Curr Microbiol (2010) 61:485–493
DOI 10.1007/s00284-010-9642-1

Endophytic Bacteria Improve Seedling Growth of Sunflower Under Water Stress, Produce Salicylic Acid, and Inhibit Growth of Pathogenic Fungi

Gabriela Forchetti · Oscar Masciarelli · María J. Izaguirre · Sergio Alemano · Daniel Alvarez · Guillermina Abdala

Received: 28 December 2009 / Accepted: 26 March 2010 / Published online: 11 April 2010

Abstract Endophytic bacterial strains SF2 (99.9% homology with *Achromobacter xylosoxidans*), and SF3 and SF4 (99.9% homology with *Bacillus pumilus*) isolated from sunflower grown under irrigation or drought were selected on the basis of plant growth-promoting bacteria (PGPB) characteristics. Aims of the study were to examine effects of inoculation with SF2, SF3, and SF4 on sunflower cultivated under water stress, to evaluate salicylic acid (SA) production by these strains in control medium or at $\Psi a = -2.03$ MPa, and to analyze effects of exogenously applied SA, jasmonic acid (JA), bacterial pellets, and bacterial supernatants on growth of pathogenic fungi *Alternaria* sp., *Sclerotinia* sp., and *Verticillum* sp. Growth response to bacterial inoculation was studied in two inbred lines (water stress-sensitive B59 and water stress-tolerant B71) and commercial hybrid Paraiso 24. Under both water stress and normal conditions, plant growth following inoculation was more strongly enhanced for Paraiso 24 and B71 than for B59. All three strains produced SA in control medium; levels for SF3 and SF4 were higher than for SF2. SA production was dramatically higher at $\Psi a = -2.03$ MPa. Exogenously applied SA or JA caused a significant reduction of growth for *Sclerotinia* and a lesser reduction for *Alternaria* and *Verticillum*. Fungal growth was more strongly inhibited by bacterial pellets than by bacterial supernatants. Our findings indicate that these endophytic bacteria enhance growth of sunflower seedlings under water stress, produce SA, and inhibit growth of pathogenic fungi. These characteristics are useful for formulation of inoculants to improve growth and yield of sunflower crops.

Abbreviations

JA	Jasmonic acid
PGPB	Plant growth-promoting bacteria, pathogenic fungi
SA	Salicylic acid

Introduction

Sunflower (*Helianthus annuus* L.) is a widely cultivated oil crop around the world. Overall, the area planted with sunflower has steadily increased because of its moderate cultivation requirements and high oil yield. In Argentina, the potential area for sunflower planting extends from Chaco in the north to La Pampa in the south. Sunflower plants are often exposed to periods of drought in this area, increasingly so toward the west. Expansion of soybean culture has affected the planting area of other crops in recent years, and sunflower has been displaced to marginal areas.

One major obstacle to high yield and production in sunflower is the lack of synchronized crop cycle due to poor weather and soil conditions [24]. Application of microorganisms is important in agriculture to minimize the use of chemical fertilizers. Inoculation with bacteria can increase plant growth, speed up seed germination, protect plants from disease, improve seedling emergence, response to external stress factors, and root growth pattern [22].

G. Forchetti · O. Masciarelli · M. J. Izaguirre · S. Alemano · G. Abdala (✉)
Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina
e-mail: gabdala@exa.unrc.edu.ar

D. Alvarez
Instituto Nacional de Tecnología Agropecuaria, INTA-EEA, Manfredi, Argentina

One possible strategy to help plants cope with environmental stresses may be the introduction of beneficial bacteria into root zone soil. The bacteria can normalize and in some cases improve plant performance under stressful conditions, and thereby preserve or enhance yield [6]. This approach improved growth of cacti [26] and the periwinkle *Catharanthus roseus* [18] under water deficit stress.

Bacteria associated with plants grown under chronically stressful conditions have coevolved with them and provide a significant benefit to the plants. These microorganisms, which may grow in the rhizosphere, rhizoplane, phylosphere, or freely in the soil, were termed "plant growth-promoting bacteria" (PGPB) by [4].

Plant growth-promoting bacteria promote plant growth in several ways: (i) directly enhance plant metabolism by increasing levels of substances that are usually in short supply; e.g., fix atmospheric nitrogen, solubilize phosphorus and iron, or enhance production of plant hormones; (ii) improve tolerance of the plant to environmental stresses such as drought and salinity; and (iii) indirectly promote plant growth by mitigating the harmful effects of phytopathogenic bacteria, fungi, nematodes, and viruses [5]. Diverse genera of bacteria (including *Achromobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Rhizobium*) have been shown to function as PGPB.

Attempts to introduce beneficial bacteria into rhizospheres of agricultural crops have generally met with varying degrees of failure, due mainly to difficulties of incorporating non-resident species into established and acclimated microbial communities. Typically, native bacteria isolated from a specific plant rhizosphere contain highly efficient genotypes to promote plant growth and perform this function better than exotic strains.

Pathogenic fungi, particularly the genera *Alternaria*, *Sclerotinia*, and *Verticillium*, cause the most serious and economically damaging diseases for sunflower in Argentina. *Alternaria* produces dark brown spots on leaves. Leaf lesions may coalesce, causing leaves to wither. Stem lesions begin as dark flecks which enlarge to form long, narrow lesions. These stem lesions often coalesce to form large blackened areas, resulting in stem breakage. *Sclerotinia* overwinters in the soil or on plant debris as sclerotia. The sclerotia germinate when they come in contact with sunflower roots. Surviving plants are smaller than healthy plants and may or may not produce seed. Symptoms are sudden wilting of the leaves, root rot, stem canker, and production of sclerotia in the stem. The initial symptoms of *Verticillium* infection are necrotic mosaic areas with yellow margins, always between the veins, that begin on the lower leaves and progress slowly upward. Affected leaves rapidly become completely dry. In a stem cross section, the vascular system is discolored brown and visible as a brown ring.

