Are drought-resistance promoting bacteria cross-compatible with different plant models?

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Marasco R, Rolli E, Vigani G, Borin S, Sorlini C, Ouzari H, Zocchi G, Daffonchio D. Are drought-resistance promoting bacteria cross-compatible with different plant models?. Plant Signalling & behaviour volume 8: fascicolo 10, anno 2013, e26741. Doi: 10.4164/psb.26741.

Abstract
The association between plant and plant growth promoting bacteria (PGPB) contributes to the successful thriving of plants in extreme environments featured by water shortage. We have recently shown that, with respect to the non-cultivated desert soil, the rhizosphere of pepper plants cultivated under desert farming hosts PGPB communities that are endowed with a large portfolio of PGP traits. Pepper plants exposed to bacterial isolates from plants cultivated under desert farming exhibited a higher tolerance to water shortage, compared with untreated control. This promotion was mediated by a larger root system (up to 40%), stimulated by the bacteria, that enhanced plant ability to uptake water from dry soil. We provide initial evidence that the nature of the interaction can have a limited level of specificity and that PGPB isolates may determine resistance to water stress in plants others than the one of the original isolation. It is apparent that, in relation to plant resistance to water stress, a feature of primary evolutionary importance for all plants, a cross-compatibility between PGPB and different plant models exists at least on a short-term.
Keywords: : arid ecosystem, drought tolerance, endosphere, plant growth promoting bacteria, plant-bacteria cross-compatibility, rhizosphere, water stress

Drought is a threat to crop productivity worldwide1

Grayson M. Agriculture and drought. Nature 2013; 501:S1; http://dx.doi.org/10.1038/501S1a; PMID: 24067757
[Crossref], [PubMed], [Google Scholar]
and is not restricted to desert regions, since global warming is determining dramatic losses in agricultural yield even at temperate latitudes.2 During water deprivation, plants rapidly modulate a series of physiological responses in order to economize the water use, such as 1) the stimulation of stomata closure through an increase of ABA content, 2) the accumulation of compatible solutes, and 3) the increased expression of aquaporins and vacuolar H+-pyrophosphatases for maintaining cell turgor through osmotic adjustments.3

Bartels D, Sunkat R. Drought and salt tolerance in plants. Crc Cr Rev Plant Sci 2005; 24:23 - 58; http://dx.doi.org/10.1080/07352680590910410
[Taylor & Francis Online], [Web of Science ®], [Google Scholar]
When drought persists, long-term adjustments of plant physiology are adopted. Ethylene levels reach concentrations that block plant growth, increasing root to shoot ratio, and in the meanwhile, a larger root system is stimulated to increase water adsorption. Despite these adaptation processes, accumulation of ROS can occur, dramatically compromising cell integrity and functionality, hampering the overall survival of the plant. The role of plant-associated microbiomes in plant adaptation to drought is emerging.4

East R. Microbiome: Soil science comes to life. Nature 2013; 501:S18 - 9; http://dx.doi.org/10.1038/501S18a; PMID: 24067761
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
Plants benefit of mutualistic association with soil microorganisms that colonize the rhizosphere and the endosphere. Mycorrhizal fungi and Plant Growth Promoting Bacteria (PGPB) are capable of modulating the physiological response to water deprivation, increasing plant survival under adverse environmental conditions.5

Redman RS, Kim YO, Woodward CJDA, Greer C, Espino L, et al. Increased Fitness of Rice Plants

to Abiotic Stress Via Habitat Adapted Symbiosis: A Strategy for Mitigating Impacts of Climate Change. PLoS ONE 2011; 6:e14823; http://dx.doi.org/10.1371/journal.pone.0014823; PMID: 21750695
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
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Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, et al. A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS ONE 2012; 7:e48479; http://dx.doi.org/10.1371/journal.pone.0048479; PMID: 23119032
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
Rapid changes in the structure of the plant-associated bacterial communities toward the selection of assemblages metabolically and physiologically adapted to drought,7

Berard A, Ben Sassi M, Renault P, Gros R. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. J Soils Sediments 2012; 12:513 - 8; http://dx.doi.org/10.1007/s11368-012-0469-1
[Crossref], [Web of Science ®], [Google Scholar]
determine the provision of differential services to plant, improving its resistance to water stress.8 We showed that the rhizosphere and endosphere of pepper plants, cultivated in a traditional desert farm in Egypt, host bacterial microbiomes whose structure is different from uncultivated soil, indicating an enrichment of specific bacterial taxa exerted by the plant.6

Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, et al. A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS ONE 2012; 7:e48479; http://dx.doi.org/10.1371/journal.pone.0048479; PMID: 23119032
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
Rhizobacteria and endophytes exhibited a large array of PGP properties, potentially acting in synergy to support plant growth under water stress. Greenhouse experiments under drought-controlled conditions confirmed the potential of these bacteria in improving plant growth during water stress. Although affected by drought, several physiological parameters, related to photosynthesis efficiency, recorded higher values in pepper plants exposed to bacteria than in those untreated. The root biomass and length of plants exposed to bacteria plants increased up to 40% suggesting that the formation of a larger root system stimulated by the association with the PGPB is responsible for a higher efficiency in the water uptake from the dry soil. These findings underline that desert farming provides critical ecosystem services in arid lands where the plant rhizosphere represents a reservoir of microbial communities endowed with multiple PGP traits that sustain plant growth during drought. To test if such promotion effect exerted by PGPB can go beyond a laboratory controlled setting, we present preliminary results about bacterial strain inoculation on pepper plants cultivated in field trials (Fig. 1A). Plants exposed to *Klebsiella oxytoca* (R5ACCd) and *Citrobacter freundii* (R16ACCd) isolates6

Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, et al. A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS ONE 2012; 7:e48479; http://dx.doi.org/10.1371/journal.pone.0048479; PMID: 23119032
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
established larger root system than uninoculated controls (Fig. 1A), supporting the idea that under water shortage, plants exposed to bacteria have enhanced chances to survive. Here we provide initial evidences to one of the intriguing questions that is rising on the role of PGPB in plant resistance to water stress. Is bacteria-mediated drought resistance a widespread feature among

different bacterial classes and different types of plants? In our experiments, we observed that it is a beneficial trait shared by different bacteria associated to different plants like olive tree and grapevine. Two isolates, a Gram-positive *Rhodococcus* and a Gram-negative *Pseudomonas* (respectively strains B-O and B-G), isolated from the roots of olive tree and of *Vitis vinifera* exhibited the ability to enhance, respectively, tomato root biomass and the whole biomass of Barbera cultivar (*Vitis vinifera*) grafted on *Vitis ripariae* x *Vitis berlandieri* SO4 rootstock under water limited conditions (Fig. 1C-D). While the photosynthesis of uninoculated control tomato plants under water stress decreased by about 80% (38.95 ± 0.35 nmol O2/ml min) compared with untreated plants (197.33 ± 4.81 nmol O2/ml min), the treatment with strain B-O limited the photosynthesis decrease to 58% (82.45 ± 24.99 nmol O2/ml min). These findings indicate that PGPB are able to stably interact with different plant systems and support their growth under water stress. Recently, we performed a large survey of bacteria associated to the grapevine root system in 3 sites located along an aridity gradient from Italy, Tunisia, and Egypt. Despite differences in the bacterial community structure, a homeostatic bacterial PGP potential, in terms of number and types of PGP traits, was observed in the 3 sites along the transect.9

Marasco R, Rolli E, Fusi M, Cherif A, Abou-Hadid A, El-Bahairy U, Borin S, Sorlini C, Daffonchio D. Plant growth promotion potential is equally represented in diverse grapevine root-associated bacterial communities from different biopedoclimatic environments. Biomed Res Int 2013; 2013:ID 491091
[Google Scholar]

Thus, independently from the structure of the associated microbiome or the geographical location, the same plant species (here grapevine) selects for different bacterial communities still able to sustain plant growth and development under different degrees of water stress, and whose composition is driven by the specific environmental setting.9 However, when environmental conditions are more selective the microbiome variability may be simplified, possibly due to a relatively limited span of taxonomic diversity within such a selective condition. For instance, in hypersaline ecosystems, where salinization is a major constraint, native halophytes such as *Salicornia* sp. retain a rhizospheric bacterial community, homogenous in taxa composition, that still show abiotic stress tolerance and a large portfolio of PGP abilities.10 Several mechanisms of drought resistance in plants have been proposed to be induced by PGPB, through the elicitation of the so-called induced systemic tolerance (IST)11

Yang J, Kloepper JW, Ryu CM. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 2009; 14:1 - 4; http://dx.doi.org/10.1016/j.tplants.2008.10.004; PMID: 19056309
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]

(Fig. 2). It has been shown that the modulation of ethylene concentration by PGPB rescues normal plant growth under stress conditions. The degradation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by bacterial ACC-deaminase activity promoted in drying soil shoot and root growth, indicating that the bacterial effects was directly mediated by ethylene signaling.12

