Cadmium modulates tomato root architecture and root hair responses to ABA-metabolising rhizobacteria

Abstract

Exogenous cadmium (Cd) stimulates ABA accumulation *in planta*, enhancing Cd tolerance by maintaining growth and limiting Cd accumulation. Since rhizobacteria that metabolise ABA may compromise Cd tolerance, tomato Ailsa-Craig plants were grown *in vitro* with or without 80 µM $CdCl_2$, and with or without ABA-metabolising *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W and the Cd-tolerant, ACC deaminase-containing *Variovorax paradoxus* 5C-2 (negative control). Root colonisation of the ABA-metabolising strains was *circa* 50% and 2 orders of magnitude less than 5C-2 without and with Cd respectively. While root Cd concentrations were independent of inoculation, only 5C-2 decreased leaf Cd concentrations. P6W decreased root ABA concentrations without Cd, and leaf ABA concentrations irrespective of Cd treatment. Although P1Y didn't affect root ABA concentrations, it decreased and increased leaf ABA concentrations with and without Cd respectively. With Cd, both ABA-metabolising strains inhibited primary root length and P1Y increased lateral root length. Without Cd, 5C-2 increased primary root length and lateral root number, while all strains increased root hair length and density. Cd increased these root hair traits in uninoculated seedlings, with bacterial addition causing further increments. To further investigate how ABA affects root (hair) traits, the ABA-deficient *flacca* mutant and its wild-type (WT) were compared at varying (0-100 µM) ABA concentrations. Although WT plants tended to have longer primary roots, *flacca* root hair length was *circa* 29% longer irrespective of exogenous ABA concentration. Higher ABA concentrations decreased primary and lateral root length and lateral root number, had no effect on root hair length but increased root hair density similarly in both genotypes. That both ABA-metabolising strains increased root hair length and density of *flacca* suggests ABA-independent regulation of these root hair traits. Despite stimulating root hair development, the ABA-metabolizing strains didn't enhance Cd concentrations *in planta* but they compromised Cd tolerance by inhibiting primary root length.

Keywords: abscisic acid, cadmium, PGPR, rhizosphere, root hairs, tomato.

Introduction

Widespread soil contamination with heavy metals, particularly cadmium (Cd), affects many plant physiological processes including root growth (Sigel et al. 2013; Li et al. 2023). Cd inhibited main and lateral root elongation and lateral root initiation of *Arabidopsis thaliana* (Vitti et al. 2013; Kohanova et al. 2018; Yemets et al. 2021). Although 5-20 µM Cd increased root hair length and density in *A. thaliana* (Vitti et al. 2013; Yemets et al. 2021), decreased root hair density was also reported (Kohanova et al. 2018). Cd has variable effects on roots of other species. While Cd inhibited root elongation, lateral root number and root hair length of rice (Yu et al. 2015), inhibition of tomato main root elongation by Cd was accompanied by enhanced lateral root formation (Liu et al. 2024) and increased root hair initiation (Carvalho et al. 2019). Thus Cd exposure has variable effects on growth of different root types in different species.

Phytohormones often regulate root morphological responses to stresses such as Cd. Cd treatment increased bean (*Phaseolus vulgaris*) root and shoot ABA concentrations, but these increments were not related to external Cd concentrations (Barcelo et al. 1986). Despite much greater root Cd accumulation, all maize tissues (roots, young and old leaves) accumulated ABA similarly, with substantial root growth inhibition but maintenance of young leaf growth (Abd Elgawad et al. 2020) suggesting ABA affects root and shoot growth differently. Exposing ABA-deficient mutants to Cd (and other stresses) evaluated ABA's role in growth regulation, although lower water status of these mutants may complicate the interpretation of differences in growth. Diminished root and shoot growth of ABA-deficient mutants, even when leaf water status is equivalent to WT plants, indicates ABA regulates growth by limiting production of the growth inhibitor ethylene (Sharp et al. 2000). Nevertheless, external Cd concentrations (10-25 µM) inhibited *in vitro* root growth of 7-day old ABA-deficient (*aba-1*), ABA-insensitive (*abi2-1* and *abi3-1*) and WT Arabidopsis seedlings similarly (Sharma and Kumar 2002), implying ABA does not mediate growth responses to Cd. Roots of the tomato ABA-deficient mutant *sitiens (sit)* accumulated approximately 30% more Cd than the WT cultivar Micro-Tom when exposed to 100 µM Cd hydroponically for 96 hours, with Cd increasing root and shoot growth of WT and *sit* similarly (Pompeu et al. 2016). Longer exposure times (2 weeks) to the same Cd concentration approximately halved shoot growth of the tomato ABA-deficient mutant *notabilis (not)* compared to the WT although endogenous Cd concentrations were similar, while *not* root growth was approximately 20% higher despite accumulating 4-fold more Cd (Chu et al. 2020). Although endogenous ABA accumulation was not measured, ABA maintained shoot growth but inhibited root growth (Chu et al. 2020). Thus the duration of Cd exposure and the growing system determines how ABA-deficient mutants respond to Cd.

Without Cd exposure, exogenous ABA treatments decreased *A. thaliana* root elongation (Gao et al. 2018), lateral root number (Guo et al. 2012) and root hair length (Rymen et al. 2017; Lombardo et al. 2018; Li et al. 2020) and density (Yao et al. 2003; Rymen et al. 2017; Li et al. 2020) although root hair density may also increase (Lombardo et al. 2018). Nevertheless, exogenous ABA stimulated rice root hair elongation (Chen et al. 2006; Wang et al. 2017) but inhibited primary root elongation (Yao et al. 2003). Applying 1 µM ABA didn't affect primary root length and lateral number of WT tomato growing in dry sand (water potential of -0.68 MPa), but rescued these variables in *not* (Zhang et al. 2021). Although *not* had longer root hairs and higher root hair density than its WT in well-watered sand (Karanja et al. 2021), whether ABA status modulates tomato root hair traits in response to Cd treatment is unknown.

