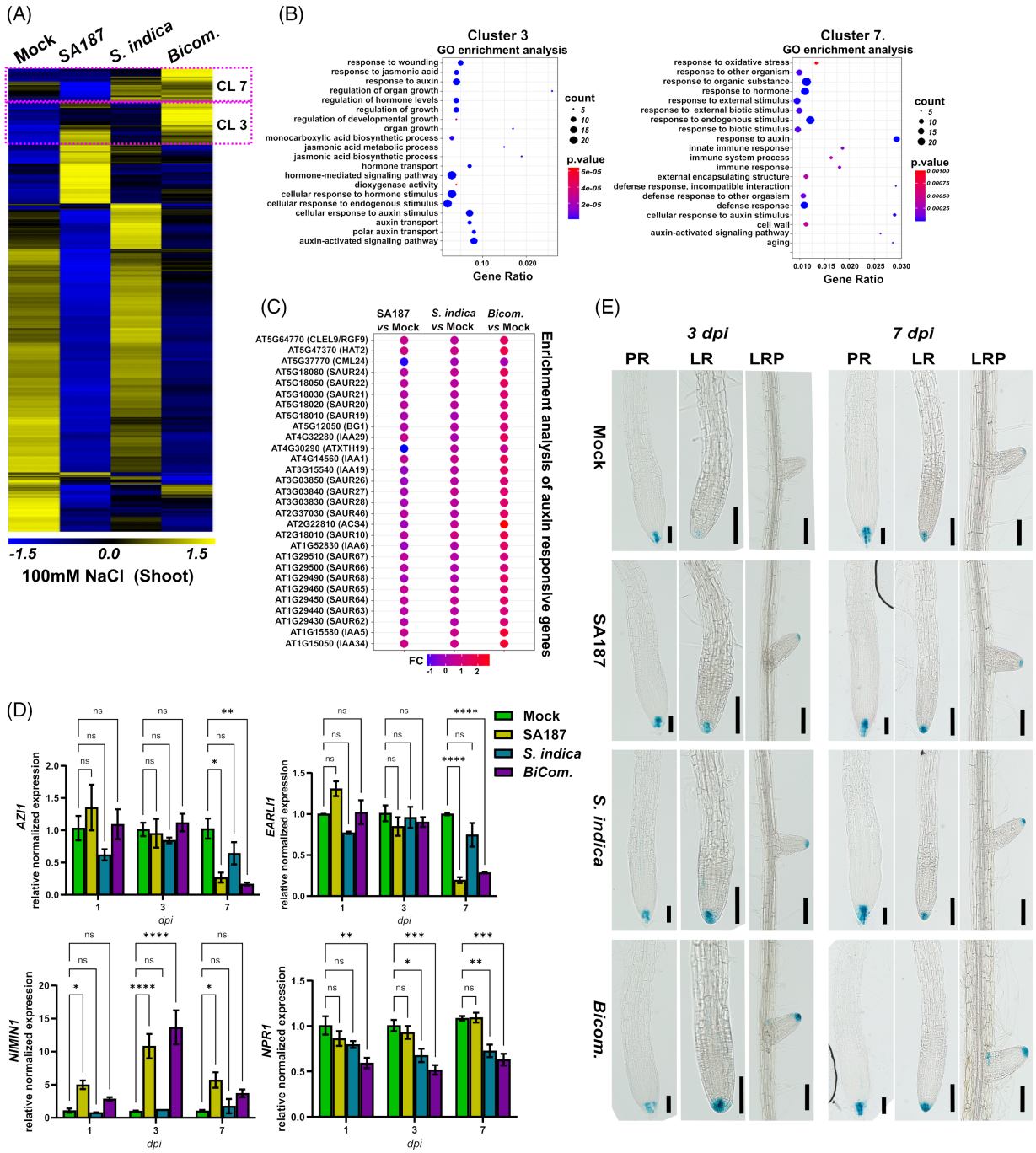

Figure 4. Differential expression of Arabidopsis genes in response to salt stress in the presence of a selected microbial combination.
(A) Hierarchical clustering of differentially expressed genes in Arabidopsis shoots upon ±100 mM NaCl treatment when co-cultivated with mock, *SA187*, *Serendipita indica*, or *Bicom*. The original FPKM values were adjusted by normalized genes/rows and subsequently processed by hierarchical clustering using the average linkage method using MeV4.0. Blue and yellow color indicate low and high expression levels, respectively. $P < 0.05$ and a fold change cut-off was set to $\log_2 > 1$ for up- and $\log_2 > -1$ for downregulated genes.
(B) GO enrichment analysis using the agriGo database (TAIR10) in clusters showing differential gene expression in *Bicom.* compared to others in salt-treated shoots. Dots denote gene count while the color indicates the *P* value.
(C) Enrichment analysis of genes involved in auxin response obtained from auxin-related GOs in cluster 3 and cluster 7 [from (B)]. The color of dots represents Log_2 fold change compared to mock.
(D) Relative normalized expression of defense-related genes after 1, 3, and 7 days of inoculation with SA187, *S. indica, and Bicom.* * indicates significant difference from WT at indicated time point. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$ as determined by Two way ANOVA with Dunnett's multiple comparisions test.
(E) Auxin reporter, pDR5:GUS, visualizing the auxin response and distribution in primary root (PR) tips, lateral root (LR) tips, and lateral root primordia (LRP) of mock- and *SA187*, *S. indica*, or *Bicom.* inoculated seedlings. Bar = 200 μm.

Plants colonized with *S. indica* show increased uptake of nutrient elements like P, N, Zn, Mg, and Fe (Bakshi et al., 2017; Bandyopadhyay et al., 2022; Gill et al., 2016; Jahandideh Mahjen Abadi et al., 2021; Wan et al., 2024). In line with these studies, we also found an increased uptake of P in shoots as well as roots independently of the salt treatment. This shows that *S. indica* increases P uptake in plants irrespective of salt stress (Figure 3D). The cell wall extract of *S. indica* activates cytosolic calcium uptake in Arabidopsis roots, which is mediated by the CNGC19 Ca^{+} transporter, thereby assisting fungal colonization of roots by altering the plant innate immune system (Jogawat et al., 2020; Vadassery et al., 2009). Our results show that under saline conditions, the elevation of Ca^{+} concentrations in shoots was similar in all microbial combinations. However, in roots of salt-treated plants, *Bicom.*-colonized plants had significantly higher Ca^{+} levels followed by *S. indica* and SA187, respectively (Figure 3E). These results suggest that the combined effect of SA187 and *S. indica* induces higher Ca^{+} levels in roots to suppress immunity, which supports enhanced fungal colonization in *Bicom.* plants (Figure 2B,C) and thus promotes increased uptake of essential nutrients like phosphate.

To further analyze the interactions of SA187, *S. indica*, and Arabidopsis at the molecular level, we studied the transcriptome of Arabidopsis colonized by single or *Bicom.* microbes under ambient and salt stress conditions. To unravel the enhanced growth phenotype of *Bicom.* plants, we looked for the unique key signatures in the transcriptome of these plants. While the majority of *Bicom.* plant transcriptome profiles reflected either SA187 or *S. indica* signatures, there were two clusters that pointed towards the activation of auxin transport and signaling, while the other also contained GO terms for immunity (Figure 4B,C).

On the basis of the transcriptome analysis, we analyzed a number of immunity-related genes over a time frame of 7 days upon plant infection either by SA187 and *S. indica* alone or by the *Bicom.* We observed a dynamic regulation of the expression of immunity-related genes at different stages of SA187 and *S. indica* interaction with Arabidopsis (Figure 4D). Importantly, compared to SA187, *S. indica* infection of roots is a much slower process and hyphae usually attempt to enter the host plant only after several days of contact with the plant (Jacobs et al., 2011). The suppression of immunity-related genes by SA187 at day 7 might therefore be an optimal time window to support hyphal infection of roots by *S. indica*.

