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Bacillus velezensis S141 improves the root growth of soybean under drought conditions

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Running head: *B. velezensis* for drought tolerance in soybean

Abstract

Bacillus velezensis S141 helps soybean establish specific symbiosis with strains of *Bradyrhizobium diazoefficiens* to form larger nodules and improve nitrogen fixation efficiency. In this study, we found that the dry weight of soybean roots increased significantly in the presence of S141 alone under drought conditions. Hence, S141 improved the root growth of soybean under limited water supply conditions. S141 can produce some auxin, which might be involved in the improved nodulation. Inactivating *IPyAD* of S141, which is required for auxin biosynthesis, did not alter the beneficial effects of S141, suggesting that the root growth was independent of auxin produced by S141. Under drought conditions, soybean exhibited some responses to resist osmotic and oxidative stresses; however, S141 was relevant to none of these responses. Although the mechanism remains unclear, S141 might produce some substances that stimulate the root growth of soybean under drought conditions.

Keywords

drought stress, plant growth-promoting rhizobacteria, soybean

Environmental problems such as global warming and radical climate change are elevating the level of stress on plants and threatening the stability of crop production. Simultaneously, the total human population is continuously increasing, demanding secure food supplies. These mutually contradictory facts necessitate addressing the difficult problem of increasing crop productivity even under stressful conditions. Crops are naturally exposed to various environmental stresses during their growth and development under natural and agricultural conditions and thus possess a certain tolerance. The molecular mechanisms underlying this tolerance have been intensively investigated (Saharan *et al*. 2022; Villalobos-López *et al*. 2022). Among the various stress conditions, drought is one of the most serious conditions affecting plant productivity. More than 80% of fresh plant biomass is composed of water, which plays an indispensable role in various physiological processes, including several aspects of plant growth, development, and metabolism. Consequently, drought stress disrupts normal plant metabolism and alters plant morphological and physiological traits (Chieb *et al*. 2023; Wahab *et al*. 2022; Ahmad *et al*. 2022b). In nature, plants are often simultaneously exposed to multiple stresses (Atkinson *et al*. 2012), and the physiological and molecular responses occurring in plants are highly complex and remain to be elucidated (Nostar *et al*. 2013; Ramegowda *et al.* 2015). It is also important to consider the possibility that not only plants but also microorganisms that exist in the vicinity of plants are similarly exposed to environmental stresses and react in some manner, which may affect the plants.

Recently, several studies have proposed that plant growth-promoting rhizobacteria (PGPR) and bacterial endophytes provide numerous direct and indirect benefits to crop plants (Sarma *et al.* 2014; Rho *et al*. 2018). It is assumed that PGPR are in closer contact with host plants and can improve stress tolerance (Zhang *et al*. 2019;

Dombrowski *et al*. 2017; Dubey *et al*. 2021; Pandey *et al*. 2016). Examples of such PGPR include *Azotobacter* (Wu *et al*. 2012; Abdiev *et al*. 2019), *Azospirillum* (Remans *et al*. 2008; Aung *et al.* 2013), *Bacillus* (Remans *et al.* 2008; Korir *et al*. 2017; Prakamhang *et al*. 2015), *Pseudomonas* (Zahir *et al*. 2011; Nascimento *et al*. 2019), *Serratia* (Pan *et al.* 2002; Zahir *et al.* 2011), *Staphylococcus* (Prakamhang *et al*. 2015), and *Streptomyces* (Htwe *et al*. 2018), all of which share some common microhabitat at the root–soil interface. These bacteria produce exopolysaccharides and reduce ethylene levels using 1-aminocyclopropane-1-carboxylic acid deaminase, which plays a vital role in supporting plant growth under salinity and drought stress conditions. Other metabolites produced by PGPR bacteria are phytohormones (including auxins, such as 3-indoleacetic acid, and cytokinins), siderophores, volatile compounds, and organic acids, which promote plant growth under stress conditions (Somasegaran *et al*. 2012). In addition to these examples, it is predictable that microorganisms produce various compounds involved in improving stress tolerance in plants, and their identification and application are anticipated.

