Lawrence Berkeley National Laboratory
LBL Publications

Title

Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance

Permalink

https://escholarship.org/uc/item/8px8z3td

Authors

Tringe, Susannah

Coleman-Derr, Devin

Publication Date

2014-06-23

Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance

Authors: Devin Coleman-Derr[1] and Susannah Tringe[1*]

1 Department of Energy Joint Genome Institute // LBNL - Walnut Creek, CA

* To whom correspondence may be addressed. Susannah G. Tringe, Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, 94598, USA. sgtringe@lbl.gov

May 22, 2014

Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance

Devin Coleman-Derr[1], Susannah G. Tringe*[1]

[1] Joint Genome Institute, Walnut Creek, CA, USA

* Correspondence: Susannah G. Tringe, Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, 94598, USA. sgtringe@lbl.gov

Keywords: symbiosis, abiotic stress, agriculture, plant growth promotion, plant-microbe interactions, drought.

Abstract

The exponential growth in world population is feeding a steadily increasing global need for arable farmland, a resource that is already in high demand. This trend has led to increased farming on subprime arid and semi-arid lands, where limited availability of water and a host of environmental stresses often severely reduce crop productivity. The conventional approach to mitigating the abiotic stresses associated with arid climes is to breed for stress-tolerant cultivars, a time and labor intensive venture that often neglects the complex ecological context of the soil environment in which the crop is grown. In recent years, studies have attempted to identify microbial symbionts capable of conferring the same stress-tolerance to their plant hosts, and new developments in genomic technologies have greatly facilitated such research. Here, we highlight many of the advantages of these symbiont-based approaches and argue in favor of the broader recognition of crop species as ecological niches for a diverse community of microorganisms that function in concert with their plant hosts and each other to thrive under fluctuating environmental conditions.

Introduction

Climate change and an increasing world population are predicted to drastically increase the global need for arable farmland, a resource that is already in high demand (Barrow et al., 2008). With the world population expected to reach 9 billion by 2050, it is estimated that the global food supply will need to increase by 70% to meet rapidly rising demand (Editorial, 2010). Changes in the global climate may well compound this challenge, as predicted increases in drought and temperature-related stresses are expected to reduce crop productivity (Grover et al., 2010; Ciais et al., 2005; Larson, 2013).

This large expansion in agricultural output will require both improvements in crop yield as well as the cultivation of additional farmland. One direct effect of this trend will be the steadily increasing prevalence of farming on marginal, arid and semi-arid lands, especially in the developing world (Köberl et al., 2011; Lantican et al., 2003). Even without considering the effects of climate change, semi-arid and arid lands often present a host of abiotic challenges to plant growth, including extreme temperatures, excess radiation, and poor nutrient and water availability (Yang et al., 2009).

The historical approach to mitigate the negative effects of abiotic stresses on crop yield has been the creation of stress-tolerant cultivars (Barrow et al., 2008; Eisenstein, 2013). Conventional breeding techniques have enabled the development of crop varietals with increased yields and greater tolerance to a variety of abiotic stresses (Atkinson and Urwin, 2012), but are both time and labor intensive; genetic engineering of crops with improved stress tolerance is faster, but comes with its own set of drawbacks. Furthermore, both methods often neglect the complex ecological context of the soil environment in which the crop is grown (Morrissey et al., 2004).

In recent years, plant-associated microbial communities have received considerable attention for their ability to confer many of the same benefits to crop productivity and stress resistance as have been achieved through plant breeding programs (Barrow et al., 2008; Tank and Saraf, 2010; Marulanda et al., 2009; Marasco et al., 2012; Mayak et al., 2004). It is now well recognized that all plants, and nearly all tissues within the plant, are inhabited by a variety of microorganisms (Berg et al., 2013; Partida-Martínez and Heil, 2011), many of which offer benefits to the host, improving nutrient uptake, preventing pathogen attack, and increasing plant growth under adverse environmental conditions (Yang et al., 2009; Turner et al., 2013). In return these microorganisms receive shelter from the surrounding environment and access to a carbon-rich food supply. The most well-studied of these symbionts include the mycorrhizal fungi, which enhance nutrients uptake (Bonfante and Anca, 2009) and root-nodulating bacteria, which fix nitrogen from the surrounding soil (Lugtenberg and Kamilova, 2009), but many other novel plant growth promoting microorganisms (PGPM) continue to be identified each year. These organisms confer stress resistance via diverse mechanisms recently reviewed elsewhere (Yang et al., 2009; Zelicourt et al., 2013; Lugtenberg and Kamilova, 2009; Nadeem et al., 2014; Grover et al., 2010). Importantly, efforts are being made to harness these naturally-occurring, soil-derived beneficial microbes for large-scale improvement of crop performance in agriculture (Nadeem et al., 2014).

