Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress

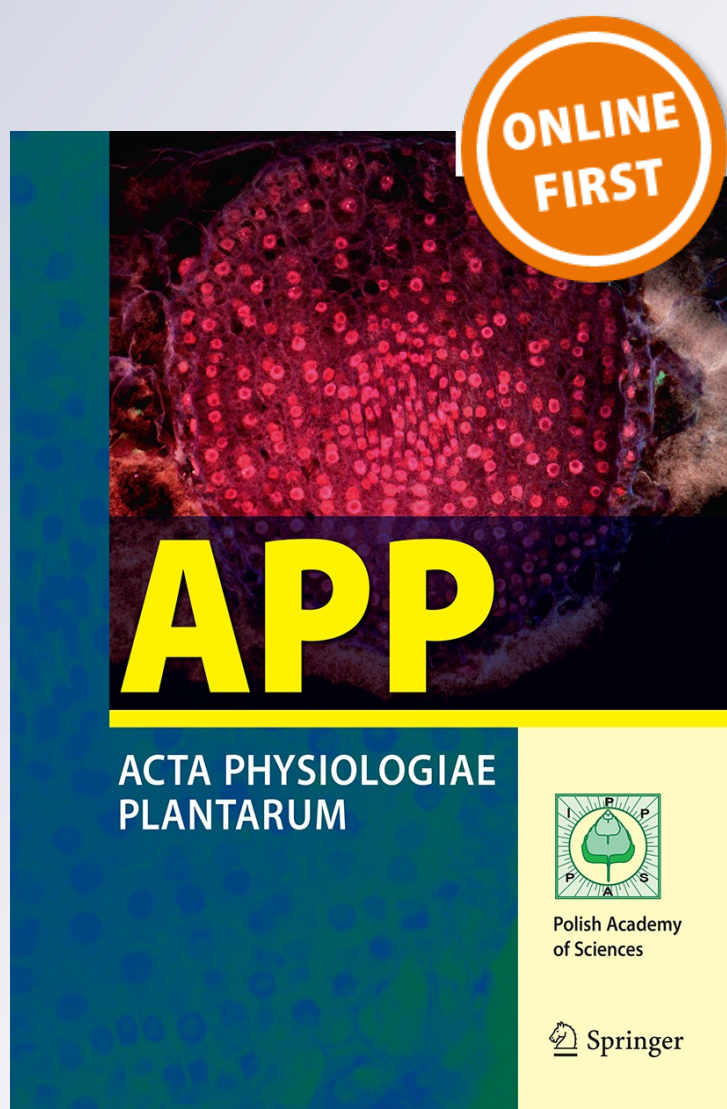## Paula Castillo, Maximiliano Escalante, Melina Gallardo, Sergio Alemano & Guillermina Abdala

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Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress

Paula Castillo · Maximiliano Escalante · Melina Gallardo · Sergio Alemano · Guillermina Abdala

Abstract The purpose of the study was to measure shoot and root dry matter (DM) and production of auxins, salicylic acid, abscisic acid, and jasmonic acid in sunflower (*Helianthus annuus* L.) seedlings cultivated under water stress and singly inoculated or co-inoculated with *Achromobacter xylosoxidans* (SF2) and *Bacillus pumilus* (SF3 and SF4) bacterial strains. Shoot DM was higher in non-stressed seedlings than in stressed seedlings for all inoculation treatments. Water stress resulted in decreased relative water content and reduction of shoot DM. Root DM was higher in stressed seedlings than in non-stressed seedlings. Salicylic acid was the most abundant phytohormone in shoots of stressed, singly inoculated and co-inoculated seedlings. High salicylic acid content in stressed seedlings suggests that this hormone plays a key role in abiotic stress. Abscisic acid was higher in stressed and co-inoculated seedlings than in non-stressed seedlings but was lower than that of salicylic acid. Auxin profile was similar to that of abscisic acid in co-inoculated seedlings. Shoot jasmonic acid content was increased in stressed seedlings co-inoculated with SF2/SF3 or SF2/SF4. Shoot hormonal profiles were different from those of root, suggesting a differential effect of bacterial inoculation on these plant organs. Our findings will be useful in future strategies to mitigate drought effects on crop plants through bacterial inoculation treatments.

Keywords Bacterial inoculation · Bacterial co-inoculation · Phytohormone · Sunflower

Abbreviations
ABA	Abscisic acid
DM	Dry matter
ET	Ethylene
IAA	Indole-3-acetic acid
JA	Jasmonic acid
RWC	Relative water content
SA	Salicylic acid

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops and is cultivated worldwide. This crop is characterized by low to medium drought sensitivity, and its productivity is strongly affected by water availability. Water stress causes irregular seed germination, poor and unsynchronized establishment of seedlings (Mwale et al. 2003), and reduction of stem length, stem diameter, and leaf area (Agele 2003; Turhan and Baser 2004). Water stress also reduces the relative water content (RWC) in leaves, and this parameter is a potential indicator of the

plant water status (Teulat et al. 2003; Bano et al. 2012; Zhang et al. 2012).

The crop rhizosphere in which Rhizobacteria develop and colonize the roots of crop plants is also affected by water stress condition. Many genera of bacteria (including *Acetobacter*, *Achromobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Herbaspirillum*, *Pseudomonas*, and *Rhizobium*) have been shown to function as plant growth promoting bacteria (PGPB) (Weller and Thomashow 1994; Glick 1995; Probanza et al. 1996; Forchetti et al. 2010). The inoculation of selected bacterial strains can mitigate the yield limitation caused by water deficit and improve the ability of crop plants to withstand water stress (Casanovas et al. 2002; Creus et al. 2004; Forchetti et al. 2010) and salt stress (Shilev et al. 2012; Dodd and Pérez-Alfocea 2012), and to better exploit the soil and absorb water and nutrients via the roots.