Belimov AA, Dodd IC, Hontzeas N, Theobald JC, Safronova VI, Davies WJ. Rhizosphere bacteria containing ACC deaminase increase yield of plants grown in drying soil via both local and systemic hormone signaling. New Phytol 2009; 181:413 - 23; http://dx.doi.org/10.1111/j.1469-8137.2008.02657.x; PMID: 19121036
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]

Furthermore, PGPB can produce hormone-like substances (e.g., auxin) that affect plant root development. The increment of root length, root surface area, and the number of root tips, leading to enhanced water and nutrient uptake, represents a key factor to improve plant survival chances when water represents the limiting growth factor.13

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang YP, Guo JH. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS ONE 2012; 7:e52565; http://dx.doi.org/10.1371/journal.pone.0052565; PMID: 23285089
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
Meanwhile, to cope with ROS deleterious effects, enzymatic and non-enzymatic antioxidant responses are stimulated by PGPB to scavenge these toxic compounds.13

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang YP, Guo JH. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS ONE 2012; 7:e52565; http://dx.doi.org/10.1371/journal.pone.0052565; PMID: 23285089
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
In the rhizosphere, where limited water content cause salt accumulation, osmotic stress, and soil hardness, PGPB produce extracellular matrices favoring soil and water stability, increasing root adhering soil, and positively influencing water potential around the root system.14

Sandhya V, Ali SKZ, Minakshi G, Reddy G, Venkateswarlu B. Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biol Fertil Soils 2009; 46:17 - 26; http://dx.doi.org/10.1007/s00374-009-0401-z
[Crossref], [Web of Science ®], [Google Scholar]
Even volatile organic compounds (VOCs) released by PGPB play a role in IST response.15

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu C-M, Kim YC. 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana.*. Mol Plant Microbe Interact 2008; 21:1067 - 75; http://dx.doi.org/10.1094/MPMI-21-8-1067; PMID: 18616403
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
VOCs released by *Bacillus subtilis* GB03 stimulated *Arabidopsis* biosynthesis of choline, a compatible solute involved in preventing water loss and maintaining cell turgor.16

Zhang H, Murzello C, Sun Y, Kim MS, Xie X, Jeter RM, Zak JC, Dowd SE, Paré PW. Choline and osmotic-stress tolerance induced in *Arabidopsis* by the soil microbe *Bacillus subtilis* (GB03). Mol Plant Microbe Interact 2010; 23:1097 - 104; http://dx.doi.org/10.1094/MPMI-23-8-1097; PMID: 20615119
[Crossref], [PubMed], [Web of Science ®], [Google Scholar]
Moreover, stomata closure was induced in *Pseudomonas chlororaphis* O6-colonized plants by 2R,3R-butanediol, a volatile metabolite able to interfere with several hormone signaling pathways including salicylic acid, ethylene, abscisic acid, and jasmonic acid.15 Our findings suggest that in relation to plant resistance to water stress, a feature of primary evolutionary importance for all plants, a cross-compatibility between PGPB and different plant models exists at least on a short term. It remains to be determined the long-term stability of such a cross-compatibility that, from an applied perspective, represents a crucial knowledge to develop bioprotective strains against drought, widely usable with different plants and cultivars.
Figure 1. PGP strains have a cross-efficacy in promoting drought resistance in different plant models. (**A**) Representative images of the promotion effect on the root system of pepper plants inoculated with R05ACCd and R16ACCd strains6

Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, et al. A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS ONE 2012; 7:e48479; http://dx.doi.org/10.1371/journal.pone.0048479; PMID: 23119032

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and field-grown for 2 months under irrigation-limited conditions, compared with the uninoculated control (labeled with "no bacteria added"). Bar = 10 cm. (**B**) Pepper root fresh weights determined on the plants of the experiment in (**A**). Values are means of 4 plants ± standard deviations. The *P* values according to the student *t*-test are indicated to evaluate the differences between the plants treated with bacteria and the non-treated controls. (**C**) B-O strain, isolated from the rhizosphere of an olive tree growing in South Tunisia, was used to inoculate tomato plants (3 plants for each treatment). After re-watering, following a 10-d induced drought by withholding irrigation, tomato plants treated with B-O strain exhibited an increase of root fresh biomass compared to untreated control. (+): uninoculated plants, properly supplied with water during the experiment; (-): uninoculated control plants subjected to drought; (B-O): plants treated with B-O isolate and subjected to water stress. (**D**) B-G strain, isolated from the root endosphere of Barbera plants, was used to inoculate SO4-grafted grapevine plants (5 plantlets for each treatment). After re-watering, following a drought event induced by reducing water irrigation to 50% of water holding capacity, grapevine plants treated with B-G strain exhibited an increase of grapevine fresh biomass compared with untreated control. (+): uninoculated plants, properly supplied with water during the experiment; (-): uninoculated control plants subjected to drought; (B-G): plants treated with B-G isolate and subjected to water stress. *: $P \leq 0.05$ according to the student *t*-test.