In this article, we will highlight some of the advantages associated with symbiont-based approaches to increasing crop resistance to abiotic stress, with a focus on engineering increased tolerance to drought, which is the most critical and prevalent factor for crop production in many parts of the world (Grayson, 2013; Castiglioni et al., 2008). We present suggestions for future directions of abiotic stress tolerance improvement in crop plants, including the use of cutting edge genomic technologies for the identification and selection of candidate symbionts and the functional modules they employ for enhancing host growth, as well as an assessment of current agronomic practices in the light of modern understanding of microbial community influence over plant phenotype. We conclude with an argument in favor of increased collaboration between conventional breeding programs and microbial-based research for crop improvement and, more generally, for a broader conceptual understanding of crop productivity as a complex product of plant genetics and microbial community function.

Limitations associated with direct engineering of increased stress tolerance into crop plants

The success of plant biotechnology programs has helped the world's food supply keep pace with the increasing rate of population growth (Morrissey et al., 2004). Novel crop varietals, with superior yields as well as increased tolerance to biotic and abiotic stresses, have been continuously produced for decades through conventional plant breeding programs, and more recently through genetic engineering (Atkinson and Urwin, 2012). Despite the undeniable success of these past efforts and their continued applicability to drought-tolerance in crop species, each of these methods has its drawbacks, which should be fully considered. Plant breeding is highly time consuming, as well as labor and cost intensive (Ashraf, 2010; Eisenstein, 2013). Additionally, in the quest for the

improvement of a particular trait, such as drought tolerance, certain (often unknown) desirable traits can be unintentionally lost from the host's gene pool during conventional breeding (Philippot et al., 2013). Perhaps the largest drawback, however, is that plant breeding only confers benefit to a single host species, and this benefit is often not easily transferable to other crop systems, as the genetic components responsible for the improvements frequently remain unidentified.

To avoid the time and labor costs associated with conventional breeding, some researchers have turned to generation of transgenic lines for producing varietals with improved plant growth regulators, antioxidants, organic osmolytes or other factors capable of increasing drought tolerance (Eisenstein, 2013). Unfortunately, the vast majority of these are developed and tested in the greenhouse, rather than in the field and claims made regarding their performance are often inflated compared to actual results in agricultural settings, due to the large array of abiotic and biotic factors left out of the initial experiments (Ashraf, 2010). Additionally, these transgenic crops often must pass rigorous food and environmental safety regulations and trials before becoming marketable, which adds additional time to the product development process (Eisenstein, 2013). Furthermore, release of a transgenic product into the marketplace does not guarantee its success, as public response to use of genetically-modified crops varies considerably from country to country (Fedoroff et al., 2010).

Both the conventional breeding and genetic engineering based approaches may rely too heavily on the assumption that plants function as autonomous organisms regulated solely by their genetic code and cellular physiology (Barrow et al., 2008), although plant-microbe interactions can heavily influence crop response to environmental conditions. Many field trials of new stress-tolerant cultivars simply have not addressed microbial influence on improved performance (Budak et al., 2013; Cooper et al., 2014; Swamy and Kumar, 2013). Greenhouse trials are often conducted with standard sterilized potting soils and sterilized soil amendments (Witt et al., 2012; Porch, 2006; Waterer et al., 2010) in an attempt to create a microbe-free growth environment, an artificial context rarely if ever found in nature (Friesen et al., 2011; Partida-Martínez and Heil, 2011). By doing so, they not only neglect one of the top determinants of phenotypic output, they may also miss vertically transmitted symbionts present within the plant seed (Barrow et al., 2008), which could lead to overestimations of the effect of host genotype on plant phenotype.

Advantages of symbiont-based approaches to improving stress tolerance

Compared with methods for directly engineering stress tolerance into the host described above, symbiont-based approaches to improving stress tolerance offer some clear advantages. First, microbial symbionts are frequently capable of conferring stress tolerance to a wide variety of diverse plant hosts, and many PGPM can confer benefits to both monocots and dicot crop species (Zhang et al., 2008; Redman et al., 2002; Timmusk and Wagner, 1999). The bacterium *Achromobacter piechaudii*, isolated from dry riverbeds of southern Israel, was capable of increasing salt and drought resistance in both pepper and tomato (Mayak et al., 2004). Using olive trees, tomato, grapevine and pepper plants, Marasco et al. have demonstrated that microbes isolated from the roots of one host species cultivated under desert farming conditions are capable of improving the growth of a different host species when grown under a water-stress regime (Marasco et al., 2013). The ability to transfer stress-resistance solutions from one crop species to another through a microbial inoculum has the potential to save years of plant breeding effort.