With regards to auxin signaling, we analyzed a number of auxin pathway mutants for their role in the interaction of SA187 and *S. indica* alone or upon *Bicom.* infection. The intimate control of auxin conjugation during the mutual plant–*S. indica* interaction is a prerequisite for the beneficial effect of the fungus (González Ortega-Villaizán et al., 2024). The loss of the beneficial effect of *S. indica* in auxin efflux and influx mutants, *pin2/eir-1* and *aux/lax1-3Q* (Figure 5A), clearly showed that the maintenance of the polar auxin transport is essential for the beneficial effect of *S. indica*. The mutant *arf7arf19* is defective in lateral root hair formation (Okushima et al., 2007), and the failure of *S. indica* to initiate a response in the *arf7arf19* double mutant (Figure 5A) again proves the key role of the auxin pathway in mediating the beneficial response to *S. indica*. The beneficial effect of SA187 is mediated via ethylene signaling in plants, where SA187 feeds KMBA, a precursor of ethylene (De Zélicourt et al., 2018). Ethylene is also essential for root colonization by *S. indica* (Khatabi et al., 2012). In line with these published data, the *acs-hept* showed a beneficial effect when treated by SA187, proving that ethylene biosynthesis is not essential for the SA187 phenotype (Figure 5B) (De Zélicourt et al., 2018). Interestingly, plants treated with *S. indica* did not show any positive effect in the *acs-hept* mutant, indicating that *S. indica* requires ethylene production and ethylene signaling for its growth promotion effect (Figure 5B).

There is increasing evidence suggesting a crosstalk between the two phytohormones auxin and ethylene (Zemlyanskaya et al., 2018). Mutants in auxin signaling (*axr1*, *axr2/iaa7*, *axr3/iaa17* and *tir1*) display ethylene insensitive phenotypes, suggesting that plant ethylene responses are influenced by auxin signaling (Zemlyanskaya et al., 2018). Auxin induces expression of several ACS genes (Tsuchisaka & Theologis, 2004), many of which contain auxin response elements (ARF binding sites) in the promoter region (Stepanova et al., 2007) suggesting these ACS genes to be direct targets of ARFs. Ethylene has been shown to regulate the polar auxin transport in roots by modulating expression of AUX1 and several PINs (Semeradova et al., 2020). Ethylene also promotes local auxin biosynthesis in root tips (Zemlyanskaya et al., 2018). This reciprocal modulation of the endogenous hormonal crosstalk fine-tunes the plants response towards external cues.

Endophytic SA187 preferentially colonizes the apoplast where the bacterially produced KMBA is converted *in planta* to ethylene via apoplastic cell wall peroxidases (De Zélicourt et al., 2018). This process is independent of the canonical ACC synthase pathway. However, *S. indica* produces auxin (IAA) in the medium and can alter the auxin levels in plants, thereby promoting plant growth (Ansari et al., 2013). *S. indica* is also known to modulate auxin signaling and ethylene biosynthesis and signaling to alleviate stress in plants (Wan et al., 2024). *S. indica* was shown to induce *ACS1* and *ACS8* (Khatabi et al., 2012) to produce ethylene to support its own colonization. We found a massive increase in *ACS4* expression levels in the *Bicom.* treated plants, confirming that the synergistic effect of the *Bicom.* is mediated by modulating ethylene and auxin pathways to enhance plant growth and salinity stress tolerance (Figure 6).