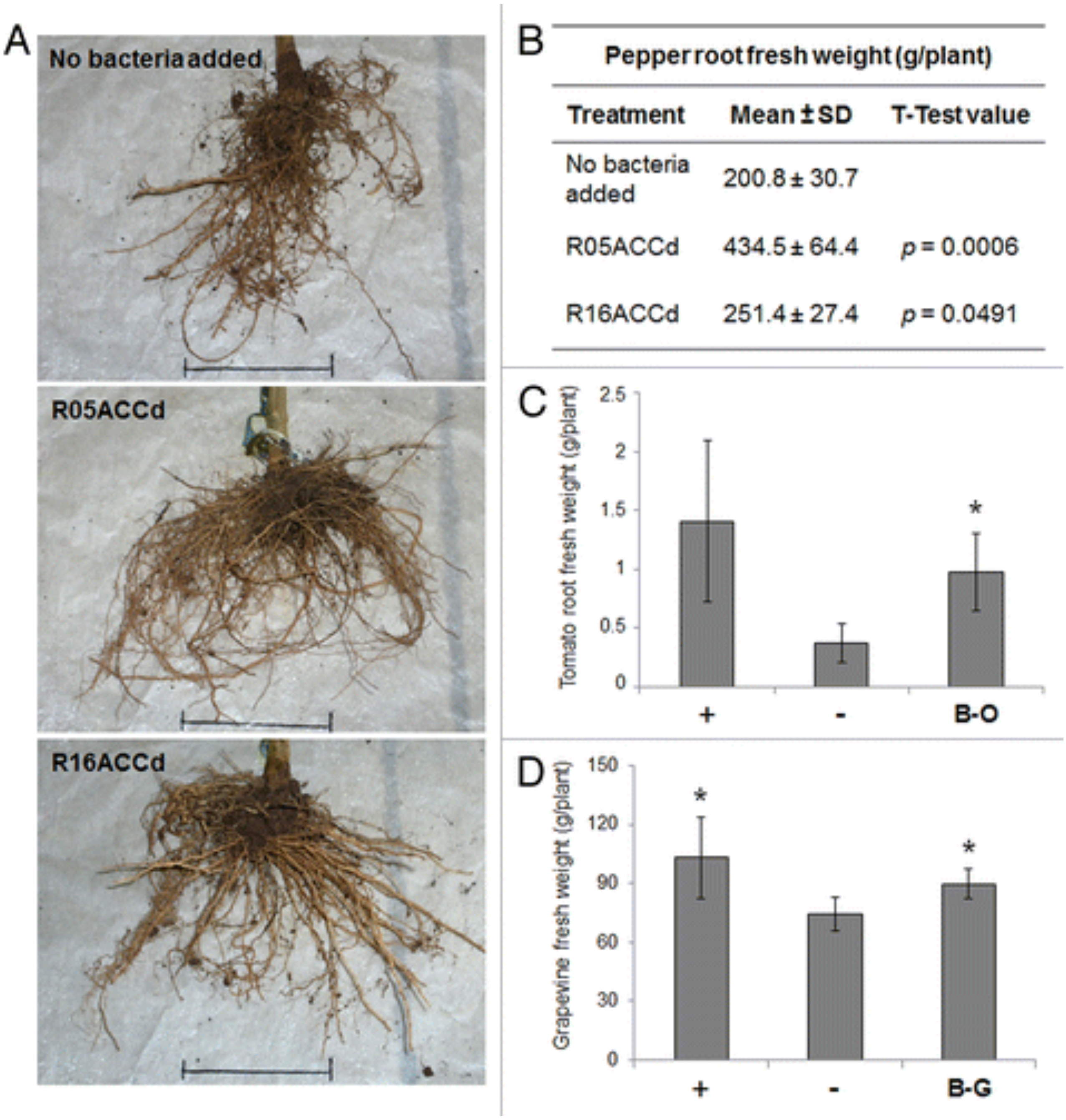
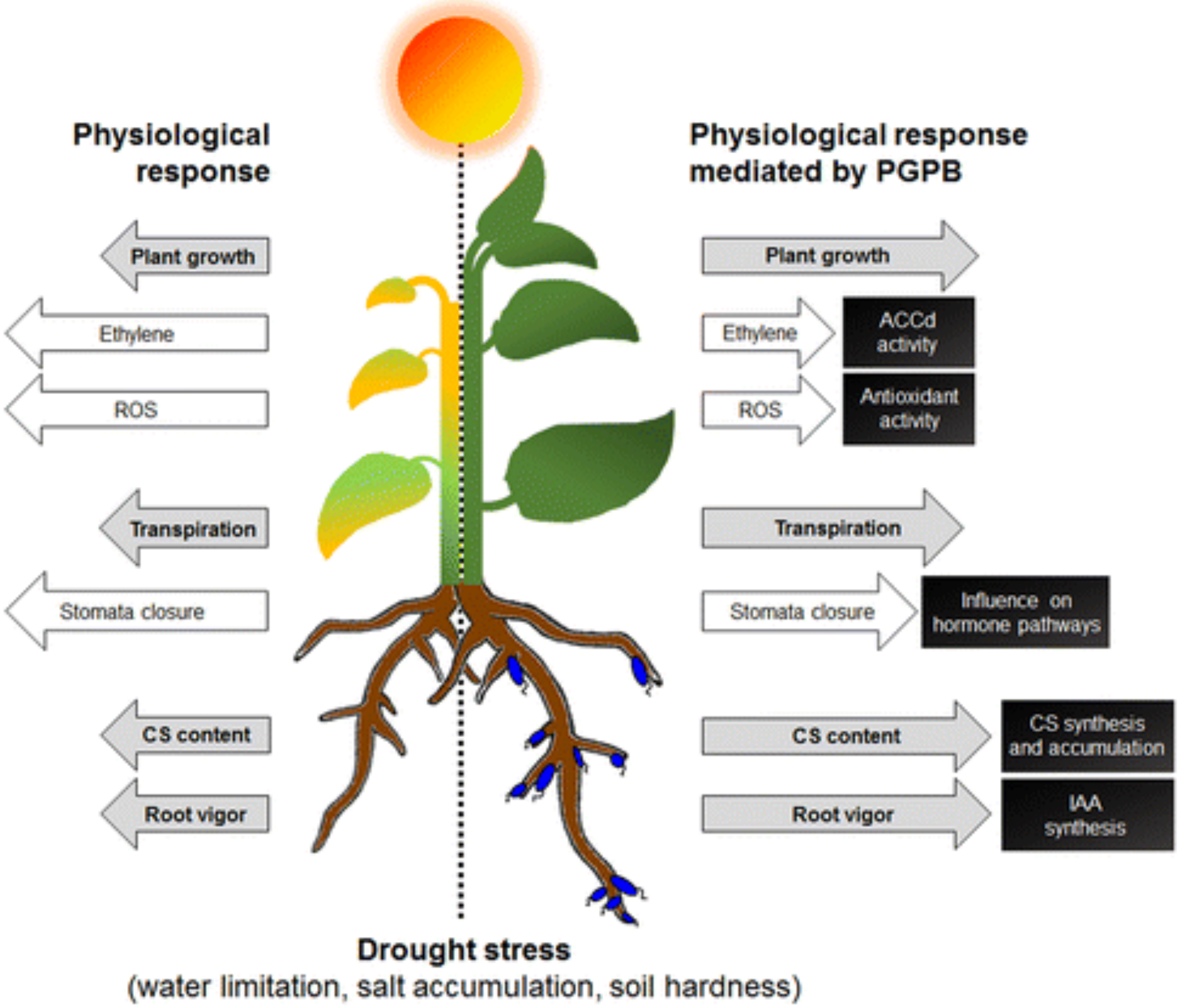


Figure 2. PGPB associated to plants stimulate growth during drought. Physiological plant processes (gray arrows) and mechanisms (white arrows) change according to stimulations determined by the associated PGPB (in blue in the plant drawing) in response to drought. On the left and the right sides of the plant drawing are reported the intensities of processes and mechanisms under water stress, in the absence or presence of the PGPB. For each process and mechanism represented in the same line, the lengths of the arrows indicate reduced/increased physiological responses. The black boxes indicate the specific promoting activities exerted by the PGPB in relation to the specific processes or mechanisms.



Abbreviations:	
ACCd	1-aminocyclopropane-1-carboxylic acid deaminase
CS	Compatible Solutes
IAA	auxins
ROS	Reactive Oxygen Species

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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