Secondly, PGPM frequently confer more than one type of abiotic and/or biotic stress tolerance (Mayak et al., 2004; Rodriguez et al., 2008), and crops grown on arid and semi-arid lands typically suffer from multiple stress factors. It has been shown that *Arabidopsis* plants in symbiosis

136 with *Paenibacillus polymyxa* have increased drought tolerance as well as improved resistance to
137 pathogen attack (Timmusk and Wagner, 1999). Waller et al. demonstrated that barley plants
138 inoculated with the fungus *Piriformospora indica* have both increased resistance to *Fusarium* and
139 *Blumeria* infections and increased salt tolerance (Waller et al., 2005). These examples of microbes
140 conferring multiple benefits are likely due to the fact that many symbionts exert their influence over
141 the plant host through manipulating plant hormone pathways (Friesen et al., 2011; Glick et al., 2007)
142 and that considerable cross-talk exists between plant stress response pathways (Atkinson and Urwin,
143 2012).

144   Thirdly, plant-associated microbial species represent a vast reservoir of genetic information
145 that has coevolved with their hosts under natural environmental conditions. These microbes can add
146 genetic flexibility to the adaptation of comparatively sessile and longer-lived plants (Barrow et al.,
147 2008). The concept of 'habitat-specific symbioses', put forth by Rodriguez et al., is one of the most
148 intriguing discoveries pertaining to microbial contributions to stress tolerance made in recent years
149 (Rodriguez et al., 2008). Their research found that salt, drought, and disease resistance were each
150 individually conferred by specific fungal symbionts that had been harvested from coastal, arid, and
151 agricultural environments, respectively. Furthermore, they found that these beneficial effects could
152 be conferred on different plant host species, including both monocots and dicots. These insights
153 suggest that the foundation for the growth-promoting effects of microbial symbionts is based on the
154 co-evolution of the association between plant and microbe under adverse environmental conditions
155 (Rodriguez et al., 2008). For the purposes of developing novel biotechnological agents for use in
156 agriculture, this study supports the idea that the optimal place to look for PGPM that confer
157 resistance to a specific environmental stress is in soils where that stress is a regular phenomenon.

158 **Future directions of abiotic stress tolerance improvement in crop plants**

159 Microbial species with plant-growth promoting capabilities are both numerous and easier to
160 characterize now than ever before. A considerable fraction of endophytes isolated from crops appear
161 to have measurable effects on host fitness (Friesen et al., 2011). Two recent studies found that more
162 than 25% of bacteria isolated from cultivated crops had plant growth promoting activities (Hassan et
163 al., 2010; Marasco et al., 2012). While the identification of microbial endophytes has been
164 challenging in the past due to the frequent lack of plant-host symptoms, localized colonization,
165 intimate integration with plant cellular structures, and lack of cultivability, recent advances in
166 genomic technologies have helped make this process faster and cheaper (Berg et al., 2013). A recent
167 technique for selective depletion of chloroplast and mitochondrial-derived 16S amplicons allows for
168 vastly increased resolution of bacterial endophyte populations derived from within plant tissues
169 (Lundberg et al., 2013). While in the past whole-genome sequencing of candidate symbionts was
170 only possible for cultivable species, it is now possible to obtain draft genomes of microbial
171 endophytes in a high-throughput fashion using single-cell sorting coupled with next-generation
172 sequencing technologies (Woyke et al., 2006). Understanding the genomic content of these PGPMs
173 will enable us to better understand the mechanisms behind the conferred stress-tolerances, as well as
174 cultivate them for experimental investigation (Pope et al., 2011).

175 As more and more genomes from plant-growth promoting microorganisms become available, our
176 ability to identify the shared genetic components or metabolites that are responsible for conferring
177 specific abiotic stress advantages increases. Through a transcriptomic analysis of the symbiosis
178 between oilseed rape and *Stenotrophomonas rhizophila*, a recent study identified spermidine as a
179 novel PGPM regulator of plant abiotic stress (Alavi et al., 2013). Identification of the genetic
180 components within PGPMs that are responsible for alleviating abiotic stress may in some cases yield
181 potential targets for transgenic modification of the host organism (Nadeem et al., 2014). Recently,

182 bacterial cold-shock proteins transformed into various plant species led to increased tolerance to a
183 variety of abiotic stresses, including cold, heat and drought (Castiglioni et al., 2008).