Several plant hormones, including salicylic acid (SA) and jasmonic acid (JA), have been reported to display antifungal activity. For example, SA reduced hyphal growth and biomass of *Fusarium oxysporum* [32], and JA inhibited spore germination of *Pyricularia oryzae* [25] and appressoria differentiation of *Erysiphe graminis* f.sp. *hordei* [28].

In a previous study [13], we isolated numerous bacterial strains from soil samples collected from sunflower grown under irrigation or drought at the end of the vegetative stage. Three strains, identified as *Bacillus* spp. (SF3 and SF4) and *Achromobacter* sp. (SF2), were selected based on nitrogen-fixing ability, phosphate-solubilization ability, proteolytic and cellulolytic activity, inhibition of pathogenic fungi, and production of JA, 12-oxo-phytodienoic acid, and abscisic acid.

In the present study, we examined (i) the ability of strains SF2, SF3, and SF4, inoculated into inbred sunflower lines B59 (sensitive to water stress), B71 (tolerant of water stress), and commercial hybrid Paraiso 24, to improve seedling growth under water stress; (ii) ability of SF2, SF3, and SF4 to produce SA under normal and stressful conditions; (iii) ability of pellets and supernatants from these three bacterial strains to inhibit growth of pathogenic fungi (*Alternaria* sp., *Sclerotinia sclerotiorum*, and *Verticillum* sp.); and (iv) effect of exogenously added SA or JA, on growth of these fungi.

Materials and Methods

Inoculation Procedure and Plant Growth

Inbred sunflower lines B59 and B71, generated by the National Institute of Agricultural Technology (INTA) of Argentina, characterized, respectively, as sensitive and tolerant to water stress [2], and the commercial hybrid Paraiso 24 were subjected to inoculation assays. Five seeds were placed in a pot containing sterile vermiculite, in a photoperiod chamber with cycle of 16-h light (28°C)/8-h dark (20°C).

At day 4, seedlings were inoculated separately with strains SF2, SF3, and SF4 (1-ml bacterial inoculum containing 10^8 cfu ml^{-1}). One milliliter LB medium was added to non-inoculated (control) seedlings. Seedlings were watered by capillary ascent with distilled water.

A group of inoculated seedlings was watered with half-strength Hoagland solution. Two other groups were watered with half-strength Hoagland solution supplemented with different polyethylene glycol (PEG) 6000 concentrations to generate $\Psi a = -0.48$ and -0.96 MPa.

At day 14 after inoculation, seedlings were harvested, and root and shoot dry matter (DM) values were recorded. Each

experiment consisted of four repetitions of each treatment, and experiments were performed in quadruplicate.

Salicylic Acid (SA) Evaluation

Twenty five milliliter LB medium from cultured bacterial strains in stationary phase (96 h) (cell density 10^{10} cfu ml^{-1}), or from cultures in medium with 13.7% PEG 6000 ($\Psi a = -2.03$ MPa) (cell density 10^8 cfu ml^{-1}), were used for SA determination. Twenty five milliliter LB medium without bacteria ($\Psi a = -1.12$ MPa) was used as control.

Cultures were centrifuged at 8000 rpm, 4°C, for 20 min, and 2-ml supernatant was filtered and acidified to pH 2.5 with HCl solution. Fifty nanograms ($^2H_4$)-SA was added as internal standard. Each sample was partitioned two times with 2-ml ethyl acetate and evaporated to dryness. The residue was dissolved in 50 µl methanol, and a 10 µl aliquot was injected on LC-MS/MS. Experiments were performed in quintuplicate.

Liquid Chromatography

Analyses were performed using an Alliance 2695 (Separation Module, Waters, USA) quaternary pump equipped with auto-sampler. A Restek C_{18} (Restek, USA) column (2.1 × 100 mm, 5 µm) was used at 28°C, with injected volume 10 µl. The binary solvent system used for elution gradient consisted of 0.2% acetic acid in H_2O (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 Spectrometry

MS/MS experiments were performed on an Micromass Quatro Ultima™ *PT* double quadrupole mass spectrometer (Micromass, Manchester City, UK). All analyses were performed using turbo ion spray source in negative ion mode with the following settings for SA: capillary voltage −3000 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 701 l h^{-1}, collision (50), and multiplier (650). MS/MS parameters were optimized in infusion experiments using individual standard solutions of SA 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 double quadrupole mass spectrometer, and mass was analyzed using the second analyzer of the instrument. In negative mode, the spectrum for SA gave deprotonated molecule $[M–H]^-$. Quantitation was performed by injection of samples in multiple reaction monitoring (MRM) mode, since many compounds could present the same nominal molecular mass. The combination of parent mass and unique fragment ions was used to selectively monitor SA in bacterial culture extracts. MRM acquisition was performed by monitoring the 137/93 and 141/97 transitions for SA and ($^2H_4$)-SA (internal standard), respectively, with dwell 1000 ms for each transition.