Forchetti et al. (2007) characterized and identified bacterial strains from roots collected from sunflower grown under irrigation or drought at the end of the vegetative stage. Three strains, identified as *Achromobacter xylosoxidan*s (SF2) and *Bacillus pumilus* (SF3 and SF4), were selected based on assays of nitrogen-fixing and phosphate-solubilization ability, proteolytic and cellulolytic activity, inhibition of pathogenic fungi, and production of the phytohormones jasmonic acid (JA), 12-oxo-phytodienoic acid (OPDA), abscisic acid (ABA), and salicylic acid (SA).

Specific responses to biotic or abiotic stresses usually depend on the coordinated action of several phytohormones, such as JA, ABA, SA, and ethylene (ET); such coordination involves a complex interplay among several pathways. Hormonal changes affect not only adaptive responses but also the normal growth of the harvestable organs and thus influence economic productivity. Plant hormones are therefore considered to be a primary component of signaling pathways controlling diverse processes (Pérez-Alfocea et al. 2011).

The phytohormone indole-3-acetic acid (IAA) affects almost every aspect of plant development and is involved in abiotic stress responses (Jain and Khurana 2009; Shibasaki et al. 2009). Several bacterial species, including *Azospirillum brasilense* and *Bradyrhizobium japonicum* (Boiero et al. 2007; Cassán et al. 2009), are able to produce IAA at concentrations sufficient to induce morphological and physiological changes in young plant tissues.

SA is another growth regulator involved in a wide range of physiological and metabolic responses in plants (Hayat et al. 2010). During the past two decades, SA has been studied intensively as a signaling molecule that mediates local and systemic defense responses against pathogens and has been reported to play a role in plant responses to abiotic stresses including drought, low and high temperatures, heavy metals, and osmotic stress (Janda et al. 1999; Rao

and Davis 1999; Molina et al. 2002, Nemeth et al. 2002; Munne-Bosch and Peñuelas 2003; Shi and Zhu 2008; Rivas-San Vicente and Plasencia 2011).

Endogenous levels of ABA and jasmonates are modified in response to abiotic stresses (Wasternack and Hause 2002; Pedranzani et al. 2003; Wasilewska et al. 2008). The key role of ABA and jasmonates as primary signaling molecules in the regulation of plant defenses has been well established (Bari and Jones 2009; Pieterse et al. 2009). These hormones generate a signal transduction network that leads to a cascade of events resulting in the physiological adaptations of plants to stresses.

Single inoculation and co-inoculation of crop plants with Rhizobacteria provides an opportunity to target alterations in plant hormone status to specific growth stages or under particular stress conditions. In the present study, we measured dry matter (DM) and the production of IAA, SA, ABA, and JA in shoots and roots of sunflower seedlings cultivated under water stress and singly inoculated or co-inoculated with the bacterial strains SF2, SF3, and SF4. The RWC in shoots was also measured. Our findings will be useful for the formulation of growth-promoting products (e.g., inoculants) that comprise these strains and phytohormones, to be added to sunflower seeds during sowing to improve seedling performance.

Materials and methods

Plant growth and inoculation procedure

Seedlings were grown in sand rather than vermiculite because the water capacity of sand (40 ml 100 g^{-1}) is less than that of vermiculite (440 ml 100 g^{-1}).

Assays of bacterial inoculation and co-inoculation were performed on the commercial hybrid Paraiso 24 (Nidera). For each treatment group, five seeds were placed in a pot containing sterile sand in a growth chamber with a cycle of 16 h light (28 °C)/8 h dark (20 °C). Four days after planting, the seedlings were singly inoculated with strain SF2 (EF465456), SF3 (EF465457), or SF4 (EF465458) (1-ml bacterial inoculum containing 10^8 cfu ml^{-1}) or co-inoculated with a combination of SF2/SF3, SF2/SF4, or SF3/SF4 (0.5 ml of each inoculum). For each experimental treatment, one group of seedlings was watered by capillary ascent with half-strength Hoagland solution and another group watered with half-strength Hoagland solution supplemented with polyethylene glycol (PEG) 6,000 at a concentration sufficient to produce $\psi a = -2.03$ MPa. Fourteen days after inoculation, the seedlings were harvested and the shoots and roots were cut off, frozen in liquid nitrogen, lyophilized, weighed (DM weight), and ground to a fine powder. Each experiment was performed

in quintuplicate, and the treatments within each experiment were performed in triplicate.

Relative water content assay

An RWC assay was performed on nine singly inoculated and co-inoculated seedlings grown under the conditions described above. A pool of leaves from the top of each plant was collected 14 days after inoculation and used for RWC determination. Fresh weight (FW), dry weight (DW), and turgid weight (TW) were measured, and RWC was calculated by the formula:

$$RWC(\%) = (FW - DW)/(TW - DW) \times 100$$

Phytohormone extraction

DM (0.2 g) was homogenized in 5 ml ultrapure water and added with 20 µl of a mixture of internal standards containing 50 ng [^{2}H$_5$]-IAA, 50 ng [^{2}H$_4$]-SA, 50 ng [^{2}H$_6$]-ABA, and 50 ng [^{2}H$_6$]-JA. Centrifugation was performed at $5,000g$ for 15 min, the pellet was discarded, the pH of the supernatant was adjusted to 2.8 with acetic acid, and the supernatant was partitioned twice against an equal volume of diethyl ether (Durgbanshi et al. 2005). The aqueous phase was discarded, and the organic fraction was evaporated. The solid residue was resuspended in 1.5 ml methanol (MeOH) and filtered through a 0.22-µm cellulose acetate filter. The organic fraction was evaporated at 35 °C under vacuum in a Speed Vac model SC110 (Savant Instruments Inc., New York, NY, USA) and resuspended in 50 µl MeOH 100 %. A 5-µl aliquot of this solution was injected into the HPLC system.