184   Investigation of the mechanisms by which plant-growth promoting microorganisms confer
185 stress-tolerance to their plant hosts is another avenue for identifying targets for direct transgenic
186 manipulation of stress response in crops. Recent technological advances in cell-type specific
187 transcriptomics (Taylor-Teeples et al., 2011), combined with an experimental system designed to
188 examine host transcription during symbiosis with PGPM, could allow for a precise dissection of the
189 genetic signaling mechanisms responsible for increased stress tolerance. An improved understanding
190 of these host mechanisms could provide potential candidate loci for transgenic or plant-breeding
191 strategies aimed at plant-host improvement (Grover et al., 2010). For example, salt tolerance induced
192 by *Bacillus subtilus* was shown to be the result of tissue specific modulation of the expression of the
193 *Arabidopsis* Na+ /K+ transporter, *HKT1* (Zhang et al., 2008). Similarly, drought resistance in
194 *Arabidopsis* as a result of inoculation with *Paenibacillus polymyxa* was related to strong upregulation
195 of the host gene *ERD15* (Timmusk and Wagner, 1999).

196   Finally, there is a need for rethinking modern agronomic practices in light of our current
197 understanding of the importance of host-associated microbial communities for plant productivity and
198 health. Current large-scale agricultural systems rely heavily on monoculture cropping systems, in
199 many cases without between-season crop rotation, which has been shown to lead to the build up of
200 specialized plant pathogens, increased disease incidence, and decreased yield (Berendsen et al., 2012;
201 Gentry et al., 2013). Research is being conducted to determine if the use of specific cover crops can
202 be used to promote and maintain a beneficial microbiome between growing seasons for important
203 crop species (East, 2013). Current methods of tilling may also negatively impact the plant microbial
204 community; alternatives, including 'conservation-' or 'zero-tillage', may have the potential to
205 promote a healthy belowground microbiome by reducing moisture loss and maintaining naturally
206 occurring strata within the soil, which helps support microbial biodiversity (East, 2013).

207 **Conclusion**
208

209 As with the plant-breeding and transgenic approaches to engineering stress-resistance in tomorrow's
210 crops, there are of course challenges associated with symbiont based strategies that will need to be
211 overcome. One potential challenge will be detangling synergistic and antagonistic effects of different
212 microorganisms within the plant microbiome (Trabelsi and Mhamdi, 2013). Research has
213 demonstrated synergistic effects of multiple PGPM (Figueiredo et al., 2008), and another study has
214 identified a virus present within a plant growth promoting fungus as the causative agent of heat
215 resistance conferred to a tropical grass (Márquez et al., 2007). A second challenge stems from the
216 fact that while many PGPM have been shown to confer their benefits across multiple host species, it
217 is clear that this is not always the case. In some studies, the host species (and even host cultivar) has
218 been shown to play a significant role in driving microbial community composition and activity
219 (Philippot et al., 2013; Ofek et al., 2013), selecting for and against particular microbial partners.
220 Additionally, interactions between the PGPM and the members of the existing microbial community
221 could alter or negate the potential beneficial effects of the microbe (Schippers et al., 1987). Due to
222 the complexity of interactions among the microbes, host, and environment, there is the potential that
223 a PGPM that confers benefit in one context may have a null, or even negative, effect in a different
224 context; therefore, considerable work will need to be done to determine the range of applicability for
225 each PGPM as a beneficial agricultural agent. A third challenge, which is equally important for both
226 symbiont and host-based methods of improving stress tolerance, will be unraveling the complex
227 relationships between the various biotic and abiotic stress responses. Research programs aimed at

developing tolerance to a particular stress do not necessarily test susceptibility to other stresses; due to the intrinsically related nature of the pathways governing stress response, later field trials have in some instances revealed increased susceptibility to other stresses (Atkinson and Urwin, 2012). Lastly, methods of microbial delivery within field settings and stable integration of PGPMs into the agricultural soil ecosystem will need improvement. While many applications of PGPMs to crops in field settings have demonstrated significant improvements to stress tolerance (Rolli et al., 2014; Mengual et al., 2014; Celebi et al., 2010), others have shown inconsistent or even negative effects (Nadeem et al., 2014). One promising method of stabilizing beneficial effects of PGPM in the field involves the inoculation of a microbial consortium of PGPM, as opposed to a single PGPM species. Combining PGPM known to grow and perform well together will likely increase the resilience of the inoculum and its beneficial effects, and additionally allow for tailoring the community to respond to specific combinations of abiotic and biotic stresses (Trabelsi and Mhamdi, 2013).