Pathogenic Fungi Growth Assay

In view of previous finding [13] that certain bacteria inhibit activity of pathogenic fungi, we evaluated the effects of bacterial pellets and supernatants (separately) on growth of *Alternaria* sp., *Sclerotinia sclerotiorum*, and *Verticillum* sp. Effects of pure SA and JA were also tested. In order to obtain pellets and supernatants of strains SF2, SF3, and SF4, 20 ml of exponential phase culture was centrifuged at 4°C, 8000 rpm for 20 min, sonicated for 10 min at 25 Hz frequency, frozen at −20°C for 30 min, and thawed. Pellets were spread in front of mycelia of each fungal species, on plates containing Malt Extract Agar. Bacterial supernatants (1 ml), SA (4 pmol ml^{-1}), or JA (2 pmol ml^{-1}) were fused separately to Malt Extract Agar at 37°C, and transferred to sterile plates.

Fungal mycelia (1 cm diameter cylinder) were introduced in the plate center and incubated for 7 days at 25°C. Plates containing fungal mycelia without pellet, supernatant, SA, or JA were used as controls.

Mycelium growth inhibition was calculated as $I = [(C - T)/C] \times 100$, where I is mycelium growth inhibition (%), C mycelium diameter in control, and T mycelium diameter in each plate. Experiments were performed in sextuplicate.

Statistical Analysis

Analysis of variance (ANOVA) test was used for statistical analysis of root and shoot DM, SA level, and antifungal effect. Data were subjected to a posteriori Multiple Range Test. A non-parametric test (Kruskal–Wallis) was used for effect of bacterial pellet on *Verticillium* (Fig. 6f) and for effect of SA/JA on *Alternaria* and *Sclerotinia* (Fig. 5a, b). Software used was Statgraphics Plus, v.3 (Manugistics, Rockville, MD, USA).

Results

Effect of SF2, SF3, and SF4 Inoculation on Seedlings Grown Under Normal and Water Stress Conditions

Monitoring the response of sunflower genotypes with differential sensitivity to water stress, in the presence versus

absence of bacterial strains, permits an assessment of early plant responses to stress and of effects on roots versus shoots.

Under normal growth condition ($\Psi a = -0.2$ MPa), commercial hybrid Paraiso 24 inoculated with all three bacterial strains showed increased shoot DM (Fig. 1a). At $\Psi a = -0.48$ MPa, inoculation of SF3 and SF4 increased shoot DM 1.29-fold and 1.43-fold, respectively, relative to non-inoculated control (Fig. 1b). At $\Psi a = -0.96$ MPa, only a slight increase in shoot DM was found in seedlings inoculated with any of the three strains (Fig. 1c).

Root DM was increased strongly by SF3 at $\Psi a = -0.2$ MPa and to a lesser degree by SF2 and SF4 (Fig. 1d). Under mild stress (Ψa $= -0.48$ MPa), SF3 and SF4 enhanced root DM (Fig. 1e). Under severe stress ($\Psi a = -0.96$ MPa), all three strains significantly increased root DM (Fig. 1f).

Inoculation of water stress-tolerant genotype B71 with the three strains markedly increased shoot DM under normal and stressful conditions (Fig. 2a–c). A similar response was observed for root DM (Fig. 2d–f). SF2 was the only strain that did not increase root DM under severe stress (Fig. 2f).

For water stress-sensitive genotype B59, SF2, and SF3 increased shoot DM of seedlings grown under normal condition (Fig. 3a). Under mild stress, SF3 and (particularly) SF4 caused a greater increase of shoot DM than did SF2 (Fig. 3b). In contrast, shoot DM was not affected by inoculation of plants under severe stress (Fig. 3c). Root DM of plants under normal growth condition was not significantly changed by inoculation (Fig. 3d). Root DM was increased markedly by all three strains under mild stress (Fig. 3e) and slightly by SF3 under severe stress (Fig. 3f).

Bacterial SA Production

Salicylic acid production was measured at 72 h, when bacterial culture reached stationary phase. Strains SF2, SF3, and SF4 grown in medium LB ($\Psi a = -1.12$ MPa) and in the presence of PEG to produce a water potential of -2.03 MPa were used for hormone evaluation.

At $\Psi a = -1.12$ MPa generated by LB culture medium, SF3 and SF4 produced significant amounts of SA. SA production by SF2 (16 pmol ml^{-1}) was lower than that of SF3 (238 pmol ml^{-1}) and SF4 (270 pmol ml^{-1}). Under water stress ($\Psi a = -2.03$ MPa), production of SA by the three strains increased dramatically (Fig. 4).

Effects of SA, JA, Bacterial Supernatants, and Pellets on Pathogenic Fungi Growth

Exogenous addition of SA or JA reduced *Alternaria* growth by ~13% (Fig. 5a) and reduced growth of *Verticillum* to a