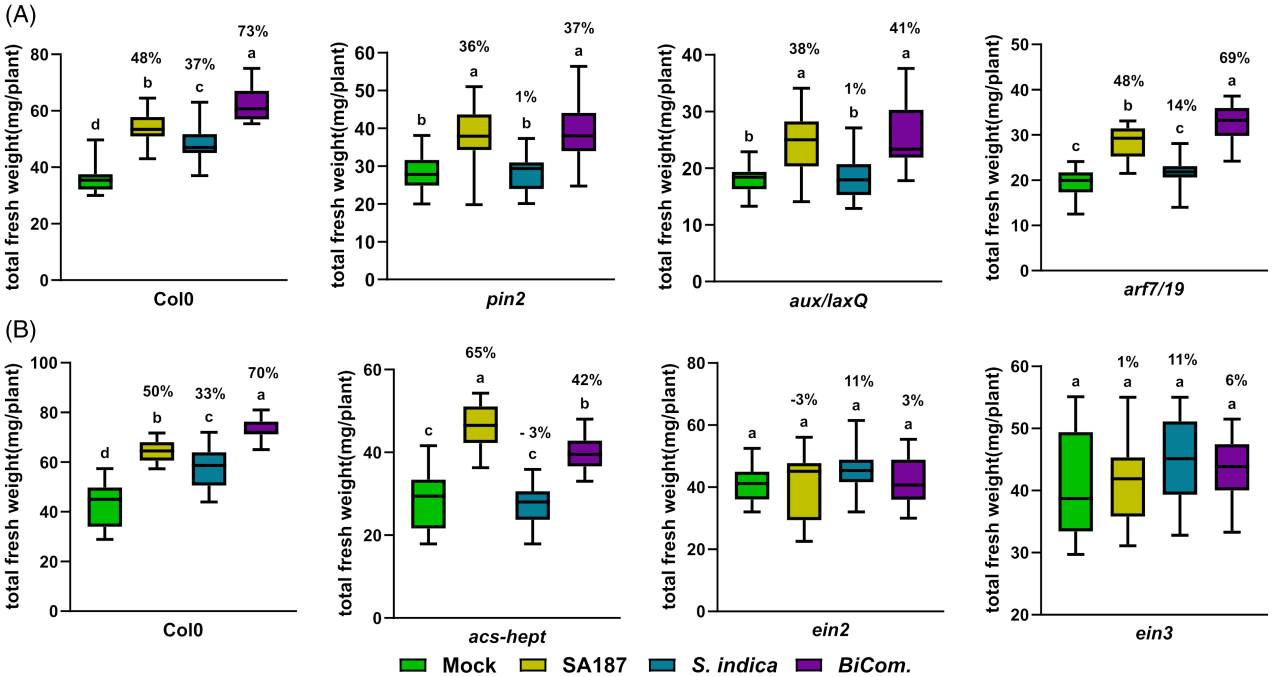

Figure 5. Effect of SA187, *Serendipita indica*, and *Bicom*. on Arabidopsis auxin and ethylene mutants.
(A) Total fresh weight of *A. thaliana* Col-0 and auxin receptor (*tir1*), transport (*pin2* and *aux/laxQ*) and auxin response (*arf7 arf19*) mutants non-colonized (Mock) or colonized with microbes upon growth for 16 days on ½ MS + 100 mM NaCl. Box plot represents data from three individual biological replicates (n = 18 for mutants, 30 for Col0). The numbers on top of each box represent the percentage change in plant biomass compared to non-colonized plants (mock). The significance difference from the mock is denoted by alphabet (One-way ANOVA, tukey test for multiple comparison).
(B) Total fresh weight of Col.0 and ethylene biosynthesis (*acs-heptuple* = *acs1-1 acs2-1 acs4-1 acs5-2 acs6-1 acs7-1 acs9-1*), signaling (*ein2* and *ein3*) mutants non-colonized (Mock) or colonized with microbes upon growth for 16 days on ½ MS + 100 mM NaCl. Box plot represents data from three individual biological replicates (n = 18–20). The numbers on top of each box represent the percentage change in plant biomass compared to non-colonized plants (mock). The significance difference from the mock is denoted by alphabet (One-way ANOVA, tukey test for multiple comparison).

Auxin and ethylene can participate in synergistic and antagonistic plant signaling pathways to regulate various growth, developmental, and defense processes. Our data show that rhizosphere consortia of bacteria and fungi can influence the endogenous phytohormone crosstalk in plants, either by providing precursors, such as KMBA, or by modulating the expression of rate limiting enzymes, such as *ACS4*, or the transport/signaling of phytohormones by *S. indica*. We also show that besides plant defense pathways might be involved in the plant–*Bicom*. interaction to mediate enhanced plant growth. Although these microbes do not support each other's growth on synthetic media, in the interaction with plants, the antagonistic behavior is turned into an interaction that is beneficial for all three partners, bacteria, fungus, and the plant host, highlighting the complexity of interactions among rhizosphere microbes in the context of plant–microbe interactions.