Liquid chromatographic analysis

HPLC analysis was performed using an Alliance 2695 (Separation Module, Waters, Milford, MA, USA) quaternary pump equipped with an auto-sampler. A Restek C18 (Restek, Bellefonte, PA, USA) column (2.1 × 100 mm, 5 µm) was used at 28 °C with injected volume 5 µl. The binary solvent system used for the elution gradient consisted of 0.2 % acetic acid in H$_2$O (solvent B) and MeOH (solvent A) at a constant flow rate of 200 µl min^{-1}. A linear gradient profile with the following proportions (v/v) of solvent A was applied [t (min), % A]: (0, 40) (25, 80), with 7 min for re-equilibration.

Mass spectrometric analysis

MS/MS was performed using a Micromass Quatro Ultima TM "*Pt*" double quadrupole mass spectrometer (Micromass, Manchester City, UK). All of the analyses were performed using a turbo ion spray source in negative ion mode with the following settings for IAA, SA, ABA, and JA: capillary voltage $-3,000$ V, energy cone 35 V, RF Lens1 (20), RF Lens2 (0.3), source temp 100 °C, de-solvation temp 380 °C, gas cone 100 l h^{-1}, gas de-solvation 70 l h^{-1}, collision (50), and multiplier (650). The MS/MS parameters were optimized in infusion experiments using individual standard solutions of IAA, SA, ABA, and JA at a concentration of 10 ng µl^{-1} diluted in mobile phase A/B (40:60, v/v). MS/MS product ions were produced by collision-activated dissociation of selected precursor ions in the collision cell of the mass spectrometer, and mass was analyzed using the second analyzer of the instrument. Quantitation was performed in the multiple reaction monitoring (MRM) mode.

Statistical analysis

The data were analyzed using the InfoStat E program. Some data were transformed to achieve normal distributions. Two-way analysis of variance (ANOVA) was used to compare the mean values ($\pm$SE) of shoot DM and IAA and ABA content. A non-parametric test (Kruskal–Wallis) was used to compare the root DM values. For the SA and JA contents, linear general and mixed models (LGMM) were used to meet the assumptions of homogeneity of variance. In the ANOVA and LGMM analyses, differences between means ($\pm$SE) were evaluated using Fisher's LSD test, with $p < 0.05$ considered to be significant.

## Results

Relative water content

Under non-stress condition, RWC was higher in singly inoculated (95.8 %) and co-inoculated seedlings (97.9 %) than in uninoculated seedlings (94.6 %). Under stress condition, RWC values were lower than under non-stress condition. The highest value was recorded for co-inoculated seedlings (80 %) following by singly inoculated seedlings (72 %) and uninoculated seedlings (70.4 %). Thus, the stress condition decreased RWC in all treatments, although bacterial co-inoculation improved the water status.

Shoot and root growth

Uninoculated seedlings under water stress condition (Fig. 1b) showed a 34 % reduction in shoot growth in comparison with non-stressed seedlings (Fig. 1a). Inoculation with strain SF2 and co-inoculation with the three strain combinations resulted in enhanced shoot growth in non-stressed seedlings (Fig. 1a). The inoculation
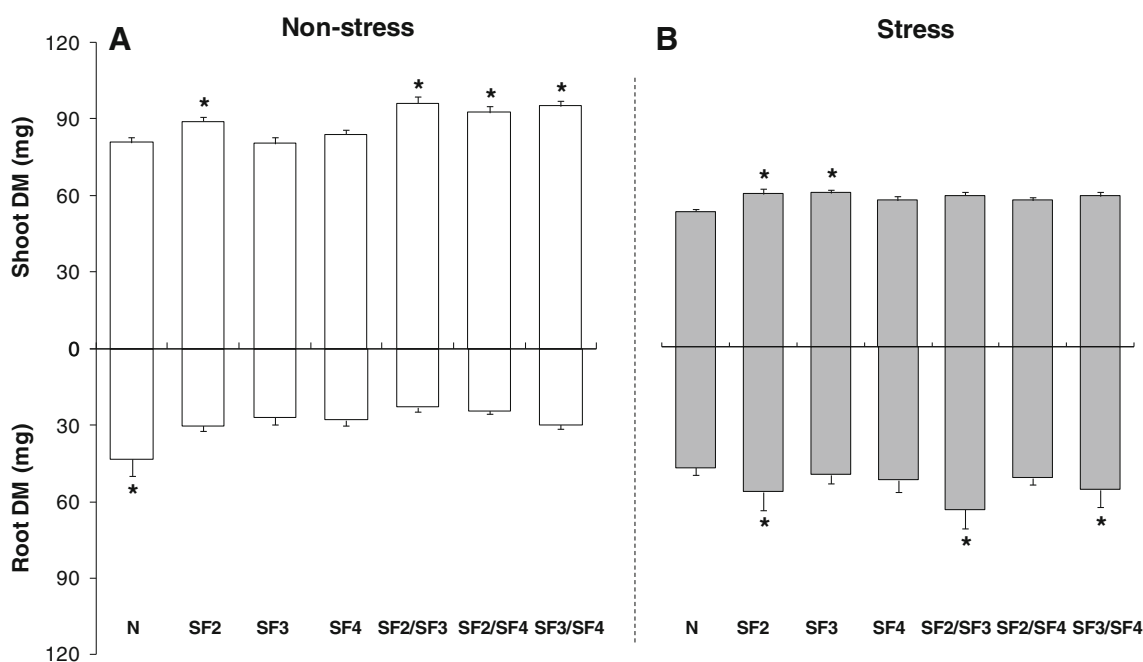
Fig. 1 Shoot and root DM of inoculated and uninoculated seedlings grown under non-stress (**a**) and stress conditions ($\psi a = -2.03$ MPa) (**b**). Experiments were performed in quintuplicate. *Bars* represent mean ± SE. Values indicated by *asterisks* are significantly different at $p < 0.05$. *N* uninoculated seedlings, *SF2* seedlings inoculated with strain SF2, *SF3* seedlings inoculated with strain SF3, *SF4* seedlings inoculated with strain SF4, *SF2/SF3* seedlings co-inoculated with strains SF2 and SF3, *SF2/SF4* seedlings co-inoculated with strains SF2 and SF4, *SF3/SF4* seedlings co-inoculated with strains SF3 and SF4