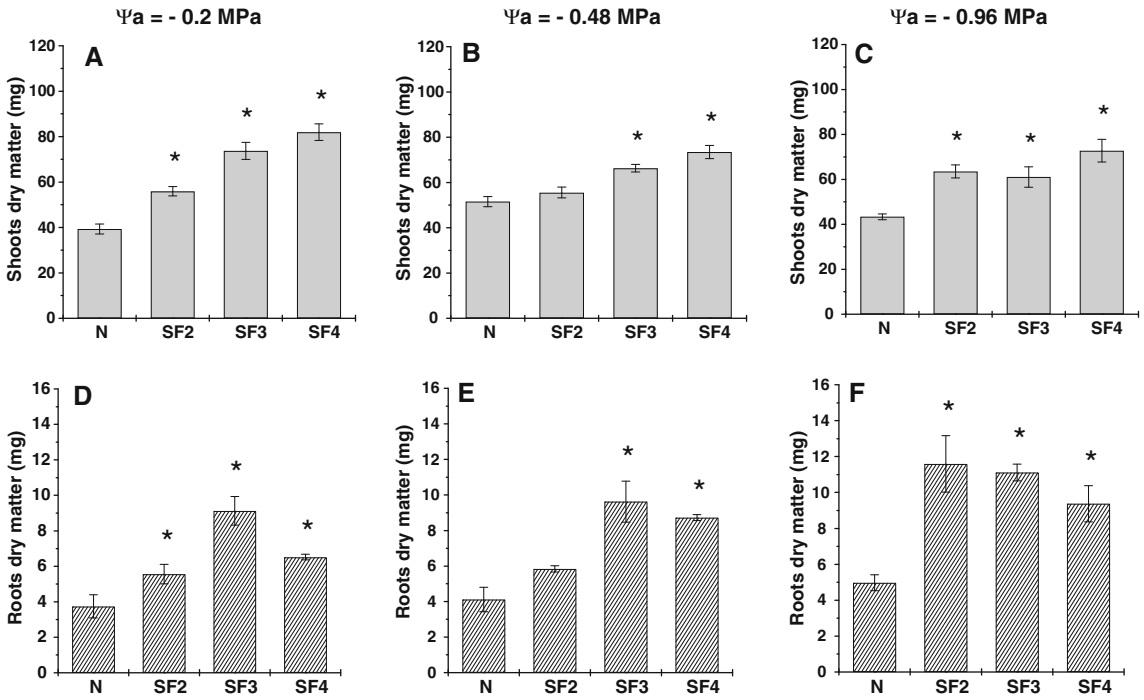

Fig. 1 Effect of bacterial inoculation on shoot and root dry matter (DM) of commercial hybrid Paraiso 24 seedlings grown under normal and stressful conditions. *N* non-inoculated. Each experiment consisted of four repetitions of each treatment, and experiments were performed in quadruplicate. * Significantly different from results for non-inoculated (control) seedlings

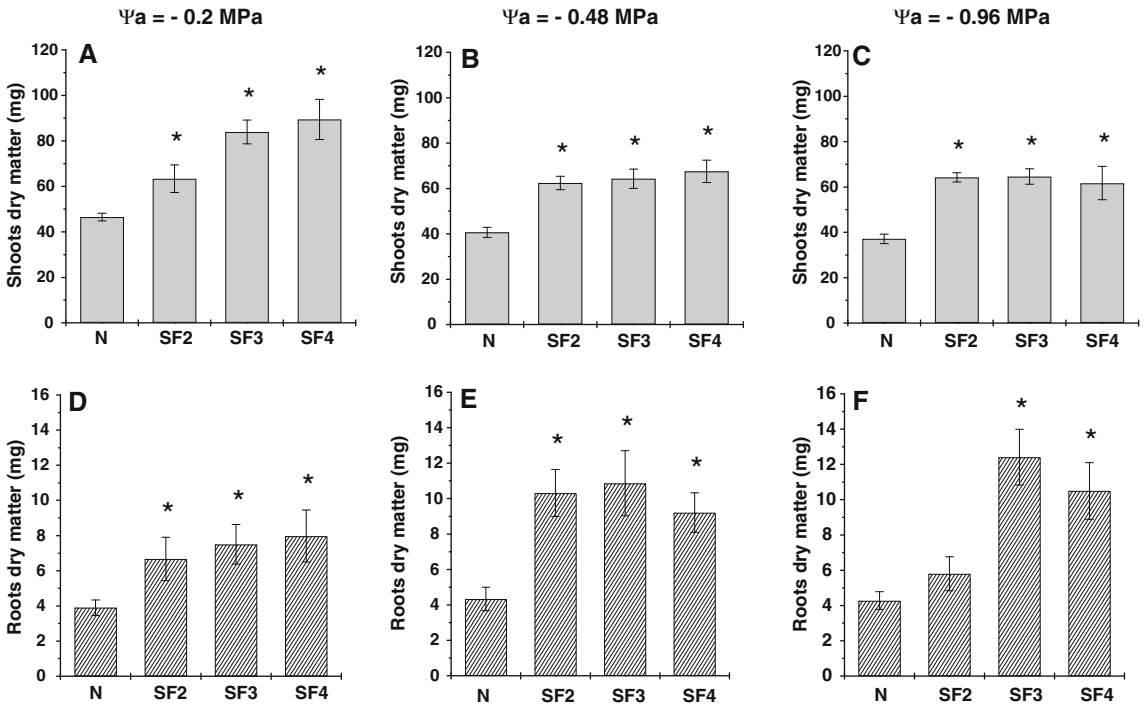

Fig. 2 Effect of bacterial inoculation on shoot and root DM of seedlings of stress-tolerant genotype B71 grown under normal and stressful conditions. Symbols and design as in Fig. 1