Plant growth-promoting rhizobacteria (PGPR) stimulate root development and root hair formation, often by modulating plant hormonal status and metabolic pathways (Vacheron et al. 2013; Singh et al. 2024). Various PGPR have repeatedly enhanced root architecture and root hairs (Online resource Table S1). Whether variation in ABA concentrations mediates these responses is less clear, as PGPR effects vary with bacterial strain, plant species and applied stress (Ahmad et al. 2022). In dry soils, the ABA-producing strains *Azospirillum brasilense* Sp 245 (Cohen et al. 2015) or *Bacillus licheniformis* Rt4M10 and *Pseudomonas fluorescens* Rt6M10 (Salomon et al. 2013) improved growth and increased foliar ABA concentrations of *A. thaliana* or grape, respectively. Inoculating soybean with ABA-producing *Bacillus subtilis* AP-3 increased root hair density although root ABA concentrations were not determined (Araujo et al. 2005). Taken together, microbial ABA production might enhance root development, but direct evidence for this hypothesis is lacking.

Inoculating Cd-treated tomato plants with ABA-producing *Az. brasilense* strain CGMCC1.10379 increased growth, decreased Cd accumulation and elevated foliar ABA concentrations (Sun et al. 2023). Drought and inoculation with *Bacillus pumilus* ACCC19290 (Liu et al. 2023) or *Ps. fluorescens* G20-18 (Mekureyaw et al. 2022) also increased tomato shoot ABA concentrations, although microbial ABA production was not measured. Tomato plants inoculated with *Leclercia adecarboxylata* MO1 mitigated the stimulating effect of 120 mM NaCl on ABA concentrations (Kang et al. 2019). Interpreting these microbial effects is difficult without measuring leaf water

status, since low leaf water status stimulates ABA biosynthesis. *B. megaterium* inoculation stimulated growth of WT tomato and didn't affect shoot ABA concentration, but inhibited growth and diminished shoot ABA concentrations of the ABA-deficient mutants *flacca* and *sitiens* (Porcel et al. 2014). However, whether these bacterial strains produced or utilized ABA was not studied.

Rhizobacteria can also mediate plant ABA status by metabolising ABA exuded from the roots. A *Corynebacterium* sp. isolated from ABA-supplemented soil metabolised ABA, but its interaction with plants was not studied (Hasegava et al. 1984). Soil microbes putatively degraded ABA into unknown compounds, but specific strains were not isolated (Hartung et al. 1996). Strains *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W were characterized as ABA-metabolising bacteria inhabiting the rhizosphere. They decreased root ABA concentrations in rice and tomato seedlings and affected plant growth (Belimov et al. 2014), with *Rhodococcus* sp. P1Y metabolising ABA into dehydrovomifoliol (Yuzikhin et al. 2021) and rhodococcal acid (Yuzikhin et al. 2022). The ABA-metabolising strain *Pseudomonas plecoglossicida* 2.4-D decreased ABA concentrations in rhizosphere soil and lettuce shoots, abolishing competitive effects in high-density plantings (Vysotskaya et al. 2023). The ABA-metabolizing strain *R. qingshengii* (BNCC203056) increased Cd, Ni and Zn uptake by *A. thaliana* and several hyperaccumulating plant species, with such bacteria proposed for phytoremediation of polluted soils (Lu et al. 2020; Wang et al. 2022). ABA modulates plant Cd uptake and tolerance (Shen et al. 2022). Thus ABA-metabolising bacteria may regulate root growth and accumulation of heavy metals from contaminated soils.

We hypothesized that Cd would elevate ABA biosynthesis *in planta* thereby inhibiting root development, but that ABA-metabolising bacteria would decrease ABA concentrations and stimulate tomato root hair formation. Thus tomato plants were treated with ABA-metabolising strains *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W, along with a negative control (*V. paradoxus* 5C-2) that does not metabolise ABA but enhances tomato growth (Belimov et al. 2014). Exogenous ABA treatment was used to further evaluate relationships between plant ABA status and impacts of ABA-metabolising bacteria on root architecture and root hair formation.

Materials and methods

Biological materials

The ABA-metabolising strains *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W (Belimov et al. 2014) were used. The Cd-tolerant PGPR strain *Variovorax paradoxus* 5C-2 having ACC deaminase activity and producing auxins, but not utilizing ABA (Belimov et al. 2014), was used as a negative control. All strains were obtained from the [redacted] and maintained on Bacto-Pseudomonas F (BPF) agar medium (Belimov et al. 2005). Seeds of tomato (*Solanum lycopersicum* Mill. cultivar Ailsa-Craig) and its ABA-deficient mutant *flacca* were obtained from the Lancaster Environment Centre (Lancaster, UK).

Cadmium tolerance and immobilization by bacteria

Tolerance to Cd was estimated visually after incubating the bacteria for 5 d at 24°C on Bacto-Pseudomonas F (BPF) agar medium containing $CdCl_2$ at various concentrations from 20 to 600 µM as previously described (Belimov et al. 2005). Immobilization of Cd was assessed by cultivating bacteria in glass test tubes with a liquid BPF medium supplemented with 80 µM $CdCl_2$ for 7 days at 24°C and shaking 150 rpm. Then the suspensions were centrifuged for 15 min at 9000 g and 4°C and supernatants used for Cd determination, as described below.

Plant culture and sampling

Gnotobiotic agar culture was previously described in detail (Belimov et al. 2022) and used here with few modifications. Briefly, Petri dishes (d = 150 mm) were filled with 100 cm^3 of the sterile nutrient solution solidified with 0.7 % plant cell culture tested agar (Sigma-Aldrich) and containing [µM]: 500 $MgSO_4$, 500 K_2HPO_4, 200 KH_2PO_4, 500 $CaCl_2$, 20 KNO_3, 5 NaFeEDTA, 0.1 $MnSO_4$, 0.1 $ZnSO_4$, 0.1 H_3BO_3, 0.01 $CoCl_2$, 0.01 $CuSO_4$, 0.01 Na_2MoO_4, 0.01 $NiCl_2$, and 0.01 KJ. The nutrient solution was supplemented either with 40 µM or 80 µM $CdCl_2$, 10-fold concentrations of ABA from 0.01 to 100 µM. To prepare inoculum, the bacteria were cultivated for 48 h at 24°C on BPF agar medium and suspended in sterile tap water up to 10^8 cells per cm^3. Bacteria were added to the nutrient solution just before solidification in final concentration of 10^6 cells per cm^3. One Petri dish was prepared for each treatment in each experiment.