treatments also enhanced shoot growth in stressed seedlings but to a lesser degree (Fig. 1b). The shoot growth of stressed seedlings inoculated with SF2 or SF3 was slightly increased (by 13.0 and 13.6 %, respectively) (Fig. 1b).

In contrast to the results for shoot growth, uninoculated seedlings under stress condition (Fig. 1b) showed a 7 % increase in root growth in comparison with non-stressed seedlings (Fig. 1a). Seedlings singly inoculated with SF2 and co-inoculated with SF2/SF3 and SF3/SF4 showed increases of root growth (17, 18, and 8 %, respectively) in comparison with uninoculated seedlings.

Indole-3-acetic acid content

The IAA content of shoots under non-stress condition was slightly higher for singly inoculated (SF3, SF4) and for co-inoculated (SF2/SF3, SF2/SF4, SF3/SF4) seedlings than for uninoculated seedlings (Fig. 2a). The root IAA content under non-stress condition was higher for SF3-inoculated and for SF2/SF3 or SF3/SF4 co-inoculated seedlings than for uninoculated seedlings (Fig. 2a). Under stress condition, the shoot IAA content of singly inoculated seedlings was not significantly different from that of uninoculated seedlings. However, was greatly increased by co-inoculation with SF2/SF3 or SF2/SF4; in particular, the latter treatment produced a sixfold increase (Fig. 2b). Under stress condition, the root IAA content was slightly

increased by single inoculation and significantly increased by co-inoculation in comparison with uninoculated seedlings. The greatest increase (5.5-fold) was found for seedlings co-inoculated with SF3/SF4 (Fig. 2b).

Salicylic acid content

Under non-stress condition, the shoot SA content of singly inoculated and co-inoculated seedlings was essentially the same as that of uninoculated seedlings (Fig. 3a). Under non-stress condition, the root SA content of SF3-inoculated and of SF2/SF3 or SF3/SF4 co-inoculated seedlings was significantly increased 2.5- and 2.0-fold, respectively, in the latter two cases (Fig. 3a). Under stress condition, the shoot SA content was unaffected by single inoculation but was significant decreased by SF3/SF4 co-inoculation (Fig. 3b). The root SA content showed a slight increase only in the case of SF2/SF3 co-inoculation (Fig. 3b). In summary, root SA content was higher than shoot SA content under non-stress condition (Fig. 3a) but was lower than shoot SA content under stress condition (Fig. 3b).