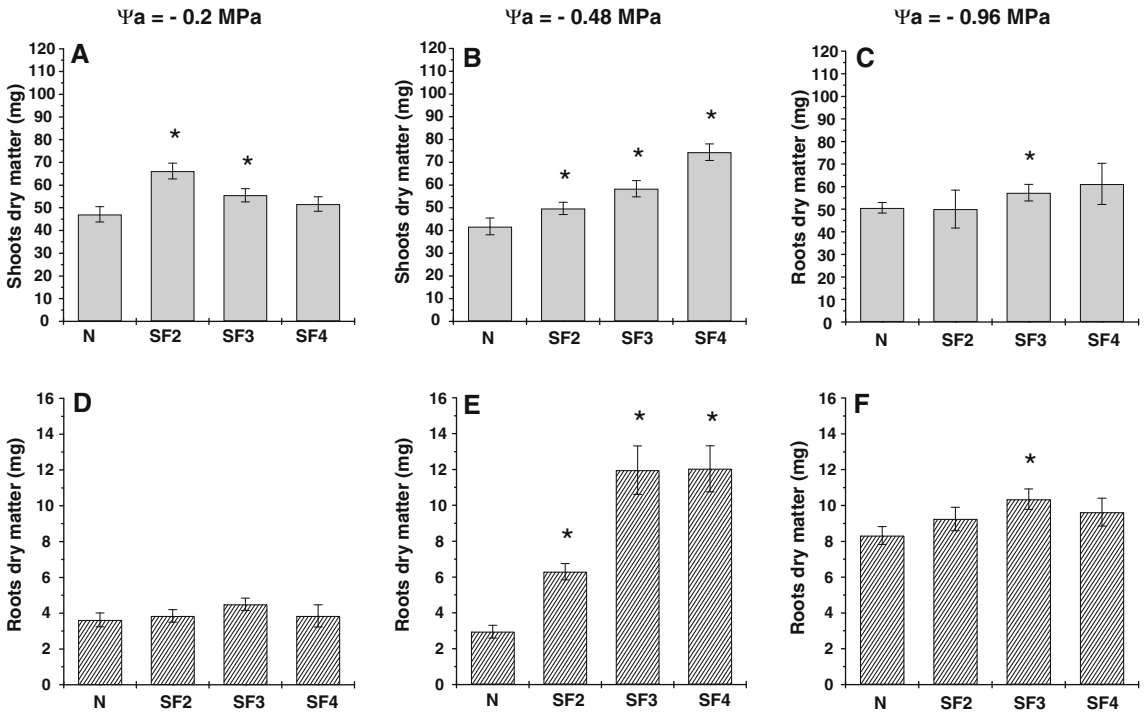

Fig. 3 Effect of bacterial inoculation on shoot and root DM of seedlings of stress-sensitive genotype B59 grown under normal and stressful conditions. Symbols and design as in Fig. 1

similar degree (Fig. 5c). Both hormones produced a greater reduction of *Sclerotinia* growth: 24% by JA; 17% by SA (Fig. 5b).

Supernatants of the three bacterial strains slightly reduced growth of *Alternaria* (Fig. 6a) and *Verticillum* (Fig. 6c). For *Sclerotinia*, growth was reduced ~43% by

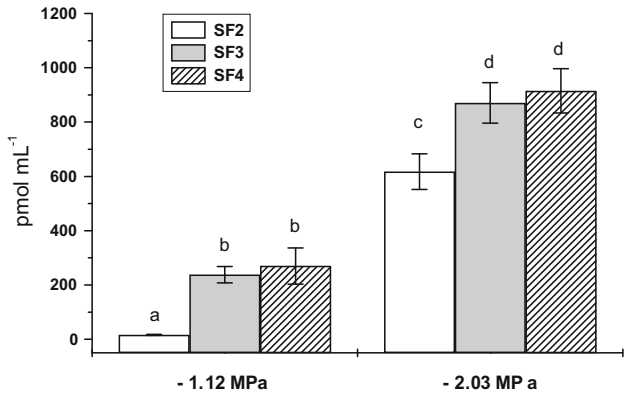

Fig. 4 Salicylic acid (SA) production by strains SF2, SF3, and SF4 grown in LB culture medium ($\Psi a = -1.12$ MPa) and in medium with PEG addition ($\Psi a = -2.03$ MPa). Experiments were performed in quintuplicate. Values indicated by *different letters* are significantly different at $P < 0.05$

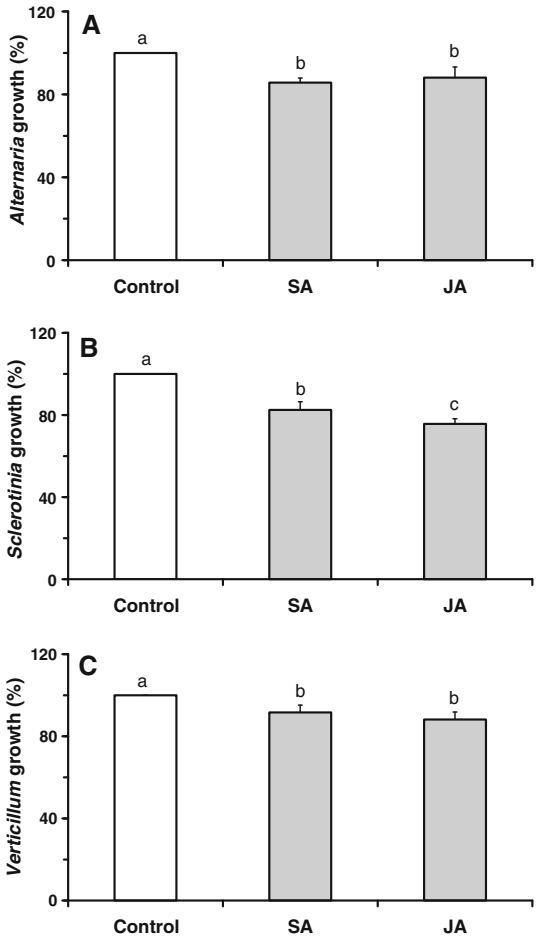

Fig. 5 Effect of exogenous salicylic and jasmonic acids on growth of pathogenic fungi in sunflower. **a** *Alternaria* spp., **b** *Sclerotinia sclerotiorum*, **c** *Verticillum dahliae*. Values indicated by *different letters* are significantly different at $P < 0.05$

supernatant of SF2, 33% by SF3, and 22% by SF4 (Fig. 6b).