Tomato seeds were surface-sterilized with 5% sodium hypochlorite for 15 min and germinated for 5 d at 25°C. Six germinated seeds were placed in a line on each Petri dish, which were incubated at a 45° angle to the vertical (Online resource Fig. S1) for 10 d in a greenhouse at a photon flux density of 400 µmol quanta m^{-2} s^{-1}, day/night cycle of 16h/8h and at 25/16°C, respectively. At harvest, the leaves were excised and rapidly frozen in liquid nitrogen, with roots removed from the agar and also frozen in liquid nitrogen after scanning. The Petri dishes were scanned and images used to determine the primary and lateral root length and lateral root number. Images of intact root hairs (Online resource Fig. S2) located on the middle of the primary root (where root hairs were fully developed) were taken using a light microscope (Axio Scope.A1, Carl Zeiss, Oberkochen, Germany) and used to determine root hair length and number. Results were expressed as mean values for each image.

Tissue samples were lyophilized, weighed and analyzed for ABA concentration via radioimmunoassay using the antibody MAC252 as previously described (Quarrie et al. 1988). The remaining roots and leaves were dried and ground, then their Cd and nutrient element content determined. Samples of bacterial supernatants, roots and leaves were digested in a mixture of concentrated HNO_3 and 38% H_2O_2 at 70°C using the digestion system DigiBlock (LabTech, Sorisole, Italy). Elemental concentrations (Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, P, S, Zn) in the digested samples was determined using an inductively coupled plasma emission spectrometer (ICPE-9000, Shimadzu, Tokyo, Japan), according to the manufacturer's instructions.

Enumeration of bacteria on roots

Fresh roots were homogenized in sterile tap water with sterile mortar and pestle. Homogenates were serially diluted in 10-fold steps and duplicate aliquots plated on BPF agar. Colony forming units (CFU) were counted after incubating plates for 5 d at 25°C.

Statistical analysis

The software STATISTICA version 10 (TIBCO Software Inc., CA, USA) was used for one- and two-way analysis of variance (ANOVA), with Fisher's least significant difference (LSD) test discriminating significant ($P<0.05$) treatment differences. Two-way analysis of variance and covariance discriminated effects of Cd, ABA, microbial treatment and their interaction (Online resource Tables S3, S4).

Results

Microbial cadmium tolerance and immobilization

When the bacteria were cultivated on BPF agar, threshold growth-inhibitory and minimum lethal concentrations were 60 µM Cd and 140 µM Cd for *Rhodococcus* sp. P1Y and 70 µM Cd and 180 µM for *Novosphingobium* sp. P6W, respectively. *V. paradoxus* 5C-2 growth was not inhibited even at 600 µM Cd. Cadmium concentration in uninoculated liquid BPF medium decreased by 14% during incubation, probably due to immobilization by components of the medium (Table 1). Although *Novosphingobium* sp. P6W had no significant effect on medium Cd concentration, *Rhodococcus* sp. P1Y and *V. paradoxus* 5C-2 decreased Cd concentration in the nutrient medium by 23% and 54% respectively. Greater Cd tolerance of *V. paradoxus* 5C-2 was associated with its ability to immobilize Cd.

Bacterial effects on plants

Root colonization of *V. paradoxus* 5C-2 was approximately twice that of the other strains (Table 2) when plants were grown on agar culture in the absence of Cd. Although 80 µM Cd did not affect root colonization of *V. paradoxus* 5C-2 (Table 2), it decreased root colonization by *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W by 22 and 12 times, respectively.

Preliminary experiments showed that 40 µM Cd inhibited lateral (but not primary) root length of WT tomato (Online resource Fig. S3B, D), but not root and leaf ABA concentrations Online resource (Fig. S3E, F). Treatment with 80 µM Cd also decreased both primary (Online resource Fig. S3A) and lateral (Online resource Fig. S3B) root length and lateral root number (Online resource Fig. S3C), but increased root ABA concentrations by 28% (Online resource Fig. S3E). Therefore further experiments used 80 µM Cd.

Without Cd, *Novosphingobium* sp. P6W decreased primary root length of WT tomato by 18% (Fig. 1A), while *V. paradoxus* 5C-2 increased primary and lateral root length (Fig. 1A, B) and lateral root number (Fig. 1C). Treatment with Cd decreased all measured root traits, particularly lateral root elongation by 3 times (Fig. 1B). Cd treatment eliminated positive effects of *V. paradoxus* 5C-2 on primary root elongation (Fig. 1A) and lateral root number (Fig. 1C), but lateral root length was about twice as long as uninoculated plants. Cadmium inhibited primary root elongation of plants inoculated with *Rhodococcus* sp. P1Y or *Novosphingobium* sp. P6W (Fig. 1A), and lateral root formation of plants inoculated with *Rhodococcus* sp. P1Y (Fig. 1C). Inoculation with *V. paradoxus* 5C-2 and *Rhodococcus* sp. P1Y increased total and mean lateral root length of Cd-treated plants (Fig. 1B, D). With Cd, microbial strains affected lateral more than primary root growth.

Irrespective of Cd treatment, all bacterial strains increased root hair length (Fig. 2A) and number (Fig. 2B) of WT plants. Root hair length was maximal with *V. paradoxus* 5C-2 and *Novosphingobium* sp. P6W without Cd, and with *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W in Cd-treated plants. Root hair density was maximal with *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W without Cd, and with *Rhodococcus* sp. P1Y in Cd-treated plants. Both Cd and microbial treatments significantly stimulated root hair formation (Fig 2 A ,B). All microbial strains increased root hair length and number (Online resource Fig. S4) of the ABA-deficient *flacca* mutant grown without Cd, although limited seed supplied precluded evaluation of Cd-treated plants.

Without Cd, strain *Novosphingobium* sp. P6W decreased root ABA concentration by 33% (Fig. 3A). Cd treatment universally increased root ABA concentrations and eliminated negative effects of *Novosphingobium* sp. P6W on root ABA concentration. Without Cd, leaf ABA concentrations of all microbial treatments were lower than the uninoculated control (Fig. 3B). While Cd treatment didn't alter leaf ABA concentrations of uninoculated and *Novosphingobium* sp. P6W-treated plants, it increased leaf ABA concentrations of *V. paradoxus* 5C-2 or *Rhodococcus* sp. P1Y-treated plants. Thus Cd treatment modulated ABA accumulation responses to different bacterial strains.