Abscisic acid content

Under non-stress condition, the ABA content of shoots and roots was essentially unaffected by single inoculation but was increased by SF2/SF3, SF2/SF4, and SF3/SF4
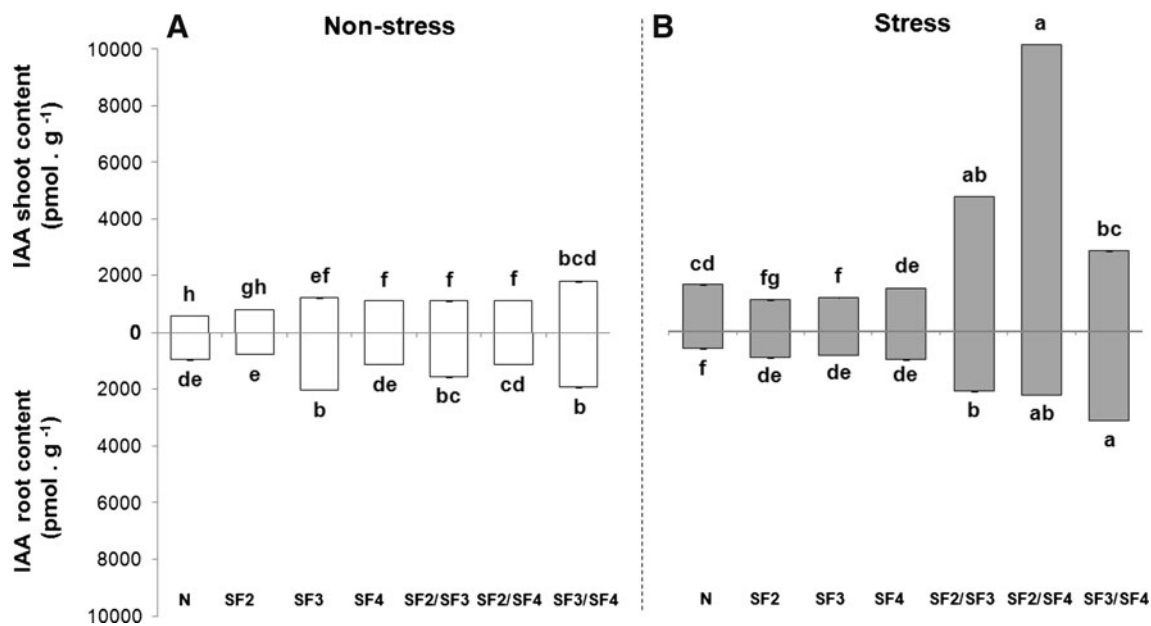
Fig. 2 IAA content in shoots and roots of inoculated and uninoculated seedlings grown under non-stress (**a**) and stress conditions (**b**). Values indicated by *different letters* are significantly different at $p < 0.05$. Experimental design and abbreviations as in Fig. 1
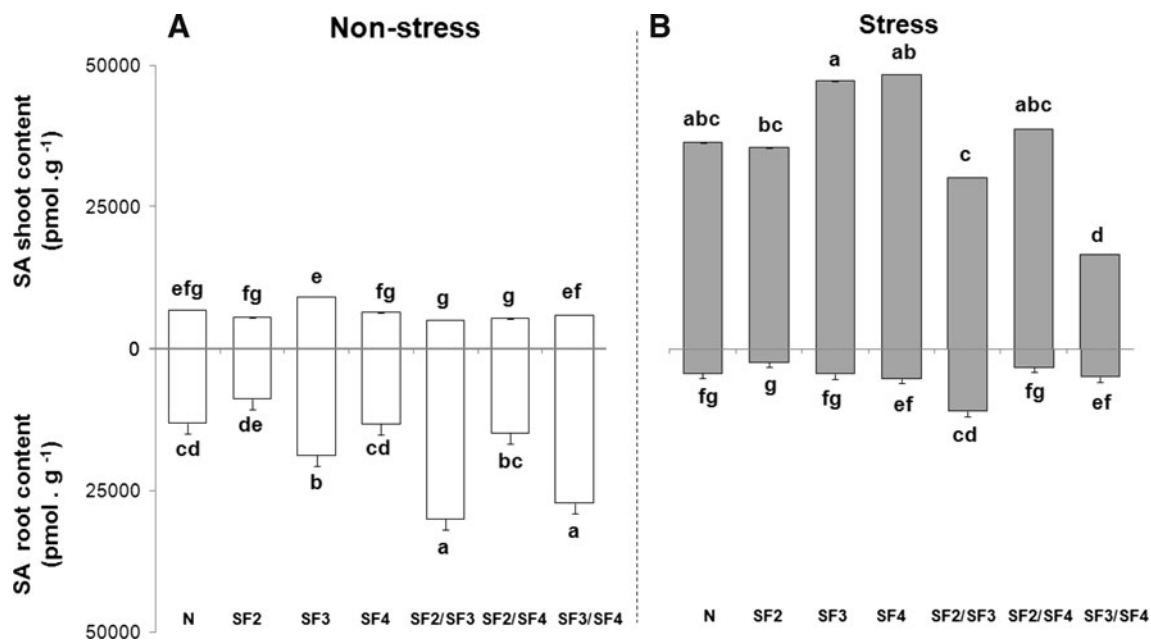
Fig. 3 SA content in shoots and roots of inoculated and uninoculated seedlings grown under non-stress (**a**) and stress conditions (**b**). Values indicated by *different letters* are significantly different at $p < 0.05$. Experimental design and abbreviations as in Fig. 1

co-inoculation (Fig. 4a). Under stress condition, the shoot ABA content was unchanged in singly inoculated seedlings but was greatly increased in SF2/SF3, SF2/SF4, and SF3/SF4 co-inoculated seedlings—sixfold in the case of SF2/SF4 (Fig. 4b). Under stress condition, the root ABA content was unaffected by single inoculation but was increased 10-, 12-, and 11-fold, respectively, in SF2/SF3, SF2/SF4, and SF3/SF4 co-inoculated seedlings (Fig. 4b). Under both non-stress and stress conditions, the shoot and root ABA contents were increased by co-inoculation, and ABA accumulation was higher in shoots than in roots.