Pellets of the three strains inhibited growth of *Alternaria* to a similar degree: ~52–53% (Fig. 6d). Growth of *Sclerotinia* was inhibited ~58% by pellets of SF4, 52% by SF3, and 50% by SF2 (Fig. 6e). Growth of *Verticillum* was inhibited ~57% by SF2, 54% by SF4, and 47% by SF3 (Fig. 6f).

Thus, the inhibitory effect on pathogenic fungi growth by bacterial pellets was considerably stronger than that of bacterial supernatant, or of pure SA or JA.

Discussion

Crop plant production and yield are highly susceptible to numerous environmental stress factors. An important strategy to normalize plant performance and preserve or enhance yield is the introduction in root zone soil of beneficial bacteria that help increase resistance to stresses. For example, inoculation of maize with *Bacillus megaterium* and *B. mucilaginous* increased growth and improved nutritional assimilation, particularly of nitrogen, phosphorus, and potassium [31]. Seed inoculation of *Jatropha curcas* (Barbados nut) with *Bacillus* spp. improved seedling vigor through a significant increase in various physiological parameters [12].

In our experiments using various sunflower genotypes [2], application of water stress to seedlings of commercial hybrid Paraiso 24 and stress-tolerant inbred line B71 in the absence of bacterial strains did not cause significant reduction of shoot or root biomass. This response could be directly related to the tolerance of these genotypes to water stress. In contrast, sensitive genotype B59 subjected to severe water stress showed dramatic increase of root biomass in the presence of bacterial strains, possibly because of enhanced water uptake.

The presence of *Bacillus* sp. (strains SF3 and SF4) and *Achromobacter* sp. (strain SF2) clearly enhanced sunflower seedling growth and increased shoot and root DM, under stressful conditions.

Under mild water stress, SF3 and SF4 increased Paraiso 24 shoot and roots DM, whereas SF2 had no effect. Under severe water stress, all three strains promoted growth of Paraiso 24 and B71 (except that SF2 had no significant effect on root DM of B71). SF2 and SF4 could not reverse the effect of severe water stress on stress-sensitive inbred line B59. Taken together, these findings suggest that *Bacillus* sp. are more effective than *Achromobacter* in helping sunflower seedlings cope with water stress conditions.

Differences were observed in growth responses of the three genotypes inoculated with SF2, SF3, and SF4. The best responses following inoculation were observed for

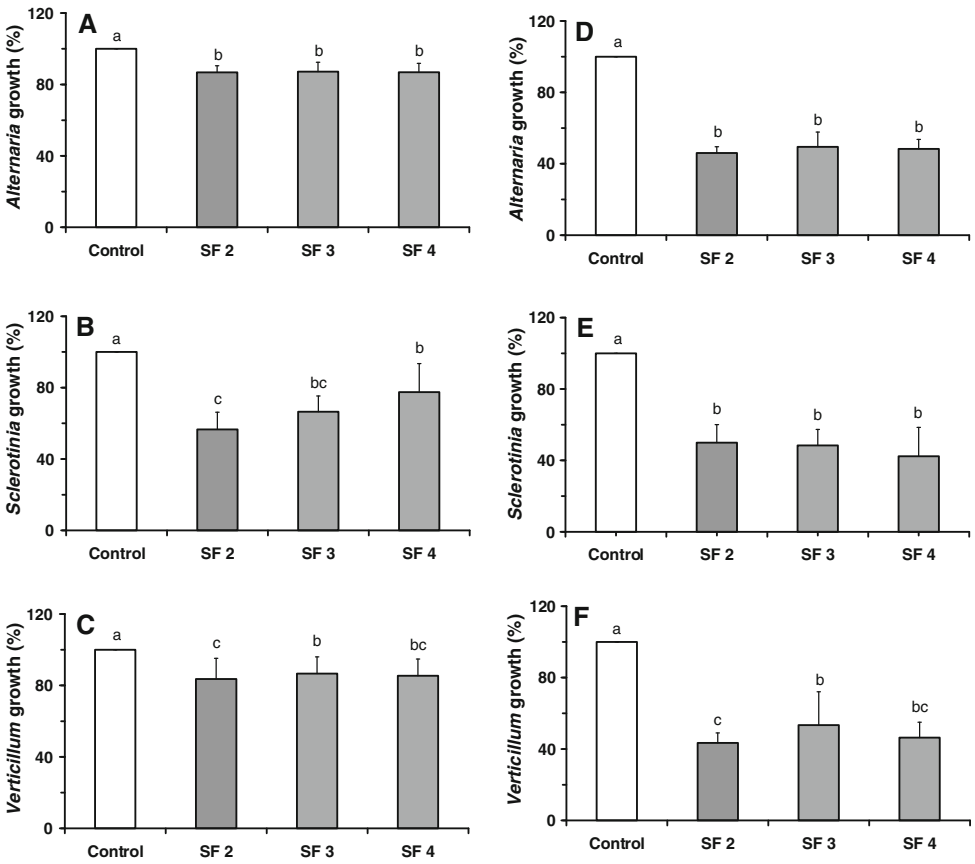

Fig. 6 Effect of SF2, SF3, and SF4 bacterial supernatants and pellets on growth of pathogenic fungi. **a–c** Bacterial supernatants, **d–f** bacterial pellets. Values indicated by *different letters* are significantly different at $P < 0.05$

Paraiso 24 and B71, under water stress as well as normal conditions. The increased root DM of inoculated Paraiso 24 and B71 growing under water stress could be a strategy to facilitate water uptake from the soil. When B59 was inoculated, shoot and root DM were increased only under mild stress condition. The differences observed in response of B59 compared to Paraiso 24 and B71 are presumed to be attributable more to sunflower genotype than to effects of the bacterial strains.