Root Cd concentrations were 6.4-fold higher than leaf Cd concentrations in uninoculated plants, and > 9-fold higher in inoculated plants. All microbial strains didn't affect root Cd concentration, while only *V. paradoxus* 5C-2 decreased foliar Cd concentration by 45% (Table 3). Cd treatment decreased root K and Mn concentrations but increased S concentration in both uninoculated and inoculated plants (Online resource Table S2). Cd treatment increased Zn concentrations but not in inoculated plants. Concentrations of K and Zn decreased, but all strains increased Mg concentration compared with Cd treatment. Both ABA-utilizing strains decreased root Fe concentration and *Novosphingobium* sp. P6W increased root P and K concentrations. *V. paradoxus* 5C-2 also increased root P concentration compared to Cd-treated plants (Online resource Table S2). Rhizobacteria and Cd did not affect leaf nutrient concentrations (Online resource Table S2).

Both cadmium and rhizobacteria significantly altered all growth parameters and ABA concentrations, except root hair length for Cd and root ABA concentration for rhizobacteria (Online resource Table S3). While Cd had no effect on total lateral root length and root ABA concentration responses to bacteria (no significant Cd x bacterial strain interaction), otherwise Cd modulated plant response to inoculation.

ABA effects on roots

ABA treatment decreased main and total lateral root length (Fig. 4A, B), and lateral root number (Fig. 4C) of WT and ABA-deficient *flacca* in a dose-dependent manner. Treatment with 0.01 µM ABA inhibited *flacca* primary root length (Fig. 4A) and lateral root length of both genotypes (Fig. 4B). Lateral root number decreased at 0.1 µM ABA for WT and at 0.01 µM ABA for *flacca*, respectively. However, ABA did not affect mean lateral root length excepting WT at 100 µM ABA (Fig. 4D), nor root hair length (Fig. 4E). Exogenous ABA significantly increased *flacca* root hair length by 29%, averaging across all ABA concentrations (Fig. 4E, Online resource Table S4). Root hair density of both tomato genotypes increased at 1 µM ABA, and especially at 10 and 100 µM ABA (Fig. 4F). Root growth of both tomato genotypes responded similarly (no genotype x ABA interactions – Online resource Table S4) to exogenous ABA.

Discussion

Environmental factors influence root system architecture and root hair development via complex regulatory networks (Vissenberg *et al.* 2020; Karlova et al. 2021), while individual PGPR strains usually possess several beneficial traits including phytohormone production and/or degradation (Dodd *et al.* 2010, Vacheron *et al.* 2013; Syrova et al. 2022; Abou Jaoude et al. 2023). The studied strains differed qualitatively and quantitatively in Cd

tolerance (Table 2) and plant growth-promoting traits including auxin production (all three), ACC deaminase activity (*V. paradoxus* 5C-2 and *Novosphingobium* sp. P6W) and ABA utilization (*Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W), with *V. paradoxus* 5C-2 consistently promoting root growth. With Cd, only *Rhodococcus* sp. P1Y enhanced total and mean lateral length but both ABA-metabolizing strains consistently enhanced root hair length and density independently of Cd concentration. Greater root hair length of the ABA-deficient *flacca* mutant (Fig. 4E) indicates ABA inhibits root hair elongation *in vitro*, while high exogenous ABA concentrations increased root hair density of both WT and *flacca* tomato and inhibited primary and lateral root length. The ABA-metabolizing strains enhanced root hair traits but not Cd uptake, but their decreased primary root growth with Cd treatment supports our hypothesis that ABA accumulation mitigates Cd toxicity.

Cadmium-bacteria interactions

Initially characterized as a Cd-tolerant PGPR with threshold growth-inhibitory and minimum lethal concentrations of 1400 µM and 3500 µM Cd respectively (Belimov et al. 2005), *V. paradoxus* 5C-2 was a negative control for *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W. These ABA-metabolizing strains had threshold growth-inhibitory Cd concentrations *in vitro* 25% and 13% lower respectively than the nutrient agar concentration (80 µM Cd) on which tomato seedling were grown. While Cd didn't limit *V. paradoxus* 5C-2's high root colonization, it severely inhibited moderate root colonisation by the ABA-metabolising strains (Table 2), likely because Cd was toxic to these bacteria. Immobilisation of Cd by *V. paradoxus* 5C-2 (Table 1) supported comparable root colonisation to growing the same tomato genotype without Cd treatment (Belimov et al. 2014).

V. paradoxus 5C-2 decreased leaf Cd concentrations *in vitro* (Table 3), as in *Medicago ciliaris* (Safronova et al. 2012), Indian mustard (Belimov et al. 2015) and pea (Belimov et al. 2020b) plants cultivated in contaminated soils. It immobilised Cd (Table 1), likely by secreting organic Cd-binding compounds causing extra-cellular precipitation with mineral anions, biosorption, bioaccumulation and compartmentalization (Halim et al. 2020; Zheng et al. 2021). Bacterial ABA metabolism did not universally affect Cd immobilisation as *Rhodococcus* sp. P1Y decreased Cd concentrations of the culture medium by 23%, but *Novosphingobium* sp. P6W had no effect. While both ABA-metabolising strains tended to diminish leaf Cd concentrations *in vitro* (Table 3), the mechanisms are not clear.

Cadmium effect on plants

Root hairs of *A. thaliana* are putatively involved in Cd accumulation (Kohanova et al. 2018), with the barley hairless mutant *brb* accumulating less Cd than its WT (Zheng et al. 2011). With Cd, all bacterial strains increased root hair length and density but root Cd concentrations were independent of rhizobacterial inoculation and leaf Cd concentrations decreased. That *Novosphingobium* sp. P6W increased root hair length and density without immobilizing Cd, yet had lower leaf Cd concentrations, suggests root hairs hardly affected Cd concentrations *in vitro*.

Cadmium enhanced root hair length and density (Fig. 2) as in other tomato cultivars and the tomato relative *Solanum pimpinellifolium* LA0122 (Carvalho et al. 2019). In *A. thaliana,* Cd stimulated root hair elongation (Vitti et al. 2013; Bahmani et al. 2016; Yemets et al. 2021) and density (Bahmani et al. 2016; Kohanova et al. 2018), but plants had fewer root hairs as root elongation was inhibited (Kohanova et al. 2018). Cd likely increases root hair traits by increasing auxin (Vitti et al. 2013; Bahmani et al. 2016) and ethylene (Abozeid et al. 2017; Bahmani et al. 2016). Since Cd treatment also increased root ABA concentrations (Fig. 3) and ABA treatment enhanced root hair density (Fig. 4F), endogenous ABA may also enhance root hair density. Testing this hypothesis requires measuring root hair traits of ABA-deficient tomato mutants grown with Cd.