Agriculture currently accounts for 70% of human fresh water use, and in many parts of the world this rate of water consumption exceeds local regeneration rates, leading to unsustainable reliance on underground aquifers that are rapidly depleting (Castiglioni et al., 2008; Jiao, 2010). Given this, it is not surprising that drought and other water-related stresses are considered by many to be the most significant threats to global agricultural security in the near future. Encouragingly, in the research conducted by Rodriguez et al., the 'habitat-specific symbionts" selected from a coastal site, a geothermal site, and an agricultural site shared one trait: the ability to confer drought resistance. Rodriguez et al. hypothesize that the ability of fungal endophytes to confer drought tolerance may be a common evolutionary relic from when plants left the ocean, as fungal symbiosis is thought to be in part responsible for the movement of plants to land (Rodriguez et al., 2008). If this turns out to be the case, proponents of symbiont-based approaches to increasing stress resistance in crop plants may do well to focus their efforts on drought and other water-related stresses.

In the future, there is a need for more collaboration between the host-focused and symbiont-focused approaches to mitigating abiotic stress in crop plants. Medical science has in recent years undergone a profound restructuring of its understanding of the microbiome housed within the body and its impact on human health (East, 2013). There is a clear parallel here for plant science, with implications that have the potential to change the face of agriculture and help us to meet the challenges confronting humanity in light of our expanding population and changing planet. The fundamental change required is a broader recognition that plants do not exist as autonomous organisms governed entirely by their genetic blueprints, but rather serve as ecological niches for diverse communities of easily overlooked microbes, which work in concert with the plant to survive in a wide range of stressful environmental conditions.

1. Acknowledgement

2. References[1]

---

[1] Provide the doi when available, and ALL complete author names.

268   Alavi, P., Starcher, M., Zachow, C., Mueller, H., and Berg, G. (2013). Root-microbe systems: the
269   effect and mode of interaction of Stress Protecting Agent (SPA) Stenotrophomonas
270   rhizophila DSM14405T. *Funct. Plant Ecol.* 4, 141. doi:10.3389/fpls.2013.00141.

271   Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.* 28, 169–
272   183. doi:10.1016/j.biotechadv.2009.11.005.

273   Atkinson, N. J., and Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from
274   genes to the field. *J. Exp. Bot.* 63, 3523–3543. doi:10.1093/jxb/ers100.

275   Barrow, J. R., Lucero, M. E., Reyes-Vera, I., and Havstad, K. M. (2008). Do symbiotic microbes have a
276   role in plant evolution, performance and response to stress? *Commun. Integr. Biol.* 1, 69–73.

277   Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M. (2012). The rhizosphere microbiome and
278   plant health. *Trends Plant Sci.* 17, 478–486. doi:10.1016/j.tplants.2012.04.001.

279   Berg, G., Zachow, C., Müller, H., Philipps, J., and Tilcher, R. (2013). Next-Generation Bio-Products
280   Sowing the Seeds of Success for Sustainable Agriculture. *Agronomy* 3, 648–656.
281   doi:10.3390/agronomy3040648.

282   Bonfante, P., and Anca, I.-A. (2009). Plants, Mycorrhizal Fungi, and Bacteria: A Network of
283   Interactions. *Annu. Rev. Microbiol.* 63, 363–383. doi:10.1146/annurev.micro.091208.073504.

284   Budak, H., Kantar, M., and Yucebilgili Kurtoglu, K. (2013). Drought Tolerance in Modern and Wild
285   Wheat. *Sci. World J.* 2013. doi:10.1155/2013/548246.

286   Castiglioni, P., Warner, D., Bensen, R. J., Anstrom, D. C., Harrison, J., Stoecker, M., Abad, M., Kumar,
287   G., Salvador, S., D'Ordine, R., et al. (2008). Bacterial RNA Chaperones Confer Abiotic Stress
288   Tolerance in Plants and Improved Grain Yield in Maize under Water-Limited Conditions.
289   *PLANT Physiol.* 147, 446–455. doi:10.1104/pp.108.118828.

290   Celebi, S. Z., Demir, S., Celebi, R., Durak, E. D., and Yilmaz, I. H. (2010). The effect of Arbuscular
291   Mycorrhizal Fungi (AMF) applications on the silage maize (Zea mays L.) yield in different
292   irrigation regimes. *Eur. J. Soil Biol.* 46, 302–305. doi:10.1016/j.ejsobi.2010.06.002.

293   Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., Aubinet, M., Buchmann, N.,
294   Bernhofer, C., Carrara, A., et al. (2005). Europe-wide reduction in primary productivity
295   caused by the heat and drought in 2003. *Nature* 437, 529–533. doi:10.1038/nature03972.