It is possible that these bacteria promote sunflower growth through production of specific plant hormones. *Bacillus pumilis* and *B. licheniformis* have been reported to enhance productions of auxins [15] and many gibberellins [16, 19]. Growth of red pepper seedlings was increased by *B. pumilus* [19] and by *B. cereus* [20]. Enhanced production of other growth regulators (e.g., such as jasmonic and abscisic acids) by SF2, SF3, and SF4 [13] could also improve plant performance under stressful soil conditions.

Several genera of bacteria, including pseudomonads, are known to synthesize SA [3, 7, 17]. We therefore considered the possibility that sunflower strains can produce SA and found that SA is synthesized by SF2, SF3, and SF4 cultured in LB medium ($\Psi a = -1.12$ MPa), and that its production increases under water stress ($\Psi a = -2.03$ MPa). Thus, SA synthesized by bacteria may play an important role not only in plant resistance against pathogen infection, but also in mediating plant reactions to abiotic stress [11, 23]. Bacteria strains that lost their ability to produce SA also showed loss of ability to induce plant resistance [10].

Exogenous application of SA alone caused 8–17% inhibition of *Alternaria*, *S. sclerotiorum*, and *Verticillum* growth, suggesting that other compounds present in bacteria act in combination with SA or JA to inhibit growth of pathogenic fungi. Different *Bacillus* species in particular produce peptides and lipopeptides such as fungicine, iturin bacillomicine, and others, which have antigungal properties [8, 9, 21]. In our study, JA was more effective than SA in inhibiting *S. sclerotiorum* growth. Consistent with our findings, other studies have shown that SA inhibits growth of *Sclerotium rolfsii* and *Sclerotinia minor* [14] and cause disruption in many compartments of the fungus *Eutypa lata* [1]. High SA concentration (270 mg l^{-1}) had a direct toxic effect on *Monilinia fructicola* and significantly inhibited mycelial growth of this fungus in vitro [29]. Exogenous SA markedly reduced hyphal growth and biomass of *Fusarium oxysporum* f.sp. *niveum* [32].

In the present study, pellets of strains SF2 and SF4 had the strongest inhibitory effect on growth of *Verticillum* spp., even though SF2 had the lowest SA level among the three strains. *Bacillus* species are well known to produce

compounds that inhibit a variety of pathogenic fungi [8, 21, 27, 30]. For example, *Bacillus* sp. strain BNM112, isolated from a sclerotium of *S. sclerotiorum* taken from sunflower capitulum, excreted metabolites that suppressed mycelial growth of numerous fungal species [27]. The antifungal effect of strains SF2, SF3, and SF4 may therefore be due in part to their production of SA, JA, and/or other antibiotic compounds. These compounds are presumably found mainly in bacterial pellet, whose inhibitory effect on fungal growth was considerably stronger than that of supernatant.

The present findings suggest that endophytic bacteria in sunflower are co-selected with the plant under the influence of environmental factors such as water stress, and that plant genotype and bacterial diversity contribute jointly to plant growth under stress conditions.

The production of bacterial SA under water stress and the suppressing effect of bacterial pellets on specific pathogenic fungi are important considerations for formulation of efficient inoculants. Bacterial inoculants promote plant growth in many ways, e.g., speed up seed germination, improve seedling emergence, root growth pattern, and response to external stress, and protect plants from disease [22]. The seed and seedling stages are crucial developmental stages in the plant life cycle and in final yield of crops. Our results suggest that sunflower plants could be productively cultivated under water stress condition, if they are inoculated with a suitable PGPB such as SF2, SF3, or SF4. The characteristics of these isolated strains and the effects of their inoculation on sunflower seedlings as described here have technological implications for formulation of inoculants to improve growth and enhance yield in sunflower crops.

Acknowledgments This work was supported by grants from SECYT-UNRC and ANPCYT to G.A., and a fellowship from CONICET to G.F.