Soybean is one of the most economically important crops, and soybean plants are so drought-sensitive that their growth and production are inhibited dramatically under drought (Kunert *et al*. 2016). Under drought conditions, several physiological and morpho-biochemical parameters are adversely affected, causing leaf wilt, reduced leaf area, root elongation, reduced chlorophyll content, and reactive oxygen species generation (Miller *et al.* 2010; Choudhury *et al*. 2017). To tolerate drought stresses, soybean accumulates a variety of osmoprotective substances and osmotic solutes such as polyamines, betaine, proline, and sugars (such as trehalose, sorbitol, and mannitol) that are generally responsible for osmotic regulation (Singh *et al*. 2022). In addition to osmotic regulation, these osmolytes play dynamic roles in maintaining membrane integrity,

mitigating ion toxicity, stabilizing proteins, protecting cellular components, scavenging reactive oxygen species, protecting antioxidant compounds, and even regulating cellular redox balance under various abiotic stress conditions (Miller *et al*. 2010; Choudhury *et al*. 2017; Singh *et al*. 2022). In general, when plants experience drought stress, there are changes in the relative water content, proline content, soluble sugars, proteins, lipid peroxidation, photosynthetic pigments, and antioxidant levels (Hare *et al*. 1998; Ashraf *et al*. 2011).

Soybean plants and their partner nitrogen-fixing rhizobia have a symbiotic relationship involving the specific organogenesis of nodules, i.e., soybean supplies carbon sources fixed by photosynthesis to rhizobia, and the rhizobia in turn fix nitrogen in the air to supply nitrogen sources to soybean. Such a mutually beneficial symbiotic relationship allows soybean to grow even in soils poor in nitrogen sources. *Bacillus velezensis* S141 is a PGPR isolated from soybean rhizosphere soil in Thailand (Prakamhang *et al*. 2015). S141 has been used in field experiments to improve the growth of soybean with symbiotic nitrogen-fixing rhizobia, such as *Bradyrhizobium diazoefficiens* USDA110 and THA6. The entire S141 genome consists of a 3.97-Mb-long circular chromosome, which encodes at least 3817 protein-coding genes. Some genes related to auxin and cytokinin biosynthesis in the S141 genome might play a role in its ability to promote plant growth (Sibponkrung *et al*. 2017; Sibponkrung *et al*. 2020). Moreover, S141 possesses β-glucosidases that can degrade isoflavone glucosides, which may contribute to the supply of isoflavones required for the initiation of the symbiotic relationship between soybean and nitrogen-fixing rhizobia (Kondo *et al.* 2023).

We have been exploring the factors responsible for the plant growth-promoting effect of S141 that stimulates the symbiotic relationship between soybean and *B.*

112 *diazoefficiens* USDA110. Nevertheless, the mechanism underlying this effect still
113 remains unclear. In this study, we explored whether there is any growth-promoting effect
114 that soybean can benefit from its one-to-one relationship with *B. velezensis* S141.
115 Soybean uninfected with rhizobia becomes chronically nitrogen-deficient, and when the
116 soybean plants in this state are subjected to drought stress, their growth is significantly
117 reduced. We found that under these harsh conditions, the presence of S141 increased the
118 dry weight of soybean plant roots. Hence, we investigated whether the auxin production
119 of S141 and any factors responsible for the drought tolerance of soybean are involved in
120 the root growth-promoting function of S141.
121
122 **Materials and methods**
123 **Bacterial strains and growth conditions**
124 The bacterial strains *B. velezensis* S141 (Aung *et al.* 2013; Prakamhang *et al*. 2014) and
125 its derivative S141ΔIPyAD (Sibponkrung *et al*. 2020) and *B. diazoefficiens* USDA110
126 (Kaneko *et al*. 2002) were used in this study. *B. velezensis* was inoculated into lysogeny
127 broth (LB) (Bertani 2004) and grown for 24 h at 28°C with shaking until the optical
128 density for the cell at 600 nm (OD_{600}) reached around 1.0. *B. diazoefficiens* was inoculated
129 into YM broth (Vincent 1970) and grown for 72 h at 28°C with shaking until theOD_{600}
130 reached around 1.0. When required, the medium was supplemented with 100 μg ml^{-1}
131 spectinomycin. The bacterial cells in the early stationary phase of cultures were harvested
132 by centrifugation at 3000 ×*g* for 10 min, washed thoroughly with sterilized 0.85% (w/w)
133 NaCl to remove the growth media, and resuspended in 0.85% NaCl to a final
134 concentration at 10^6 cells ml^{-1} as the cell suspension applied to soybean seedlings.

Soybean plant growth experiments

Seeds of soybean (*Glycine max* cv. Enrei) were successively treated with 70% ethanol for 60 s and 2% sodium hypochlorite for 3 min and then rinsed 10 times with sterilized water. The seeds were incubated on sterilized 7.5% agar for 2 days until the emergence of seedlings. The cell suspension containing 10^6 ml^{-1} cells of S141, its derivative, and USDA110 in sterilized 0.85% NaCl was used to inoculate the seedlings. As the control experiment, sterilized 0.85% NaCl was used instead of the inoculation suspension. Each of the modified Leonard jars (Blauenfeldt *et al*. 1994) contained approximately 300 ml of sterilized vermiculite, in which a seedling was grown. The soybean plant growth experiments were octuplicate for each condition.