296   Cooper, M., Gho, C., Leafgren, R., Tang, T., and Messina, C. (2014). Breeding drought-tolerant maize
297   hybrids for the US corn-belt: discovery to product. *J. Exp. Bot.*, eru064.
298   doi:10.1093/jxb/eru064.

299   East, R. (2013). Microbiome: Soil science comes to life. *Nature* 501, S18–S19.
300   doi:doi:10.1038/501S18a.

301   Editorial (2010). How to feed a hungry world. *Nature* 466, 531–532. doi:10.1038/466531a.

302   Eisenstein, M. (2013). Discovery in a dry spell. *Nature* 501, S7–S9. doi:10.1038/501S7a.

303   Fedoroff, N. V., Battisti, D. S., Beachy, R. N., Cooper, P. J. M., Fischhoff, D. A., Hodges, C. N., Knauf,
304   V. C., Lobell, D., Mazur, B. J., Molden, D., et al. (2010). Radically Rethinking Agriculture for
305   the 21st Century. *Science* 327, 833–834. doi:10.1126/science.1186834.

306   Figueiredo, M. V. B., Burity, H. A., Martínez, C. R., and Chanway, C. P. (2008). Alleviation of drought
307   stress in the common bean (Phaseolus vulgaris L.) by co-inoculation with Paenibacillus
308   polymyxa and Rhizobium tropici. *Appl. Soil Ecol.* 40, 182–188.
309   doi:10.1016/j.apsoil.2008.04.005.

310   Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., and Martinez-Romero, E.
311   (2011). Microbially Mediated Plant Functional Traits. *Annu. Rev. Ecol. Evol. Syst.* 42, 23–46.
312   doi:10.1146/annurev-ecolsys-102710-145039.

313   Gentry, L. F., Ruffo, M. L., and Below, F. E. (2013). Identifying Factors Controlling the Continuous
314   Corn Yield Penalty. *Agron. J.* 105, 295. doi:10.2134/agronj2012.0246.

315   Glick, B. R., Cheng, Z., Czarny, J., and Duan, J. (2007). Promotion of plant growth by ACC deaminase-
316   producing soil bacteria. *Eur. J. Plant Pathol.* 119, 329–339. doi:10.1007/s10658-007-9162-4.

317   Grayson, M. (2013). Agriculture and Drought. *Nature* 501, S1. doi:10.1038/501S1a.

318   Grover, M., Ali, S. Z., Sandhya, V., Rasul, A., and Venkateswarlu, B. (2010). Role of microorganisms in
319   adaptation of agriculture crops to abiotic stresses. *World J. Microbiol. Biotechnol.* 27, 1231–
320   1240. doi:10.1007/s11274-010-0572-7.

321   Hassan, M. N., Afghan, S., and Hafeez, F. Y. (2010). Suppression of red rot caused by Colletotrichum
322   falcatum on sugarcane plants using plant growth-promoting rhizobacteria. *BioControl* 55,
323   531–542. doi:10.1007/s10526-010-9268-z.

324   Jiao, L. (2010). Water Shortages Loom as Northern China's Aquifers Are Sucked Dry. *Science* 328,
325   1462–1463. doi:10.1126/science.328.5985.1462-a.

326   Köberl, M., Müller, H., Ramadan, E. M., and Berg, G. (2011). Desert Farming Benefits from Microbial
327   Potential in Arid Soils and Promotes Diversity and Plant Health. *PLoS ONE* 6, e24452.
328   doi:10.1371/journal.pone.0024452.

329   Lantican, M. a., Pingali, P. l., and Rajaram, S. (2003). Is research on marginal lands catching up? The
330   case of unfavourable wheat growing environments. *Agric. Econ.* 29, 353–361.
331   doi:10.1111/j.1574-0862.2003.tb00171.x.

332   Larson, C. (2013). Losing Arable Land, China Faces Stark Choice: Adapt or Go Hungry. *Science* 339,
333   644–645. doi:10.1126/science.339.6120.644.

334   Lugtenberg, B., and Kamilova, F. (2009). Plant-Growth-Promoting Rhizobacteria. *Annu. Rev.*
335   *Microbiol.* 63, 541–556. doi:10.1146/annurev.micro.62.081307.162918.

336 Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., and Dangl, J. L. (2013). Practical
337   innovations for high-throughput amplicon sequencing. *Nat. Methods* 10, 999–1002.
338   doi:10.1038/nmeth.2634.

339 Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., Abou-Hadid, A. F., El-Behairy, U.
340   A., Sorlini, C., Cherif, A., et al. (2012). A Drought Resistance-Promoting Microbiome Is
341   Selected by Root System under Desert Farming. *PLoS ONE* 7, e48479.
342   doi:10.1371/journal.pone.0048479.