References

1. Amborabé BE, Fleurat-Lessard P, Chollet JF, Roblin G (2002) Antifungal effects of salicylic acid, other benzoic acid derivatives towards *Eutypa lata*: structure–activity relationship. Plant Physiol Biochem 40:1051–1060
2. Andrade A, Vigliocco A, Alemano S, Alvarez D, Abdala G (2009) Differential accumulation of ABA, its catabolites in drought-sensitive, drought-tolerant sunflower seeds. Seed Sci Res 19:201–211
3. Ankenbauer RG, Cox CD (1988) Isolation and characterization of *Pseudomonas aeruginosa* mutants requiring salicylic acid for pyochelin biosynthesis. J Bacteriol 170:5364–5367
4. Bashan Y, de-Bashan LE (2005) Bacteria/plant growth-promotion. In: Hillel D (ed) Encyclopedia of soils in the environment, vol 1. Elsevier, Oxford, pp 103–115
5. Bashan Y, Puente M, Bashan L, Hernández P (2008) Environmental uses of plant growth-promoting bacteria. In: Ait Barka E, Clément C (eds) Plant–microbe interactions. Research Signpost, Trivandrum, Kerala, India, Chap 4, pp 69–93
6. Bensalim S, Nowak J, Asiedu SK (1998) A plant growth promoting rhizobacterium, temperature effects on performance of 18 clones of potato. Am J Potato Res 75:145–152
7. Buysens S, Heungens K, Poppe J, Höfte M (1996) Involvement of pyochelin, pyoverdine in suppression of Phytium-induced damping-off tomato by Pesudomonas aeruginosa, 7NSK2. Appl Environ Microbiol 62:865–871
8. Chitarra GS, Breeuwer P, Nout MJR, van Aelst AC, Rombouts FM, Abee T (2003) An antifugal compound produced by Bacillus subtilis YM 10–20 inhibits germination of Penicillium roqueforti conidiospores. J Appl Microbiol 94:159–166
9. Cho S-J, Lee SK, Cha BJ, Kim YH, Shin K-S (2003) Detection, characterization of the Gloeosporium gloeosporioides growth inhibitory compound iturin A from Bacillus subtilis strain KS03. FEMS Microbiol Lett 223:47–51
10. De Meyer G, Hofte M (1997) Salicylic acid produced by the rhizobacterium Pseudomonas aeruginosa 7NSK2 induces resistance to leaf infection by + *Botrytis cinerea* on bean. Phytopathology 87:588–593
11. De Meyer G (1999) Role of salicylic acid and systemic resistance to disease induced by the root-colonizing bacterium *Pseudomonas aeruginosa* 7NSK2. Thesis submitted in the fulfillment of the requirements for the degree of Doctor (Ph.D.) in Applied Biological Sciences-Agronomy, Gent University
12. Desai S, Narayanaiah Ch, Kranti Kumari Ch, Reddy M, Gnanamanickam S, Rajeswara Rao G, Venkateswarlu B (2007) Seed inoculation with Bacillus spp. Improves seedling vigour in oil-seed plant *Jatropha curcas* L. Biol Fertil Soils 44:229–234
13. Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.) isolation characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76(5):1145–1152
14. Georgiou CD, Tairis N, Sotiropoulou A (2000) Hdroxyl radical scavengers inhibit lateral-type sclerotial differentiation, growth in phytopathogenic fungi. Mycologia 92:25–834
15. Gutiérrez-Mañero FJ, Acero N, Lucas J, Probanza A (1996) The influence of native rhizobacteria on European alder (*Agnus glutinosa* L. Gaertn.) growth II. Characterization and biological assays of metabolites from growth inhibiting bacteria. Plant Soil 182:67–74
16. Gutiérrez-Mañero FJ, Ramos-Solano B, Probanza A, Mehouachi J, Tadeo F, Talon M (2001) The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plant 111:206–211
17. Hudson AT, Bentley R (1970) Utilization of shikimic acid for the formation of mycobactin S and salicylic acid by *Mycobacterium smegmatis*. Biochemistry 9:3984–3987
18. Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R, Panneerselvam R (2007) Pseudomonas fluorescens enhances biomass yield, ajmalicine production in Catharantus roseus under water deficit stress. Colloids Surf B 60:1–11
19. Joo GJ, Kim YM, Lee IJ, Song KS, Rhee IK (2004) Growth promotion of red pepper plug seedlings, the production of gibberellins by Bacillus cereus, *Bacillus macroides* and *Bacillus pumilus*. Biotechnol Lett 26:487–491
20. Joo GJ, Kim YM, Kim JT, Rhee IK, Kim JH, Lee IJ (2005) Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. J Microbiol 43(6):510–515
21. Kajimura Y, Sugiyama M, Kaneda M (1995) Bacillopeptins, new cyclic lipopeptide antibiotics from *Bacillus subtilis* FR-2. J Antibiot 48:1095–1103

22. Lugtenberg B, Chin-A-Woeng T, Bloemberg G (2002) Microbe–plant interactions: principles and mechanisms. Antonie van Leeuwenhoek 81:373–383
23. Maurhofer M, Reimmann C, Schmidli-Sacherer P, Heeb S, Hass D, Défago G (1998) Salicylic acid biosynthetic genes expressed in Pseudomonas fluorescens strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology 88:678–684
24. Mwale SS, Hamusimbi C, Mwansa V (2003) Germination, emergence and growth of sunflower (*Helianthus annuus* L.) in response to osmotic seed priming. Seed Sci Technol 31:199–206
25. Neto GC, Kono Y, Hyakutake H, Watanabe M, Suzuki Y, Sakurai A (1991) Isolation, identification of (-)-jasmonic acid from wild rice Oryza officinalis, as an antifungal substance. Agric Biol Chem 55:3097–3098
26. Puente M, Li C, Bashan Y (2004) Microbial populations, activities in the rizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedling. Plant Biol 6:643–650
27. Souto GI, Correa OS, Montecchia MS, Kerber NL, Pucheu NL, Bachur M, García AF (2004) Genetic, functional characterization of a Bacillus sp. strain excreting surfactin and antifungal metabolites partially identified as iturin-like compounds. J Appl Microbiol 97:1247–1256
28. Schweizer P, Gees R, Mosinger E (1993) Effect of jasmonic aicd on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f.sp. hordei. Plant Physiol 102:503–511
29. Yao HJ, Tian SP (2005) Effects on pre-and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biol Technol 35:253–262
30. Yu GY, Sinclair JB, Hartman GL, Bertagnolli BL (2002) Production of iturin A by Bacillus amyloliquefaciens suppressing *Rhizoctonia solani*. Soil Biol Biochem 34:955–963
31. Wu SC, Caob ZH, Lib ZG, Cheunga KC, Wonga MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166
32. Wu H-S, Raza W, Fan J-Q, Sun Y-G, Bao W, Liu D-Y, Huang Q-W, Mao Z-S, Shen Q-R, Miao W-G (2008) Antibiotic effect of exogenously applied salicylic acid on in vitro soilborne pathogen, *Fusarium oxysporum* f.sp. niveum. Chemosphere 74:45–50