EXPERIMENTAL PROCEDURES

S. indica and SA187 growth culture and conditions

Prior to every experiment, the SA187 was cultured on Luria broth (LB; Sigma; St. Louis, MO, USA) plates for 2 days at 28°C in dark, and a single colony was used for further plant assays. For each experiment, the *S. indica* was isolated from Arabidopsis Col0 WT plants infected with *S. indica* (Johnson et al., 2011). The isolated *S. indica* cultures were grown on potato dextrose agar (PDA; Millipore, Darmstadt, Germany) for the subsequent 3–4 weeks at 24°C in the dark before being used for plant assays. Further, the fungal and bacterial interaction was studied in ½ MS, LB, and PDA medium.

Plant material and stress assays

Arabidopsis thaliana Col-0 was used as WT in this study. The ethylene mutant lines used in this study were published previously in different studies and have been used in response to SA187 (De Zélicourt et al., 2018). The auxin mutants published elsewhere were kindly provided by Dr. Ikram Blilou, KAUST.

The Arabidopsis seeds were surface sterilized for 10 min in 70% ethanol + 0.05% Triton X-100, washed three times with 100% ethanol, and dried on sterilized filter paper in a clean bench. The seeds were then spread on ½ MS plates prepared by adding 10^8 cfu ml^{-1} SA187 or equivalent ml of LB as control plates, as described earlier, stratified for 2 days, followed by growing for 5 days in plant growth chambers (Percival Scientific) under 16 h:8 h light:dark conditions at 22°C (Alwutayd et al., 2023). The salt stress was applied as described earlier (Rawat et al., 2023). Briefly, 5-day-old seedlings with equivalent root lengths were transferred to fresh ½ MS ± 100 mM NaCl plates. For inoculating *S. indica*, a hole was made next to the root tip by removing the media from the plate and was replaced