The irrigation conditions were controlled in a growth chamber as a 16-h light:8-h dark cycle at 28°C/22°C with 80% relative humidity. Plants were watered regularly with N-free nutrient solution (Zhang *et al*. 1996; Somasegaran *et al.* 2012) and grown for 35 days after inoculation (DAI).

The drought conditions were a 16-h light:8-h dark cycle at 28°C/22°C with 50% relative humidity. The plants were grown for 45 DAI, with water withheld after 14 DAI, and only 100 ml of nitrogen-free nutrient solution was applied once every 2 weeks.

Morphological, physiological, plant parameter analyses

The harvested plant samples were frozen in liquid nitrogen and stored at −80°C for further analysis. To measure the dry weights of the total plant and root, the harvested samples were incubated in a hot air oven set at 65°C for 4 days before weighing, as described elsewhere (Somasegaran *et al.* 2012).

The effect of bacterial inoculation on the stomatal development of the host plant was analyzed using the conventional stomatal imprint technique (Hepworth *et al*. 2015). The

harvested leaves were from the youngest trifoliate formed after the imposed drought period. Dental silicone impression material "Fusion II Extra Wash Type" (Gc Dental Co., Tokyo, Japan) was applied and left to set before removing the leaf and applying clear nail varnish to the resin at the maximum leaf width, followed by observation under a light microscope (Olympus BX51; Tokyo, Japan). Eight plants per condition, one leaf per plant, and one area per leaf were examined.

Biochemical analyses

Leaves were subjected to the estimation of photosynthetic pigments, including chlorophyll a, chlorophyll b, and total chlorophyll, which were measured using the standard protocol (Hiscox *et al.* 1979). Approximately 0.1 mg of the leaf was placed into a vial containing 1 ml of DMSO to extract chlorophyll at 65°C without grinding. The extract in DMSO was assayed immediately.

For the estimation of total proline, approximately 0.1–0.5 g of plant material was homogenized in 3 ml of 40% ethanol, followed by centrifugation at 2500 rpm for 1 min. The acid–ninhydrin reaction mixture was prepared, which involved agitation until the mixture dissolved. Then, 50 µl of the homogenate was mixed with 100 µl of the acid–ninhydrin solution, containing 1% (w/v) ninhydrin, 60% (v/v) acetic acid, and 20% (v/v) ethanol, in a test tube and incubated for 20 min at 95°C. Then, the mixture was cooled to room temperature and centrifuged at 2500 rpm for 1 min to measure absorbance at 520 nm of the supernatant (Carillo *et al*. 2011).

For measuring the total soluble sugar (TSS) content, 0.5 g of plant material was homogenized in 1 ml of ethanol. Then, 100 µl of the ethanol extract was mixed with 3 ml of anthrone reagent freshly prepared using 150 mg of anthrone in 100 ml of 72% (w/w) H_2SO_4 (Irigoyen *et al*. 1992) and incubated in a boiling water bath for 10 min. After

cooling to room temperature, the absorbance at 625 nm was determined.

H_2O_2 and malonaldehyde (MDA) content analyses

H_2O_2 content was determined as described previously (Velikova *et al*. 2000). Briefly, 500 mg of leaf was homogenized in 1 ml of 0.1% (w/v) trichloroacetic acid (TCA), followed by centrifugation at 12,000 rpm for 15 min. Then, 0.5 ml of the supernatant was mixed with 1 ml of 1 M potassium iodide and 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) to measure the absorbance at 390 nm.

The content of MDA, as a product of lipid peroxidation in leaves, was measured by estimating the formation of thiobarbituric acid (TBA) reactive substances. MDA equivalents were determined as described previously (Hodges *et al*. 1999). Plant tissue samples were homogenized in 80% ethanol, followed by centrifugation at 3000 ×*g* for 10 min. A 1-ml aliquot of the supernatant diluted appropriately was added into a test tube containing 1 ml of either the solution TBA (20.0% (w/v) TCA and 0.01% butylated hydroxytoluene) or the solution + TBA (the solution −TBA supplemented with 0.65% TBA), mixed vigorously, heated at 95°C for 25 min, cooled down to room temperature, and centrifuged at 33,000 ×*g* for 10 min. The resulting supernatant was subjected to the measurement of absorbances at 440, 532, and 600 nm (Abs440, Abs532, and Abs600, respectively). The MDA equivalents were calculated as follows:

1) $[(Abs532_{+TBA} - Abs600_{+TBA}) - (Abs532_{-TBA} - Abs600_{-TBA})] = A$

2) $[(Abs440_{+TBA} - Abs600_{+TBA})\ 0.0571] = B$

3) MDA equivalents $(nmol \cdot ml^{-1}) = [(A - B)/157{,}000]10^6$

Results

S141 improved the root growth of soybean plants under drought conditions

Soybean plants were inoculated with S141 and USDA110 and grown under irrigated and drought conditions, after which the dry weights of shoot, root, and total plant were measured (Figure 1). The irrigated and drought conditions exerted no significant effects on shoot growth, and S141 alone did not improve shoot growth as no significant difference was detected in its presence and absence (Figure 1a). When USDA110 was co-inoculated, the shoot growth improved obviously due to the symbiotic nitrogen-fixing nodulation by almost four and three times in the irrigated and drought conditions, respectively (Figure 1a and b). Under our experimental conditions different from those in the field (Sibponkrung *et al*. 2020), there was no obvious growth improvement by the co-inoculation of S141 and USDA110 due to unknown reasons. Regarding root growth, S141 alone resulted in a significant improvement under drought conditions and an increasing trend under irrigated conditions (Figure 1c). Nevertheless, these positive effects disappeared when USDA110 was co-inoculated (Figure 1d). Interestingly, root growth was not as improved as shoot growth even in the presence of USDA110. This trend of plant growth improvement with the inoculation of S141 alone was also observed in the total plant weight under drought conditions (Figure 1e). However, this trend disappeared again by co-inoculation with USDA110 in the present laboratory conditions (Figure 1f).

These results indicated that S141 improved the root growth of soybean plants, which was detectable in the absence of symbiotic nitrogen fixation with USDA110 under drought conditions. Furthermore, this positive effect of S141 was more pronounced under drought conditions. Based on these results, we believe that the function of S141 was not potent but detectable only under the harsh conditions of nitrogen starvation plus drought

under our laboratory settings. We also infer that the growth-promoting effect of S141 on soybean observed in the field (Sibponkrung *et al*. 2020) may not be replicated indoors.

Auxin production in S141 was independent of the positive effects on soybean root growth

Auxin-secreting PGPR might contribute to the drought stress tolerance of plants (Ahmad *et al*. 2022a). A mutant strain of S141 generated in our previous study, S141ΔIPyAD, produced only 3.833% of the auxin amount produced by the wild-type strain, and co-inoculation of S141ΔIPyAD and USDA110 caused a reduction in the dry weight of soybean due to less efficient nodulation and nitrogen fixation than that with co-inoculation of the parent strain S141 and USDA110 (Sibponkrung *et al*. 2020). We compared S141 and S141ΔIPyAD for inoculation alone to soybean grown under irrigated and drought conditions (Figure 2). Under irrigated conditions, S141ΔIPyAD caused no significant change in the dry weights of shoots, roots, and total plant. A similar trend was observed under drought conditions. Both S141 and S141ΔIPyAD improved the plant growth better under drought conditions than under no-inoculation and irrigated conditions. These findings indicated that auxin production in S141 was not associated with the improved root growth of soybeans under drought conditions without nodulation.

The positive effect of S141 was irrelevant to any known chemical and biological changes in soybean under drought conditions

Under drought conditions, plants exhibit several cellular responses to resist osmotic and oxidative stresses. TSS and proline accumulate to maintain intracellular osmotic pressure, and H_2O_2 and MDA are the indicators of oxidative stress. We measured the accumulation of these substances in soybean leaves subjected to drought stress (Figure 3).

For TSS content (Figure 3a), the highest value was observed under drought

253 conditions (26.7 ± 2.9 g/g FW) with no-inoculation, which was significantly different
254 from that under both irrigated conditions with no-inoculation (15.8 ± 1.0 g/g FW) and
255 with S141 inoculation (20.7 ± 2.2 g/g FW). However, there was no difference in the TSS
256 content under drought conditions in the presence of S141 (22.3 ± 2.2 g/g FW) and
257 S141ΔIPyAD (23.3 ± 3.4 g/g FW). Conversely, there was no significant difference in
258 proline accumulation under any conditions (Figure 3b). H_2O_2 levels became lower under
259 drought conditions (34.1 ± 4.7 and 33.1 ± 6.2 μg/g FW with no-inoculation and S141,
260 respectively) than under irrigated conditions (48.7 ± 6.2 and 53.6 ± 8.3 μg/g FW,
261 respectively) (Figure 3c). Under any conditions, lipid peroxidation in leaves was not
262 measurable as MDA levels were always less than the detection limit (data not shown).
263 Leaves of drought-stressed plants show changes in chlorophyll content and
264 stomatal density in. In this study, the chlorophyll content was higher under drought
265 conditions; however, no significant differences were observed in the presence and
266 absence of S141 (Figure 3d). There was also no significant difference in stomatal density
267 under any conditions and combinations (Figure 3e).
268 Altogether, these findings indicated that the positive effects of S141 on soybean
269 root growth were not related to known chemical and biological changes in plants under
270 drought conditions.
271