Jasmonic acid content

Under non-stress condition, the shoot JA content was significantly decreased by single inoculation but was unaffected
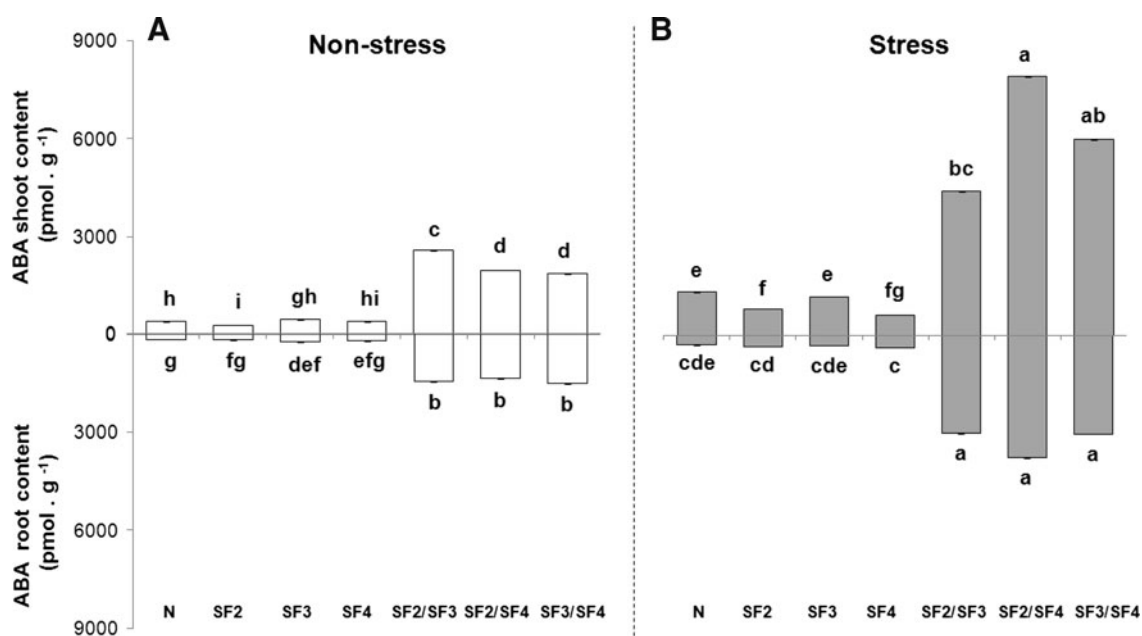
Fig. 4 ABA content in shoots and roots of inoculated and uninoculated seedlings grown under non-stress (**a**) and stress conditions (**b**). Values indicated by *different letters* are significantly different at $p < 0.05$. Experimental design and abbreviations as in Fig. 1
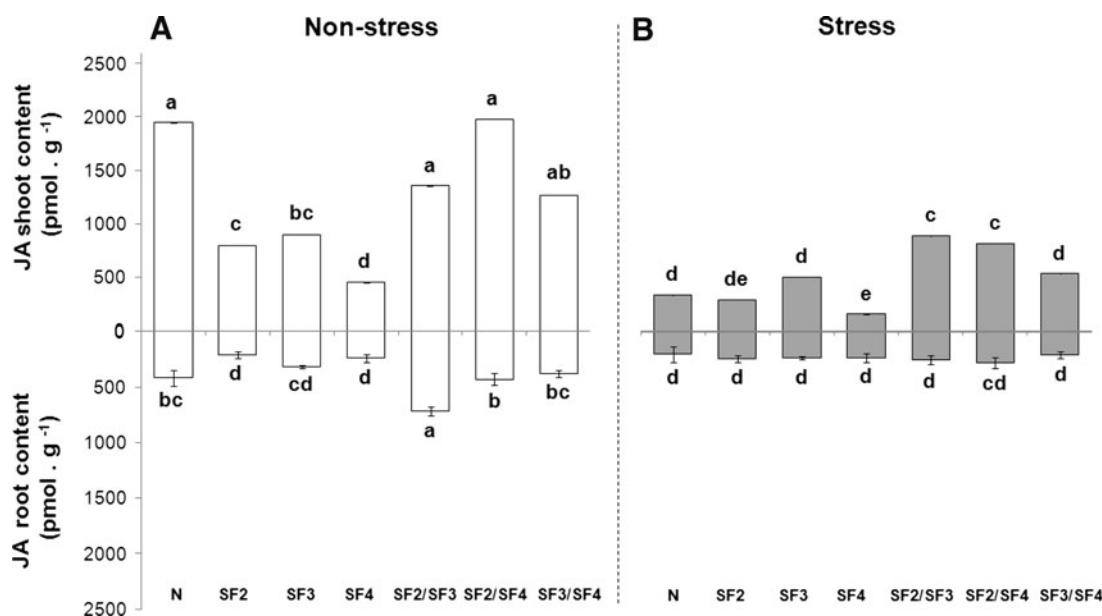
Fig. 5 JA content in shoots and roots of inoculated and uninoculated seedlings grown under non-stress (**a**) and stress conditions (**b**). Values indicated by *different letters* are significantly different at $p < 0.05$. Experimental design and abbreviations as in Fig. 1

by co-inoculation (Fig. 5a). Under non-stress condition, the root JA content was slightly decreased by single inoculation with SF2 or SF4 but was increased ∼twofold by SF2/SF3 co-inoculation (Fig. 5a). Under stress condition, the shoot JA content was increased by SF2/SF3 or SF2/SF4 co-inoculation (Fig. 5b), whereas the root JA content was unaffected by single inoculation or by co-inoculation (Fig. 5b).

In summary, the most abundant phytohormone in the shoots of seedlings grown under stress condition was SA, which reached concentrations of 20,000–45,000 pmol g^{-1}. ABA was the most abundant hormone in the roots of seedlings grown under stress condition, but reached concentrations (500–4,000 pmol g^{-1}) that were lower than those of SA.

Discussion

Single inoculation or co-inoculation with PGPB or other soil microorganisms can positively affect plant growth, as reflected by increased DM in various plant organs. We previously reported the growth response of two inbred sunflower lines (water stress-sensitive B59 and water stress-tolerant B71) and commercial hybrid Paraiso 24 to inoculation with bacterial strains SF2, SF3, and SF4. Seedlings were grown in vermiculite medium under water stress and normal conditions. The shoot DM and root DM following inoculation were more strongly enhanced for Paraiso 24 and B71 than for B59 (Forchetti et al. 2010).