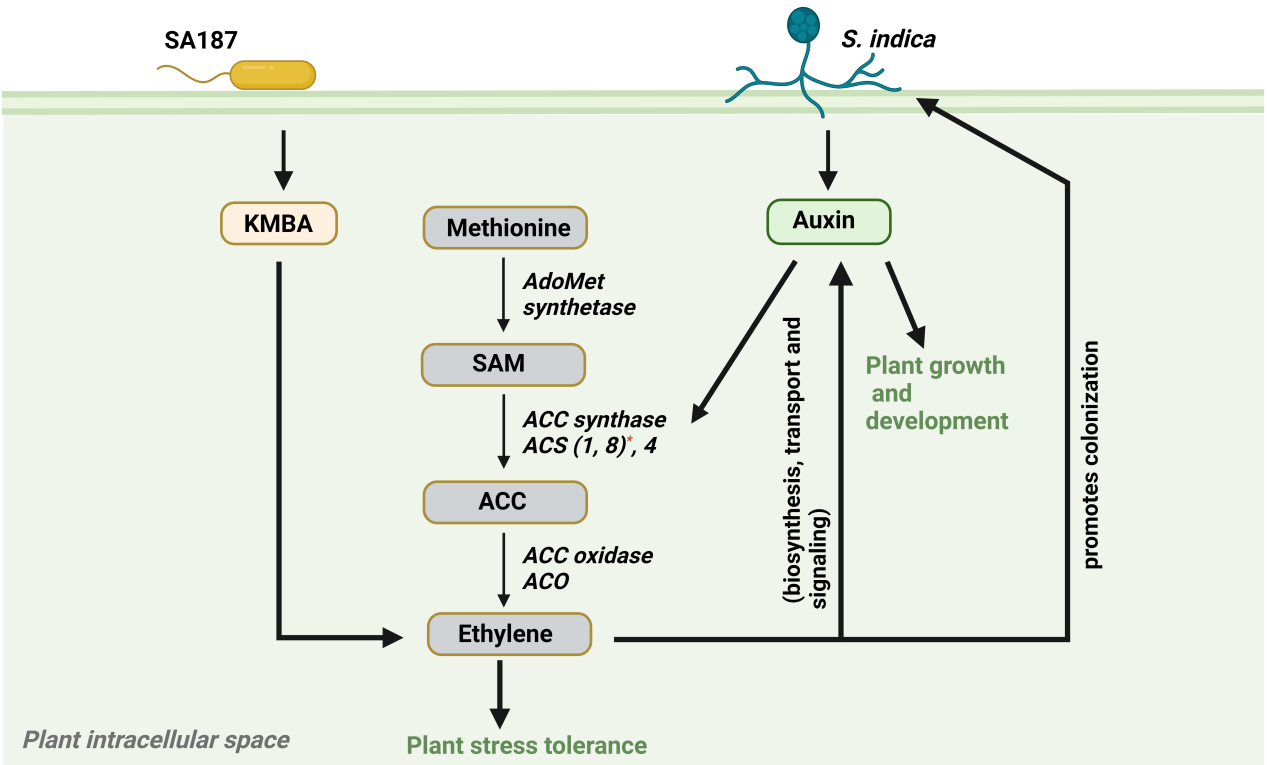

Figure 6. Mechanism of combined mode of action of SA187 and *Serendipita indica* to promote plant growth and salinity tolerance in Arabidopsis.
The canonical ethylene biosynthesis pathway in plants utilizes methionine to produce SAM, which gets converted to the ethylene precursor ACC and later into ethylene. SA187 provides an ethylene precursor KMBA (De Zélicourt et al., 2018) to the plants, which is also utilized for ethylene production. Ethylene regulates auxin responses by modulating auxin biosynthesis, transport, and signaling (Qin et al., 2019; Swarup et al., 2002; Zemlyanskaya et al., 2018). Ethylene, in turn, promotes *S. indica* colonization in plants (Khatabi et al., 2012). *S. indica* modulates auxin levels, signaling, and response in plants, thus promoting plant growth and development. Simultaneously, *S. indica* induces expression of ACS genes *ACS1** and *ACS8** (Khatabi et al., 2012) and *ACS4* (this study), enzymes playing a significant role in ethylene biosynthesis. This figure was created using www.biorender.com.

with a 3–4-week-old *S. indica* plug (Figure 1A). The plants were grown for another 16 days before harvesting for biomass quantification. The primary root length and lateral root densities were measured at day 9 after transferring to stress plates using ImageJ.

In vitro interaction assay between SA187 and *S. indica*

In vitro interaction was studied by inoculating a plug of 2-day-old SA187 (streaked freshly from glycerol stock) alongside the *S. indica* plug (picked from a 3–4 week old culture of *S. indica* on PDA plate) on nutrient-rich LB, PDA, and minimal ½ MS plates. The interaction between both the microbes was observed after 4 and 10 days of inoculation.

Bacterial colonization

Roots of 16-day-old plants colonized either SA187 alone or with *Bicom.* grown on ½ MS ± 100 mM NaCl, were collected in tubes, weighed, and ground for 2 min in 500 µl extraction buffer (10 mM $MgCl_2$ + 0.01% silwet77) using Tissue lyser II (Qiagen, Germany). Samples were vortexed and diluted 10-fold before plating on LB agar plates. Colony forming units (CFUs) were counted after overnight incubation at 28°C. The number of CFU was determined by normalizing with root fresh weight.

Histochemical staining

Fungal staining on roots colonized with *S. indica* was performed as described in (Vahabi et al., 2011). Briefly, root sections of 16-day-old plants colonized either by *S. indica* alone or with *Bicom.* were gently washed in distilled water, stained with cotton blue for 1 min, and observed with Axio Imager 2 (Carl Zeiss, Germany).

GUS staining of Arabidopsis seedlings was performed as described in De Zélicourt et al. (2018).