272 **Discussion**

273 We investigated the effects of *B. velezensis* S141 on soybean growth under irrigated and
274 drought conditions. Under irrigated conditions, S141 exerted no effect when inoculated
275 alone or co-inoculated with USDA110. In a previous study, soybean growth in the field
276 was promoted by co-inoculation with USDA110 and S141 compared with that by

277 USDA110 alone (Sibponkrung *et al*. 2020), which was unclear under the present
278 laboratory conditions. Considering these results, the positive effects of S141 in the field
279 could involve additional outdoor factors that cannot be reproduced in the laboratory
280 chamber. However, under drought conditions, we found that inoculation of S141 alone
281 improved the root growth significantly, suggesting that S141 functions to elevate
282 tolerance to drought stress in soybean plants without the symbiotic nitrogen fixation by
283 USDA110. Remarkably, the dry weight of roots increased by approximately 1.5 times
284 more with S141 inoculation under drought conditions than with no-inoculation under
285 irrigated conditions (Figure 1c). Probably, S141 itself and its culture medium were not
286 utilized as nitrogen sources because the bacteria were small in number and washed
287 thoroughly before the inoculation. If S141 provides nitrogen sources, soybean root
288 growth under sole inoculum irrigation conditions should be much better; however, the
289 results are the opposite. Moreover, S141 does not fix nitrogen, and it is even more
290 inconceivable for it to supply water. Therefore, we hypothesized that S141 stimulated the
291 inherent stress tolerance of plants under the conditions of limited water without
292 nodulation. We then considered whether S141 might support soybean growth by
293 producing phytohormones and regulators to improve tolerance to drought conditions.
294 As S141 can produce some auxin (Sibponkrung *et al*. 2020), we conducted
295 experiments using a mutant strain, S141ΔIPyAD, which is defective in auxin production.
296 However, we observed no significant difference between the parental strain S141 and the
297 mutant strain S141ΔIPyAD under either irrigated or drought conditions. In other words,
298 the presence of S141ΔIPyAD under drought conditions also increased the root dry weight
299 more than that under no-inoculation and irrigated conditions. These findings indicated
300 that the auxin production of S141 was independent of the mechanism of improving the

301 tolerance of soybean under drought conditions.

302 Plants exhibit various physiological and molecular responses under drought
303 conditions to overcome osmotic and oxidative stresses (Ahmad *et al*. 2022b). Therefore,
304 we analyzed whether any of the known drought-stress responses could be modulated by
305 S141 inoculation. The levels of TSS accumulated as osmotic regulators under drought
306 conditions, with or without S141 inoculation, exceeded those under irrigated conditions.
307 This result confirmed that drought conditions promoted TSS accumulation in soybean,
308 which occurred independently of S141 inoculation. Proline and H_2O_2 levels also showed
309 a response to drought stress; however, these levels also did not differ significantly in the
310 presence and absence of S141 or auxin production capacity of S141. For unknown reasons,
311 we could not measure MDA accumulation, another indicator of oxidative stress tolerance,
312 because the values were too small to detect. The chlorophyll content and stomatal density
313 also showed some changes under drought conditions; however, the effects of S141
314 inoculation on these parameters were not obvious. The root growth promotion effect of
315 S141 is at least independent of the known abovementioned resistance factors.
316 Nevertheless, soybean could possess other stress resistance mechanisms, and their
317 relevance remains to be explored.

318 When nodulation is adequate, the soybean plant may adapt to the drought
319 conditions without S141, and in fact, we observed no effect of S141 when co-inoculated
320 with USAD110 for nodulation. S141 improved soybean root growth under drought
321 conditions only without the co-inoculation of USDA110 for nodulation. S141 might
322 produce some substances that will enhance the root growth of soybeans to survive the
323 extreme stress from drought without nodulation. Otherwise, S141 might eliminate some
324 root growth inhibitors in the soybean rhizosphere. To clarify the effects of S141, we

would plan to examine whether S141 also helps tolerate other environmental stresses (e.g., high salt and low temperature) together with the lack of nodulation. In particular, both high salt and drought stresses have in common that make water uptake difficult for plants. To counter the detrimental effects of high salt, some plants enjoy a bacterial partner that contains the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which degrades ACC (the precursor to ethylene in all higher plants) (Orozco-Mosqueda *et al*. 2020). ACC deaminase lowers ACC levels, prevents excessive increases in ethylene synthesis, and is one of the most efficient agents to confer plant salt stress tolerance. Therefore, it would be interesting to determine whether S141 has ACC deaminase activity.