Despite positively affecting root hair traits, Cd inhibited tomato root development, especially lateral root formation (Fig. 1C) which decreased by 30%, as in hydroponically grown tomato where 75 µM Cd approximately halved the number of lateral roots (Liu et al. 2024). Enhanced lateral root formation at lower Cd concentrations (10-50 µM Cd) was ascribed to increased auxin diffusion into the root stele, where lateral roots are initiated (Liu et al. 2024). Since all strains produced auxin, bacterial impacts on lateral root number were attributed to bacterial ACC deaminase and ABA metabolism. Without Cd, only ACC deaminase-containing *V. paradoxus* 5C-2 promoted lateral root length and number suggesting an ABA-independent effect. With Cd, all strains increased lateral root length without or negatively (*Rhodococcus* sp. P1Y) affecting lateral root number. That Cd only inhibited lateral root number following inoculation with ABA-metabolising *Rhodococcus* sp. P1Y (lacking ACC deaminase) suggests bacterial ACC deaminase mediates lateral root formation. However, Arabidopsis lateral root number was

independent of bacterial ACC deaminase status (Contesto et al. 2008) and the highest mean and total lateral root length of Cd-treated plants with *Rhodococcus* sp. P1Y suggests ABA constrains lateral root growth, as exogenous ABA treatment confirms (Fig. 4B).

ABA-Cd Interactions

Exogenous ABA inhibited lateral root length primarily by decreasing lateral root number (Fig. 4B, C) of both WT and *flc* plants. Similarly, exogenous ABA inhibited peanut primary root length and lateral root number (Guo et al. 2012). Nevertheless, the ABA-deficient *not* tomato mutant had fewer lateral roots independent of soil drying, which exogenous ABA application rescued (Zhang et al. 2021). This implies an optimal endogenous ABA concentration maintains root growth. Cadmium suppression of primary and lateral root length coincided with root ABA accumulation (cf. Fig 1A, B; 3A), with ABA-metabolising bacteria inhibiting primary root elongation but enhancing lateral root length. Despite *not* accumulating 4-fold more Cd than the WT in 100 μM Cd, its greater root biomass accumulation (Chu et al. 2020) may be sustained by greater lateral root growth, although more detailed root architectural measurements are needed.

Increased exogenous ABA concentrations didn't affect root hair length (Fig. 4E) but increased root hair density of both tomato genotypes (Fig. 4F). Although ABA treatment decreased root hair length of *A. thaliana* (Rymen et al. 2017; Lombardo et al. 2018; Li et al. 2020), ABA negatively (Rymen et al. 2017; Li et al. 2020) or positively (Lombardo et al. 2018) affected root hair density. Likewise, exogenous ABA inhibited (Yao et al. 2003) or promoted (Chen et al. 2006; Wang et al. 2017) rice root hair formation. Whether ABA accumulation mediates tomato root responses to Cd toxicity is less certain as the ABA-metabolising strains didn't alter root ABA concentrations (Fig. 2A). Opposing effects of Cd (Fig. 2A) and ABA (Fig. 4E) on root hair traits suggests their response to Cd can be ABA-independent, with Cd disturbing root hair formation by affecting Ca transport and function in roots (Fan et al. 2011).

Root hair density of ABA-treated plants negatively correlated with primary root length of Ailsa-Craig ($r = -0.97$, $p = 0.001$, $n = 6$) or *flacca* ($r = -0.93$, $p = 0.006$, $n = 6$) (Fig. 5), with inoculated WT plants also showing this negative correlation ($r = -0.71$, $p = 0.049$, $n = 8$). Likewise, treating the Micro-Tom cultivar with chemical ethylene inhibitors (Ag^{+} and Co^{2+}) and ACC-utilizing PGPR (*V. paradoxus* 5C-2 and *P. brassicacearum* Am3 and T8-1) established similar correlations (Belimov et al. 2022) suggesting root growth inhibition increased root hair density. Although root hair formation was not correlated with the other parameters measured (Online resource Table S5), root ABA concentration and lateral root length were negatively correlated ($r = -0.73$, $p = 0.041$, $n = 8$) after inoculation (Online resource Table S5), as when exogenous ABA was supplied (Fig. 5). These common responses across different inhibitor and bacterial treatments implies common regulatory mechanisms.

Bacterial effects on root hairs

Without Cd, ABA-metabolising strains stimulated root hair length and density of WT plants (Fig. 2) and the ABA-deficient *flacca* mutant (Online resource Fig. S4). However, bacterial effects on root hairs did not correlate with ABA concentrations *in planta* (Online resource Table S5). These bacteria decreased tomato root ABA concentrations (Belimov et al. 2014), consistent with ABA inhibiting root hair elongation and root hair density. Moreover, root hair length and density of the ABA-deficient *not* mutant was greater than WT plants grown in well-watered sand (Karanja et al. 2021). While endogenous ABA may directly inhibit root hair development, ABA-deficient tomato mutants also show increased foliar (Sharp et al. 2000; Dodd et al. 2009) and possibly root ethylene evolution. Unlike ABA, ethylene promotes root hair length and density, with ACC-deaminase rhizobacterial mutants (allowing greater root ethylene production) enhancing *A. thaliana* root hair length (Contesto et al. 2008). While stimulatory effects of the ACC-deaminase containing *V. paradoxus* 5C-2 on root hair length and density were Cd-independent (Fig 2A, B), and when Micro-Tom seedlings were inoculated with *V. paradoxus* 5C-2 or *Pseudomonas brassicacearum* Am3 (Belimov et al. 2022), ethylene production by *Az. brazilense* was associated with enhanced root hair formation (Ribaudo et al. 2006). Nevertheless, these strains also produce IAA (Belimov et al. 2005, 2014) which positively regulates root hair development (Pitts et al. 1998; Li et al. 2022). Thus multiple hormonal changes could regulate root hair traits following bacterial inoculation.