343 Marasco, R., Rolli, E., Vigani, G., Borin, S., Sorlini, C., Ouzari, H., Zocchi, G., and Daffonchio, D. (2013).
344   Are drought-resistance promoting bacteria cross-compatible with different plant models?
345   *Plant Signal. Behav.* 8, e26741. doi:10.4161/psb.26741.

346 Márquez, L. M., Redman, R. S., Rodriguez, R. J., and Roossinck, M. J. (2007). A Virus in a Fungus in a
347   Plant: Three-Way Symbiosis Required for Thermal Tolerance. *Science* 315, 513–515.
348   doi:10.1126/science.1136237.

349 Marulanda, A., Barea, J.-M., and Azcón, R. (2009). Stimulation of Plant Growth and Drought
350   Tolerance by Native Microorganisms (AM Fungi and Bacteria) from Dry Environments:
351   Mechanisms Related to Bacterial Effectiveness. *J. Plant Growth Regul.* 28, 115–124.
352   doi:10.1007/s00344-009-9079-6.

353 Mayak, S., Tirosh, T., and Glick, B. R. (2004). Plant growth-promoting bacteria that confer resistance
354   to water stress in tomatoes and peppers. *Plant Sci.* 166, 525–530.
355   doi:10.1016/j.plantsci.2003.10.025.

356 Mengual, C., Schoebitz, M., Azcón, R., and Roldán, A. (2014). Microbial inoculants and organic
357   amendment improves plant establishment and soil rehabilitation under semiarid conditions.
358   *J. Environ. Manage.* 134, 1–7. doi:10.1016/j.jenvman.2014.01.008.

359 Morrissey, J. P., Dow, J. M., Mark, G. L., and O'Gara, F. (2004). Are microbes at the root of a solution
360   to world food production? *EMBO Rep.* 5, 922–926.

361 Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., and Ashraf, M. (2014). The role of mycorrhizae
362   and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under
363   stressful environments. *Biotechnol. Adv.* doi:10.1016/j.biotechadv.2013.12.005.

364 Ofek, M., Voronov-Goldman, M., Hadar, Y., and Minz, D. (2013). Host signature effect on plant root-
365   associated microbiomes revealed through analyses of resident *vs* . active communities: Host
366   effect on plant root-associated microbiomes. *Environ. Microbiol.* doi:10.1111/1462-
367   2920.12228.

368 Partida-Martínez, L. P., and Heil, M. (2011). The Microbe-Free Plant: Fact or Artifact? *Front. Plant
369   Sci.* 2. doi:10.3389/fpls.2011.00100.

370 Philippot, L., Raaijmakers, J. M., Lemanceau, P., and van der Putten, W. H. (2013). Going back to the
371   roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.*
372   doi:10.1038/nrmicro3109.

373 Pope, P. B., Smith, W., Denman, S. E., Tringe, S. G., Barry, K., Hugenholtz, P., McSweeney, C. S.,
374   McHardy, A. C., and Morrison, M. (2011). Isolation of Succinivibrionaceae Implicated in Low
375   Methane Emissions from Tammar Wallabies. *Science* 333, 646–648.
376   doi:10.1126/science.1205760.

377 Porch, T. G. (2006). Application of Stress Indices for Heat Tolerance Screening of Common Bean. *J.*
378   *Agron. Crop Sci.* 192, 390–394. doi:10.1111/j.1439-037X.2006.00229.x.

379 Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J., and Henson, J. M. (2002).
380   Thermotolerance generated by plant/fungal symbiosis. *Science* 298, 1581–1581.

381 Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y.-O., and
382   Redman, R. S. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *ISME J.* 2,
383   404–416. doi:10.1038/ismej.2007.106.

384 Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M. L., Gandolfi, C., Casati, E.,
385   Previtali, F., Gerbino, R., et al. (2014). Improved plant resistance to drought is promoted by
386   the root-associated microbiome as a water stress-dependent trait: Root bacteria protect
387   plants from drought. *Environ. Microbiol.* doi:10.1111/1462-2920.12439.

388 Schippers, B., Bakker, A. W., and Bakker, P. A. (1987). Interactions of deleterious and beneficial
389   rhizosphere microorganisms and the effect of cropping practices. *Annu. Rev. Phytopathol.*
390   25, 339–358.

391 Swamy, B. P. M., and Kumar, A. (2013). Genomics-based precision breeding approaches to improve
392   drought tolerance in rice. *Biotechnol. Adv.* 31, 1308–1318.
393   doi:10.1016/j.biotechadv.2013.05.004.