It is true that plants benefit from nitrogen fixation by nodulation. However, nodulation and nitrogen source supply are not synonymous. At the least, we need to count the burden of organogenesis to form nodules. In other words, nodulation causes various physiological changes in the plant that are more complex than a simple supply of nitrogen sources. At this moment, we do not distinguish whether the root growth-promoting effect of S141 under drought conditions is directly due to nitrogen source limitation or to the lack of nodulation. It would be necessary to redo all the experiments conducted in this study by adding a nitrogen source. However, nitrogen sources are known to suppress nodulation, which complicates the original PGPR effect of S141 to promote nodulation. Further studies are required to understand how S141 could promote the root growth of soybeans under drought conditions.

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Author's Contribution

K. Y. and N. T. share the correspondence of this publication. T. K. and K. Y. conceived and directed the study and prepared the manuscript. T. K. conducted the experiments in collaboration with S. S. and P. T. under the supervision of N. T. and N. B. S. I. reviewed the experiments and data. No AI was involved in this study and the manuscript preparation.

Conflict of Interest

The authors declare no competing interests.

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Data Availability

Experimental materials and data related to this study can be disclosed upon proper request.

Figure legends

Figure 1. Dry weights of shoot (a and b), root (c and d), and total plant (e and f) of soybean grown under irrigated (wet) and drought (dry) conditions with *B. velezensis* S141, *B. diazoefficiens* USDA110, or both (141+110) and without both (no-inoc). Values are mean ± SE of octuplicate experiments statistically analyzed using Tukey's test. Significant differences ($p < 0.05$) are represented with respective letters.

Figure 2. Dry weights of shoot (a), root (b), and total plant (c) of soybean grown under irrigated (wet) and drought (dry) conditions with *B. velezensis* S141 or S141ΔIPyAD (IPyAD) and without both (no-inoc). Values are mean ± SE of octuplicate experiments statistically analyzed using Tukey's test. Significant differences ($p < 0.05$) are represented with respective letters.

Figure 3. Biochemical and morphological parameters of soybean plants grown under irrigated (wet) and drought (dry) conditions with *B. velezensis* S141 or S141ΔIPyAD (IPyAD) and without both (no-inoc), including TSS content (a), proline content (b), H_2O_2 content (c), total chlorophyll content (d), and average stomatal density (e). Values are mean ± SE of octuplicate experiments statistically analyzed using Tukey's test. Significant differences ($p < 0.05$) are represented with respective letters.

513 **Caption to Graphical Abstract**

514 The dry weight of soybean roots significantly increased under drought conditions with
515 *Bacillus berezensis* S141, which promotes soybean root growth under limited water
516 supply conditions.

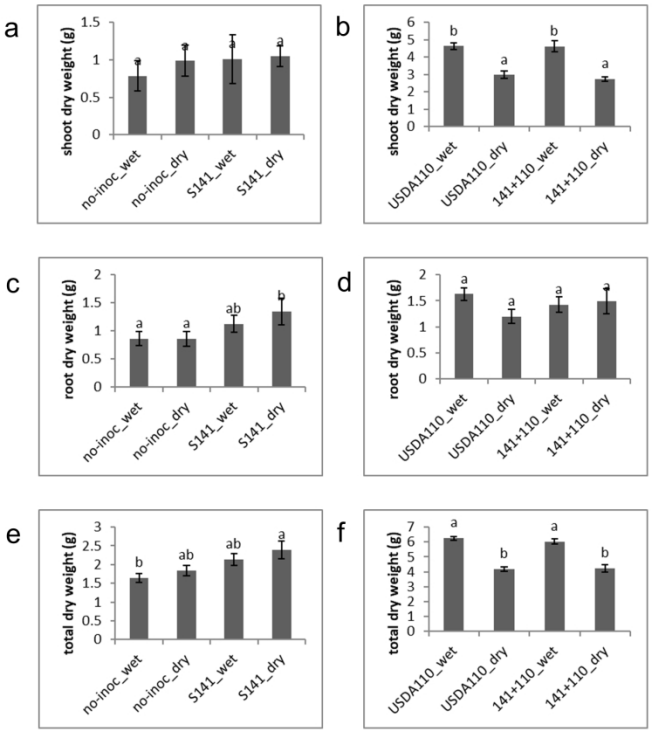

Fig. 1. Kondo *et al.*

Figure 1. Dry weights of shoot (a and b), root (c and d), and total plant (e and f) of soybean grown under irrigated (wet) and drought (dry) conditions with B. velezensis S141, B. diazoefficiens USDA110, or both (141+110) and without both (no-inoc). Values are mean ± SE of octuplicate experiments statistically analyzed using Tukey's test. Significant differences ($p < 0.05$) are represented with respective letters.