RNA sequencing and qRT-PCR

Total RNA from root and shoot samples were extracted from 16-day old plants either or not inoculated with SA187, *S. indica* and *Bicom.* using Nucleospin RNA plant kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. The RNA concentration was accessed by using Nanodorp-6000 spectrophotometer, Qubit 2.0 fluorometer with RNA BR assay kit (Invitrogen, Waltham, MA, USA) and total RNA integrity was verified using 2100-Bioanalyzer. RNA-Seq was carried out using Illumina TruSeq standard mRNA Library Preparation protocol as per manufacturer's instructions for 50-bp-end sequencing. The pooled libraries were sequenced on Illumina HiSeq4000 platform. After data trimming and alignment, differentially expressed genes (DEGs) were determined through DESeq2.

The AgriGO analysis tool was used functional categorization of DEGs (Tian et al., 2017). Three biological replicates per condition were used for RNA-Seq studies. Dot plots of enriched GO terms in clusters were created in RStudio using ggplot2 library.

To study *in vivo* interaction, the microbes were grown with plants, as described above. The effect of SA187 on *S. indica* colonization and vice a versa was determined with qPCR by quantifying the expression of *PiEF-H* (Bakshi et al., 2015) (fw – CGCAGAATACAAGGAGGCC, rv – CGTATCGTAGCTCGCCTGC) normalized to plant GADPH (fw – GAGCTGACTACGTTGTTGAG, rv – GGAGACAATGTCAAGGTCGG) and *infB* (Andrés-Barrao et al., 2021) (fw – GAAACGCGAATCCGCTAACC, rv – TGGGCAGTCCTGGTCATTTC) normalized to ubiquitin (fw – GGCCTTGTATAATCCCTGATGA, rv – AAAGAGATAACAGGAACGGAAA). Gene expression levels were calculated using Bio-Rad CFX manager software.

Measurement of ion contents

Rosettes and roots samples weighed and dried for 2 days at 60°C and were weighed again to determine the tissue dry weight. Samples were then digested in 2 ml of freshly prepared 1% HNO_3 (nitric acid; Sigma Aldrich, St. Louis, MO, USA) for another 2 days, and the ion concentration was determined using inductively coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer Optima 8300, Waltham, MA, USA).

AUTHOR CONTRIBUTIONS

AR conducted the majority of the experiments presented in this study. BH performed bacterial CFU counts. NP, HA, ASR performed ICP-OES analysis. HH conceptualized and supervised the project and helped in data analysis. AR and HH wrote the manuscript. HH reviewed and edited the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGEMENTS

This work is a part of the DARWIN21 project (http://www.darwin21.org). We are thankful to Prof. Ralf Oelmüller, Friedrich Schiller University – Jena, for kindly sharing the *S. indica* strain with us. We thank Dr. Ikram Blilou for providing the seeds of auxin mutants. We thank Dr. Katja Froehlich and Dr. Sabiha Parween for their technical support, and all members of the Hirt lab for useful discussions. The authors are grateful to the KAUST growth facility and Analytical Core Lab for their technical support. This work was supported by KAUST funding BAS/1/1062-01-01.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

RNA-Seq data presented in this study were deposited to GEO database under accession number GSE277462, and a token has been created to allow review while it remains in private status. We have initiated the process to make this data available publicly.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Growth of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized plants grown under ½ MS (control) or treated with ½ MS + 100 mM NaCl conditions for 16 days.

Figure S2. Hierarchical clusters of up- and downregulated genes in Arabidopsis shoots upon ±100 mM NaCl treatment when co-cultivated with mock, *SA187*, *S. indica* or *Bicom.* based on the RNA-Seq analysis.

Figure S3. Hierarchical clusters of up- and downregulated genes in Arabidopsis roots upon ±100 mM NaCl treatment when co-cultivated with mock, *SA187*, *S. indica* or *Bicom.* based on the RNA-Seq analysis.

Figure S4. Total fresh weight of auxin and ethylene mutants non-colonized (Mock) or colonized with microbes upon growth for 16 days on ½ MS (Control) plates.

Figure S5. Growth of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized WT, *pin2*, *aux/laxQ* and *arf7/19* plants grown on ½ MS + 100 mM NaCl conditions for 16 days.

Figure S6. Growth of non-colonized (mock) and SA187, *S. indica*, *Bicom.* colonized WT, *acs-hept*, *ein2* and *ein3* plants grown on ½ MS + 100 mM NaCl conditions for 16 days.

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