Without inoculation, Cd treatment increased root hair length and density (Fig. 2) while increasing root ABA concentration (Fig. 3A). Furthermore, Cd attenuated the stimulatory effects of *Novosphingobium* sp. P6W and *V. paradoxus* 5C-2 on root hair length (Fig. 2A) while amplifying the stimulatory effects of *Rhodococcus* sp. P1Y on root hair length (Fig. 2A) and density (Fig. 2B). The ABA- and IAA-producing *Az. brasilense* strain Az39 (Perrig

et al. 2007) enhanced wheat root hair development irrespective of Cd treatment (Vasquez et al. 2021). That root hair stimulation by *Novosphingobium* sp. P6W and *Rhodococcus* sp. P1Y occurred despite similar root ABA concentrations to Cd-treated uninoculated controls (Fig. 3A) suggests their impacts were independent of their ability to utilize ABA and/or decrease ABA concentrations.

Cadmium mediates plant response to bacteria

Previous reports on ABA-metabolising bacteria such as *Corynebacterium* sp. (Hasegava et al. 1984), *Rhodococcus qingshengii* BNCC203056 (Lu et al. 2020) and *Ps. plecoglossicida* 2.4-D (Vysotskaya et al. 2023) did not study root architecture and root hairs. Without Cd, both ABA-metabolising strains had little effect on root architecture, with only *Novosphingobium* sp. P6W decreasing primary root elongation (Fig. 1A) as previously reported (Belimov et al. 2014). This strain also decreased lateral root length and number of the ABA-deficient *flacca* mutant more than its WT, suggesting ABA involvement in plant-bacteria interactions (Belimov et al. 2014).

Similarly, growth-promoting effects of *Novosphingobium* sp. P6W and *Rhodococcus* sp. P1Y on tomato leaves correlated with decreased leaf ABA concentrations *in vitro* (Belimov et al. 2014). These results were confirmed without Cd, but only *Novosphingobium* sp. P6W decreased leaf ABA concentrations with Cd (Fig. 3B). ABA-induced stomatal closure should restrict leaf Cd accumulation by limiting transpiration fluxes (Zhao et al. 2023), thus the ABA-metabolizing strain *R. qingshengii* (BNCC203056) decreased root and shoot ABA concentrations but increased Arabidopsis shoot growth and Cd concentrations in transpiring conditions (Lu et al. 2020). That shoot Cd accumulation with bacterial inoculation was even greater in the *abi1/hab1/abi2* ABA-insensitive mutant, that grows less than WT plants (Rubio et al. 2009), suggests additional Cd accumulation due to less growth dilution (Lu et al. 2020). Although *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W had no significant effect on endogenous Cd concentrations, they decreased root Zn concentrations but maintained Cu and Ni concentrations (Table 3). Growing plants *in vitro* at high humidity minimized ABA's effects in mediating metal uptake by regulating transpiration, thus *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W didn't alter root and leaf Cd concentrations (Table 3), as when peas were grown hydroponically (Belimov et al. 2020a). Future experiments with tomato and/or *A. thaliana* plants grown in Cd-contaminated soil will determine whether these strains affect Cd accumulation of transpiring plants.

Conclusion

While Cd induced root ABA accumulation and modulated the effect of ABA-metabolising rhizobacteria on tomato root architecture and root hairs, responses depended on the inoculated strain and variable measured. Generally, ABA-metabolising strains affected root architecture more with, than without, Cd. Enhanced root hair elongation and density following inoculation with ABA-metabolising rhizobacteria were not always correlated with lower root ABA concentrations. Although unable to metabolise ABA, the ACC deaminase-containing negative control *V. paradoxus* 5C-2 affected growth and endogenous ABA concentrations similarly. While ABA-metabolising rhizobacteria have been proposed to enhance phytoremediation by boosting shoot metal accumulation and growth, low Cd tolerance of the studied strains suggests their limited utility in soil remediation.

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Table 1 Effect of rhizobacteria on Cd concentration in the liquid BPF medium supplemented with 80 µM $CdCl_2$

Treatments	Cd concentration, µM
Uninoculated control	69.1 ± 0.4 c
V. paradoxus 5C-2	31.8 ± 1.0 a
Rhodococcus sp. P1Y	53.2 ± 2.1 b
Novosphingobium sp. P6W	66.5 ± 0.6 c

Cd concentrations were measured after 7 days. Data are means ± SE. Different letters indicate significant differences (Fisher's LSD test, $p < 0.05$, n = 2) between treatments

Table 2 Colonization of tomato genotype Ailsa Craig roots by the introduced bacteria (10^3 CFU mg^{-1} root FW)

Strain	Untreated plants	Treated with 80 µM $CdCl_2$
V. paradoxus 5C-2	164 ± 20 c	149 ± 27 c
Rhodococcus sp. P1Y	65 ± 9 b	3 ± 1 a
Novosphingobium sp. P6W	82 ± 10 b	7 ± 2 a

Different letters indicate significant differences (Fisher's LSD test, $p < 0.05$, n = 6) between treatments both within and between columns