The water stress resulted in decreased RWC and reduction of sunflower shoot DM. RWC in plant leaves has been considered to be a potential indicator of plant water status because it is involved in the metabolic activity in tissues. A decline in RWC reflects a loss of turgor that results in limited cell expansion and consequent reduced growth in plants (Ashraf 2010; Lu et al. 2010). The decreases in shoot DM observed in this study, can therefore, be attributed to reduced RWC. We also observed a greater development of the root system (measured as increased root DM) in seedlings grown under water stress, as an adaptive mechanism in response to drought. Increases in root DM have been reported previously in sunflower (Tahir et al. 2002) and in *Catharanthus roseus* (Jaleel et al. 2008).

Bacterial treatments applied to sunflower seedlings under stress condition increased the root DM, thereby enhancing shoot water content (RWC) and shoot development in dry soil. Other mechanisms of plant growth promotion by Rhizobacteria, such as phytohormone production, N fixation, and enhanced mineral uptake, may also contribute to improved adaptation of inoculated plants to a water deficit (Dodd and Pérez-Alfocea 2012).

Under non-stress condition, we observed increases of shoot DM in seedlings singly inoculated with SF2 or co-inoculated with the three strain combinations. Consistent with this finding, inoculation of seeds with *Azospirillum brasilense* SM increased total DM in sorghum and shoot length in mung bean (Malhotra and Srivastava 2009). In contrast, Figueiredo et al. (2008) did not observe significant differences in DM when the common bean was singly inoculated or co-inoculated with *Rhizobium tropici* and/or *Paenibacillus polymixa.*

The observed plant growth promotion by numerous Rhizobacteria that contain 1-aminocyclopropane-carboxylic acid-deaminase (ACCd), including SF2, SF3 and SF4, particularly when the plants are subjected to environmental stresses, most likely occurs through stimulation of stress-induced ET production (Glick et al. 2007; Belimov et al. 2009). Because ET often acts as a growth inhibitor, it seems likely that decreased ACC levels in plants (due to the presence of ACCd-containing Rhizobacteria, as in sunflower strains) lowered ET production, thereby increasing shoot growth under soil water deficit (Arshad et al. 2008; Belimov et al. 2009).

Auxin production is clearly a widespread bacterial function. As much as 80 % of species within certain Rhizobacterial genera produce auxin (Ahmad et al. 2008). Numerous PGPB have the ability to produce IAA and thereby affect plant development. In the present study, the increased shoot IAA content in seedlings singly inoculated with SF3 or SF4 or co-inoculated with each of the three bacterial combinations under non-stress condition was associated with increased shoot DM in the co-inoculated but not the singly inoculated seedlings, suggesting that co-inoculation produces a synergistic effect.

Spaepen et al. (2008) reported that the up-regulation of bacterial IAA production in *A. brasilense* Sp245 increased shoot DM by at least 10 % in comparison with the WT strain. Single inoculation or co-inoculation of soybean and corn with *A. brasilense* Az39 and *Bradyrhizobium japonicum* E109 caused significant increases of shoot length, shoot DM, and root DM. Single inoculation or co-inoculation with bacteria promoted seedling growth through IAA and GA_3 production (Cassán et al. 2009). In spite of many studies, it remains unclear whether the effects of these bacteria on shoot growth result from direct long-distance IAA signaling as proposed by Dodd et al. (2010).

In water-stressed *Trifolium repens* inoculated with *Pseudomonas putida*, *Bacillus megaterium*, or other *Pseudomonas* sp., Marulanda et al. (2009) observed increased shoot DM in all of the inoculated plants and increased root DM in the plants inoculated with *P. putida* or *B. megaterium*, and concluded that these two bacterial species can produce IAA under stress condition.

We observed differential responses to single inoculation and co-inoculation under stress condition. In seedlings singly inoculated with SF2 or SF3, shoot DM was slightly increased but IAA content was unchanged. The high IAA content in seedlings co-inoculated with SF2/SF3 or SF2/SF4 was not associated with a notable change in shoot DM. However, we found a correlation between increases in the IAA content of seedlings inoculated with SF2 and co-inoculated with SF2/SF3 or SF3/SF4 and root DM. In regard to bacterial IAA production and root growth, Ali et al. (2009) reported that IAA synthesized by *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Escherichia*, and *Micrococcus* caused a decrease of wheat root length, although these bacteria also caused an increase of lateral root number. The increase in root growth observed in the present study may therefore reflect the fact that the DM parameter includes the total root system (main and lateral roots).

The ability of certain microorganisms to produce IAA under stress conditions may explain their effectiveness in promoting growth and increasing water stress tolerance in certain plants. In the present study, a positive correlation between IAA content and seedling growth was found only for certain treatments, particularly co-inoculation.

SA appears to have important regulatory functions in plants and has been associated with various physiological processes (Arfan et al. 2007), such as the induction of stomatal apertures and conductance (Khan et al. 2003; Hao et al. 2011). However, the effects of SA are variable depending on the plant species (Horváth et al. 2007). In the present study, certain growth conditions and bacterial treatments caused remarkable changes in SA profiles. Single inoculation of non-stressed seedlings with SF3 caused a significant increase of root SA content. Such increases may be due in part to the ability of bacteria to synthesize SA under normal and stress conditions (Forchetti et al. 2010).