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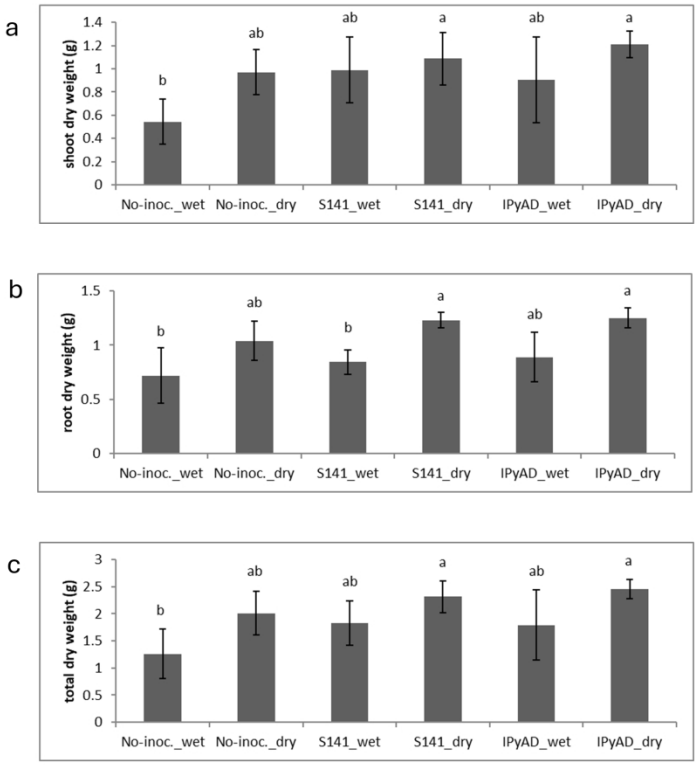

Fig. 2. Kondo *et al.*

Figure 2. Dry weights of shoot (a), root (b), and total plant (c) of soybean grown under irrigated (wet) and drought (dry) conditions with B. velezensis S141 or S141ΔIPyAD (IPyAD) and without both (no-inoc). Values are mean ± SE of octuplicate experiments statistically analyzed using Tukey's test. Significant differences ($p < 0.05$) are represented with respective letters.

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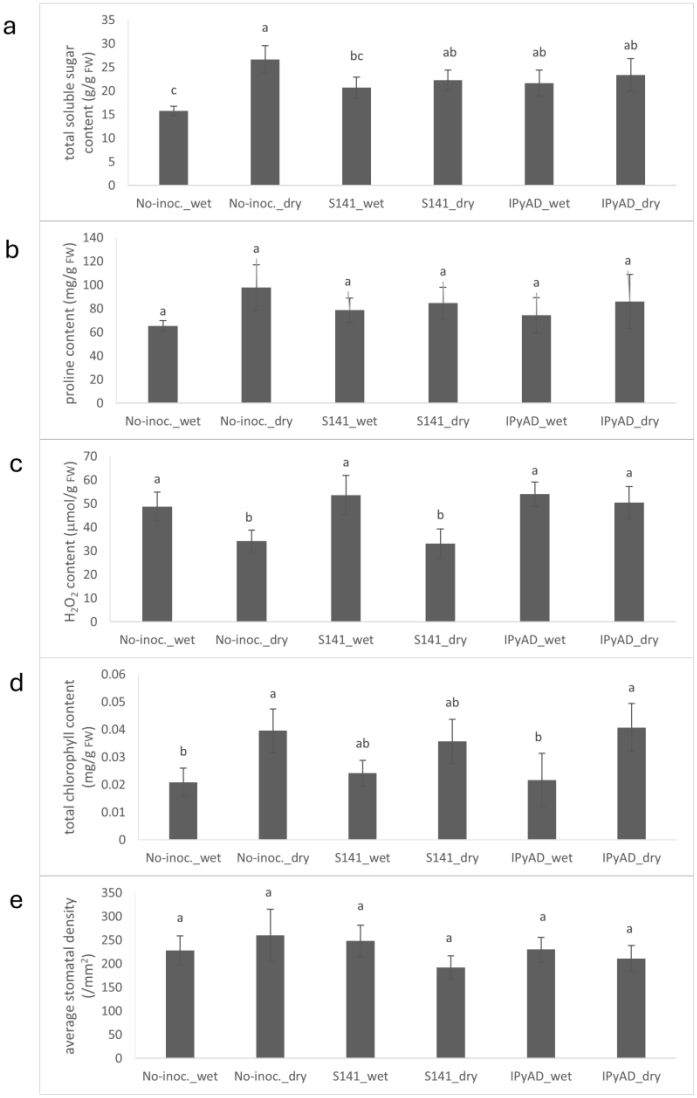