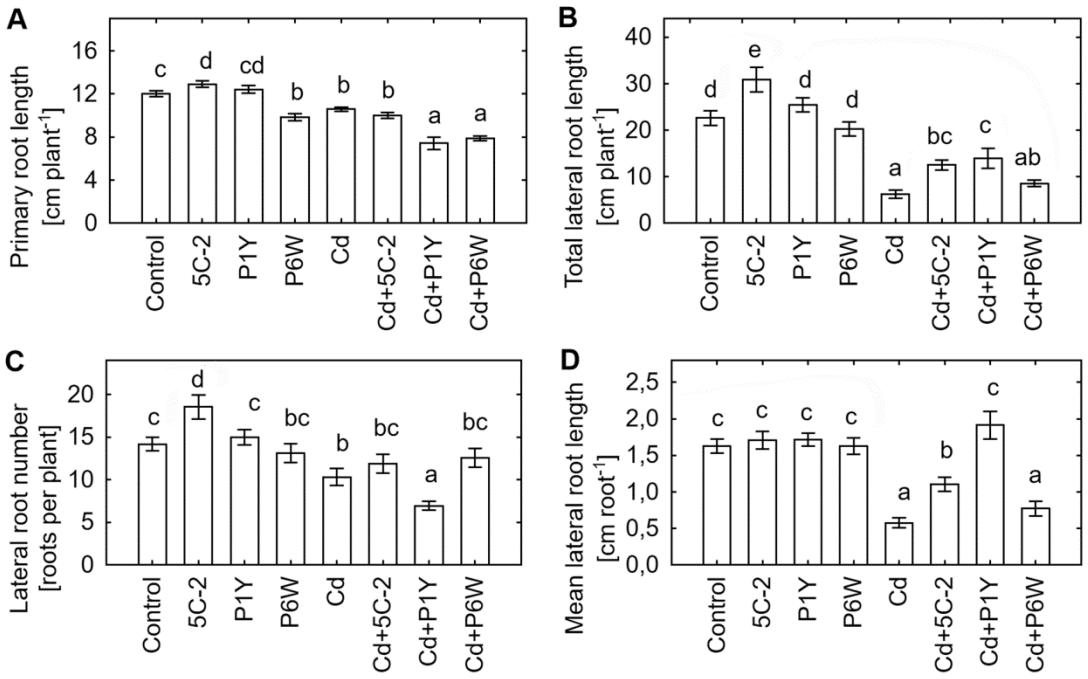

Fig. 1 Effect of rhizobacteria and cadmium on primary root length (A), total lateral root length (B), lateral root number (C) and mean lateral root length (D) of tomato genotype Ailsa Craig. Treatments: Control – uninoculated control; 5C-2 – *V. paradoxus* 5C-2; P1Y – *Rhodococcus* sp. P1Y; P6W – *Novosphingobium* sp. P6W; Cd – 80 µM $CdCl_2$; 5C-2+Cd - 80 µM $CdCl_2$ and *V. paradoxus* 5C-2; P1Y+Cd - 80 µM $CdCl_2$ and *Rhodococcus* sp. P1Y; P6W+Cd - 80 µM $CdCl_2$ and *Novosphingobium* sp. P6W. Bars are means ± SE, with different letters indicating significant differences (Fisher's LSD test, $p < 0.05$, n varied from 12 to 20 depending on treatment and parameter) between treatments

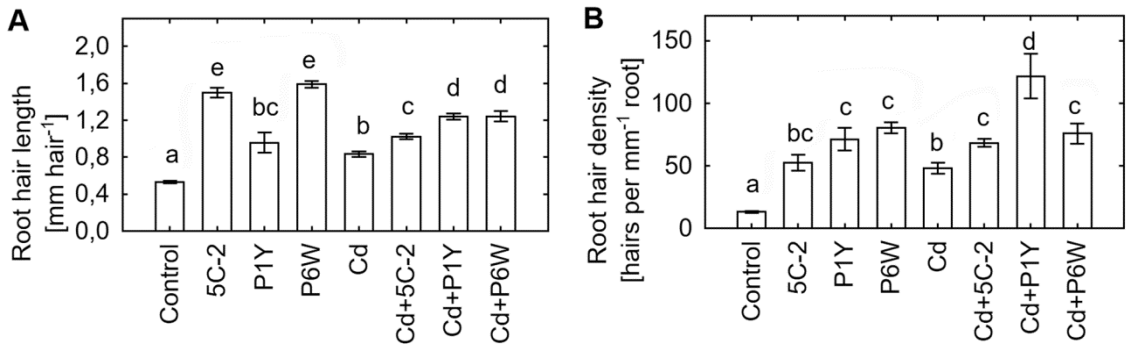

Fig. 2 Effect of rhizobacteria and cadmium on root hair length (A) and density (B) of tomato genotype Ailsa Craig. Treatments: Control – uninoculated control; 5C-2 – *V. paradoxus* 5C-2; P1Y – *Rhodococcus* sp. P1Y; P6W – *Novosphingobium* sp. P6W; Cd – 80 µM $CdCl_2$; 5C-2+Cd - 80 µM $CdCl_2$ and *V. paradoxus* 5C-2; P1Y+Cd - 80 µM $CdCl_2$ and *Rhodococcus* sp. P1Y; P6W+Cd - 80 µM $CdCl_2$ and *Novosphingobium* sp. P6W. Bars are means ± SE, with different letters indicating significant differences (Fisher's LSD test, $p < 0.05$, n varied from 18 to 38 depending on treatment) between treatments

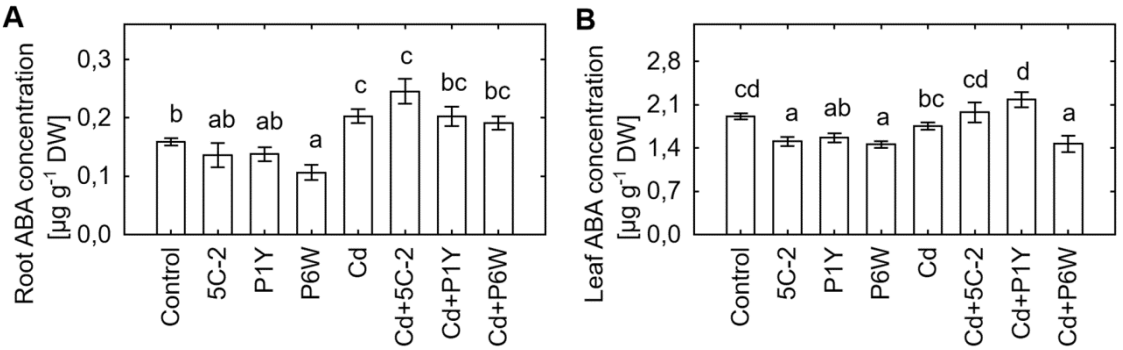

Fig. 3 Effect of rhizobacteria and cadmium on root (A) and leaf (B) ABA concentrations of tomato genotype Ailsa Craig. Treatments: Control – uninoculated control; 5C-2 – *V. paradoxus* 5C-2; P1Y – *Rhodococcus* sp. P1Y; P6W – *Novosphingobium* sp. P6W; Cd – 80 µM $CdCl_2$; 5C-2+Cd - 80 µM $CdCl_2$ and *V. paradoxus* 5C-2; P1Y+Cd - 80 µM $CdCl_2$ and *Rhodococcus* sp. P1Y; P6W+Cd - 80 µM $CdCl_2$ and *Novosphingobium* sp. P6W. Bars are means ± SE, with different letters indicating significant differences (Fisher's LSD test, $p < 0.05$, n varied from 4 to 12 depending on treatment and parameter) between treatments

Table 3 Effect of rhizobacteria on concentration of Cd in tomato roots and shoots (µg Cd g^{-1} plant DW)