Sunflower seedlings under stress condition showed a much higher SA content in shoots than in roots. SA is synthesized through two distinct pathways depending on the plant species: the isochorismate (IC) pathway and the phenylalanine (Phe) pathway (Dempsey et al. 2011). These pathways both originate from chorismate (Lee et al. 1995; Ribnicky et al. 1998) and have isochorismate synthase (ICS) and phenylalanine ammonia lyase (PAL), respectively, as the major intermediate enzymes (Catinot et al. 2008; Uppalapati et al. 2007). Klambt (1962) proposed that SA in sunflower is synthesized from benzoate, which originates from the PAL pathway. The ICS pathway is responsible for the synthesis of >95 % of SA in certain plant species (Catinot et al. 2008; Garcion et al. 2008; Chen et al. 2009). Our finding of a higher SA content in shoots than in roots suggests that water stress in sunflower may preferentially activate the ICS pathway rather than the PAL pathway. ICS is located in chloroplasts (Garcion et al. 2008), suggesting that SA is synthesized primarily in this organelle (Fragniere et al. 2011). This concept could explain the higher SA content in sunflower shoots vs. roots. Another possible explanation is that SA is recirculated between roots and shoots, as was reported for ABA in *P. sativum* by Jiang et al. (2012).

The shoot SA content was threefold higher in stressed vs. non-stressed seedlings. This finding, taken together with those of Munne-Bosch and Peñuelas (2003) in *Pisctacea lentiscus*, Chini et al. (2004) in *Arabidopsis*, and Bandurska and Stroinski (2005) in barley, suggests that SA content is directly related to abiotic stress, particularly water stress, not solely to biotic stress. The effect of SA in relation to drought tolerance in plants is not always obvious, but SA is a promising compound for the reduction of abiotic stress (Horváth et al. 2007).

In regard to ABA, Zhang et al. (2012) reported that drought stress reduced leaf RWC and increased ABA content in drought-sensitive and drought-tolerant maize cultivars. These authors suggested that endogenous ABA modulated water relations and plant growth under drought, especially in the sensitive cultivar. Similarly, shoot RWC in the present study was reduced in uninoculated and singly inoculated seedlings under water stress, in association with increased ABA content.

Several Rhizobacteria produce ABA in culture media or mediate plant ABA status (Dodd et al. 2010). To the best of our knowledge, there have been no studies on the biochemical mechanism of bacterial ABA production. Marasco and Schmidt-Dannert (2008) suggested a possible mechanism based on the existence of bacterial genome sequences for carotenoid cleavage oxygenase homologues.

B. japonicum USDA110 (Boiero et al. 2007), *A. brasilense* Az39 and Cd (Perrig et al. 2007), and *A. brasilense* sp. 245 (Cohen et al. 2008) have been shown to produce ABA in vitro under normal conditions. ABA content in *Arabidopsis thaliana* inoculated with *A. brasilense* was twice as high as in uninoculated plants. Cohen et al. (2009) reported that aseptic inoculation of *Zea mays* seedlings with *A. lipoferum* USA59b increased the tissue ABA concentrations twofold in inoculated and well-irrigated plants, indicating that ABA-producing PGPR can potentially augment plant ABA concentrations. Our finding that bacterial co-inoculation greatly increased ABA content in both non-stressed and stressed sunflower seedlings suggests that this increase may result (at least in part) from bacterial production. Increases in RWC and ABA content resulting from bacterial co-inoculation may allow the plant to react more quickly and/or effectively to stress and to survive the stress condition for a longer time.

The high RWC in maize (Casanovas et al. 2002) and wheat (Creus et al. 2004) inoculated with *Azospirillum* sp. under stress condition may result from bacterial ABA that induces stomatal closure and mitigates drought stress. As suggested by Dodd et al. (2010), it would be interesting to investigate whether the effect of bacterial inoculation on plant water content is related to bacterial ABA production or to alteration of the sensitivity of physiological processes such as stomatal closure.

Jasmonates are well known to play roles in protecting plants from biotic and abiotic stresses. The involvement of JA in responses to abiotic stresses (e.g., drought and salinity) has been reported in several species (Creelman and Mullet 1995; Wasternack 2007). We found that shoot JA content was lower in stressed than in non-stressed seedlings. Similarly, Hamayun et al. (2010) observed a decrease of JA in soybean leaves under drought stress. In *A. thaliana* at the early stage of moderate drought, JA in combination with a high ABA level stimulated the

preparatory responses necessary for drought acclimation, e.g., stomatal closure and cell wall modification (Harb et al. 2010). These authors concluded that a high JA concentration is probably not required under drought stress.

Many recent studies have addressed the crosstalk between the JA and ABA signal transduction pathways. De Ollas et al. (2012) reported a clear link between JA and ABA at the biosynthetic level in citrus. These authors found a transient increase in JA levels that preceded the ABA accumulation in roots induced by drought stress. Similar results were obtained for citrus leaves under soil flooding (Arbona and Gómez-Cadenas 2008), papaya under moderate drought stress (Mahouachi et al. 2007), and *Arabidopsis* under severe drought (Arbona et al. 2010). In each of these cases, the JA accumulation preceded or occurred simultaneously with the ABA increase, supporting the idea of a temporal connection in the action of the two hormones. Similarly, in the present study, low JA accumulation occurred simultaneously with increased ABA in shoots of co-inoculated seedlings under stress condition. Differences at the onset of the hormonal accumulation among the various experimental systems most likely reflect the differing conditions of abiotic stress, the plant material used, the time of plant growth after stress treatment, and (in our case) the bacterial inoculation.

The basic information presented here on changes in hormone levels produced by the action of *Achromobacter xylosoxidans* (SF2) and *Bacillus pumilus* (SF3 and SF4) strains on sunflower seedlings under water stress will be useful in designing appropriate hormonal profiles to more efficiently mitigate the effect of water stress on sunflower crops. Further studies are needed to assay the effects of bacterial single inoculation and co-inoculation on sunflower growth and productivity under field conditions.

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