Fig. 3. Kondo *et al.*

Figure 3. Biochemical and morphological parameters of soybean plants grown under irrigated (wet) and drought (dry) conditions with B. velezensis S141 or S141ΔIPyAD (IPyAD) and without both (no-inoc), including TSS content (a), proline content (b), H2O2 content (c), total chlorophyll content (d), and average stomatal density (e). Values are mean ± SE of octuplicate experiments statistically analyzed using Tukey's test. Significant differences ($p < 0.05$) are represented with respective letters.

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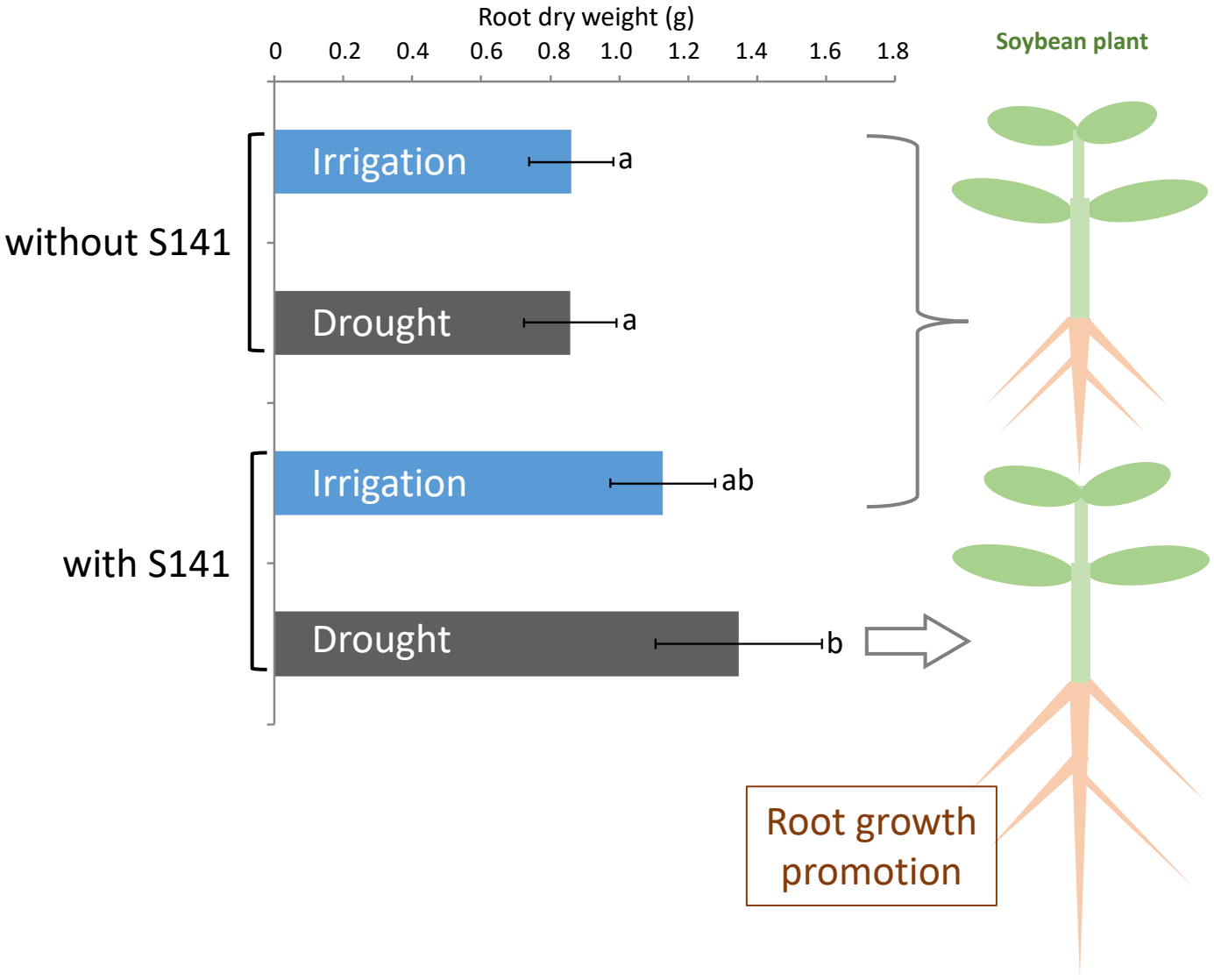

Graphical abstract