394 Tank, N., and Saraf, M. (2010). Salinity-resistant plant growth promoting rhizobacteria ameliorates
395   sodium chloride stress on tomato plants. *J. Plant Interact.* 5, 51–58.
396   doi:10.1080/17429140903125848.

397 Taylor-Teeples, M., Ron, M., and Brady, S. M. (2011). Novel biological insights revealed from cell
398   type-specific expression profiling. *Curr. Opin. Plant Biol.* 14, 601–607.
399   doi:10.1016/j.pbi.2011.05.007.

400 Timmusk, S., and Wagner, E. G. H. (1999). The plant-growth-promoting rhizobacterium Paenibacillus
401   polymyxa induces changes in Arabidopsis thaliana gene expression: a possible connection
402   between biotic and abiotic stress responses. *Mol. Plant. Microbe Interact.* 12, 951–959.

403 Trabelsi, D., and Mhamdi, R. (2013). Microbial Inoculants and Their Impact on Soil Microbial
404   Communities: A Review. *BioMed Res. Int.* 2013. doi:10.1155/2013/863240.

405 Turner, T. R., James, E. K., and Poole, P. S. (2013). The plant microbiome. *Genome Biol.* 14, 209.

406 Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R.,
407   Neumann, C., and von Wettstein, D. (2005). The endophytic fungus Piriformospora indica

408   reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl.*
409   *Acad. Sci. U. S. A.* 102, 13386–13391.

410 Waterer, D., Benning, N. T., Wu, G., Luo, X., Liu, X., Gusta, M., McHughen, A., and Gusta, L. V.
411   (2010). Evaluation of abiotic stress tolerance of genetically modified potatoes (Solanum
412   tuberosum cv. Desiree). *Mol. Breed.* 25, 527–540. doi:10.1007/s11032-009-9351-2.

413 Witt, S., Galicia, L., Lisec, J., Cairns, J., Tiessen, A., Araus, J. L., Palacios-Rojas, N., and Fernie, A. R.
414   (2012). Metabolic and Phenotypic Responses of Greenhouse-Grown Maize Hybrids to
415   Experimentally Controlled Drought Stress. *Mol. Plant* 5, 401–417. doi:10.1093/mp/ssr102.

416 Woyke, T., Teeling, H., Ivanova, N. N., Huntemann, M., Richter, M., Gloeckner, F. O., Boffelli, D.,
417   Anderson, I. J., Barry, K. W., Shapiro, H. J., et al. (2006). Symbiosis insights through
418   metagenomic analysis of a microbial consortium. *Nature* 443, 950–955.
419   doi:10.1038/nature05192.

420 Yang, J., Kloepper, J. W., and Ryu, C.-M. (2009). Rhizosphere bacteria help plants tolerate abiotic
421   stress. *Trends Plant Sci.* 14, 1–4. doi:10.1016/j.tplants.2008.10.004.

422 Zelicourt, A. de, Al-Yousif, M., and Hirt, H. (2013). Rhizosphere Microbes as Essential Partners for
423   Plant Stress Tolerance. *Mol. Plant* 6, 242–245. doi:10.1093/mp/sst028.

424 Zhang, H., Kim, M.-S., Sun, Y., Dowd, S. E., Shi, H., and Paré, P. W. (2008). Soil bacteria confer plant
425   salt tolerance by tissue-specific regulation of the sodium transporter HKT1. *Mol. Plant.*
426   *Microbe Interact.* 21, 737–744.

427

428   **3.   Figure legends**

429   **Figure 1. Advantages of symbiont-based approaches to improving stress tolerance in crops.**
430   Plant-growth promoting microbes are capable of conferring benefits to multiple species of plant
431   hosts, and of offering improved tolerance to multiple stresses simultaneously. Inoculations with
432   combinations of PGPM can be tailored to specific environmental conditions. Dissection of plant-
433   microbe interactions during symbiosis has the potential to reveal both the microbial and host genetic
434   components responsible for improved stress tolerance; these may serve as targets for plant-
435   breeding/genetic-engineering based approaches to improving stress tolerance in the host.

Figure 1. **Advantages of Symbiont-Based approaches to improving stress tolerance in crops**. Plant-growth promoting microbes are capable of conferring benefits to multiple species of plant hosts, and of offering improved tolerance to multiple stresses simultaneously. Inoculations with combinations of PGPM can be tailored to specific environmental conditions. Dissection of plant-microbe interactions during symbiosis has the potential to reveal both the microbial and host genetic components responsible for improved stress tolerance; these may serve as targets for plant-breeding/genetic-engineering based approaches to improving stress tolerance in crops.