Treatments	Roots	Leaves
Uninoculated control	Nd	Nd
80 µM $CdCl_2$	4.9 ± 0.5 a	0.77 ± 0.11 b
80 µM $CdCl_2$ + *Rhodococcus* sp. P1Y	5.2 ± 0.2 a	0.56 ± 0.14 ab
80 µM $CdCl_2$ + *Novosphingobium* sp. P6W	5.7 ± 0.4 a	0.54 ± 0.06 ab
80 µM $CdCl_2$ + *V. paradoxus* 5C-2	5.3 ± 0.1 a	0.42 ± 0.03 a

Different letters indicate significant differences (Fisher's LSD test, $p < 0.05$, n =3) between treatments within columns. Nd stands for not detected

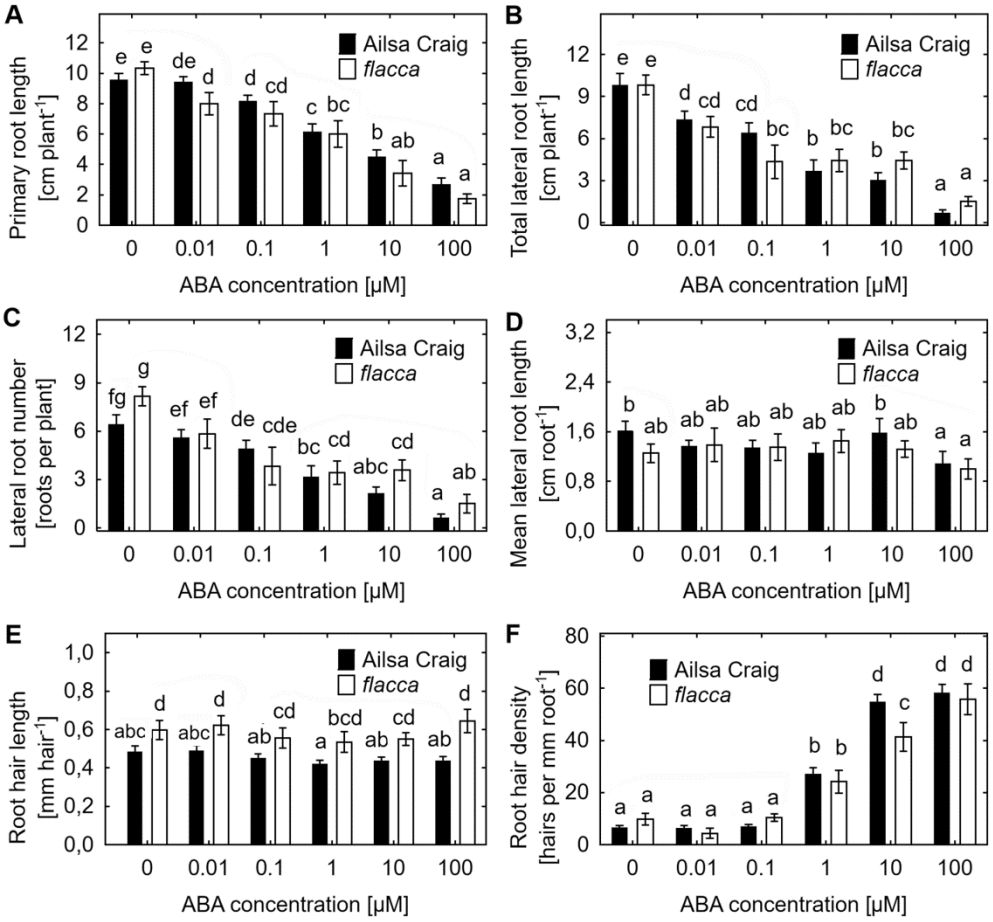

Fig. 4 Effect of abscisic acid (ABA) supplemented to the nutrient agar on primary root length (A), total lateral root length (B), lateral root number (C), mean lateral root length (D), root hair length (E) and root hair density (F) of tomato genotypes Ailsa Craig and *flacca*. Bars are means ± SE, with different letters indicating significant differences (Fisher's LSD test, $p < 0.05$, n varied from 6 to 12 depending on genotype and the parameter measured). between treatments

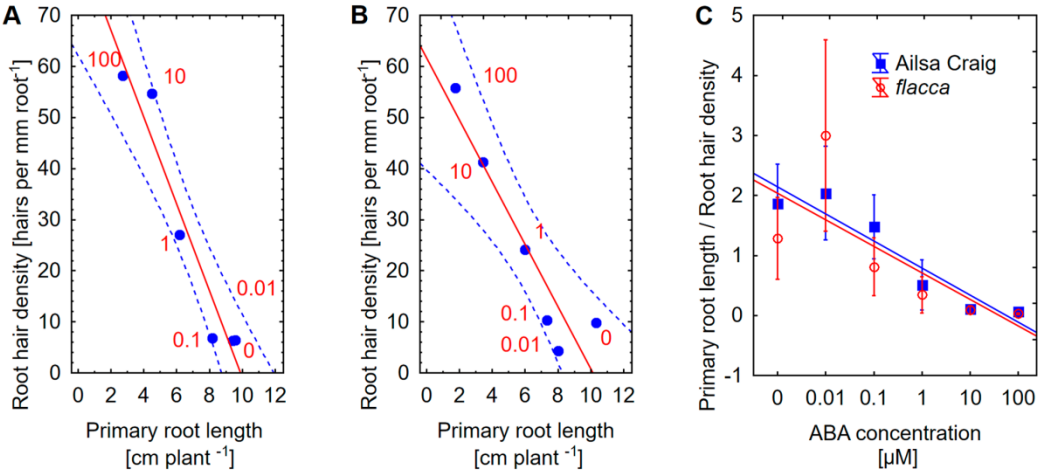

Fig. 5 Scatterplot showing correlations between mean root hair density *versus* primary root length of Ailsa-Craig (A) and *flacca* (B) plants and the ratio of primary root length to root hair density (C) of both genotypes *versus* exogenous ABA concentration. Panels A and B show mean values with blue symbols, ABA concentrations with red numbers; linear regressions with red lines and 95% confidence intervals with blue dotted lines. Panel C symbols are means ± confidence interval ($p = 0.05$, n varied from 6 to 12 depending on genotype and the parameter measured)