

Rubén Bottini · Fabricio Cassán · Patricia Piccoli

Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase

Received: 2 February 2004 / Revised: 8 June 2004 / Accepted: 11 June 2004 / Published online: 28 July 2004
© Springer-Verlag 2004

Abstract This review focuses on studies with bacteria for which biosynthesis/production of the plant hormones gibberellins have been demonstrated. Actual data on gibberellin metabolism by bacteria are analyzed in comparison with the biosynthetic pathways known for vascular plants and fungi. The potential involvement of gibberellins produced by symbiotic and soil-endophytic microorganisms in plant growth promotion and yield increase is also discussed.

Gibberellins as plant hormones

Gibberellins are tetracyclic diterpenoid acids that are involved in a number of developmental and physiological processes in plants (Crozier et al. 2000; Davies 1995). These processes include seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth (King and Evans 2003; Pharis and King 1985; Sponsel 2003). Gibberellins are also implicated in promotion of root growth, root hair abundance, inhibition of floral bud differentiation in woody angiosperms, regulation of vegetative and reproductive bud dormancy and delay of senescence in many organs of a range of plant species (Bottini and Luna 1993; Fulchieri et al. 1993; Reinoso et al. 2002; Tanimoto 1987). In most (if

not all) of these processes gibberellins act in combination with other phytohormones and additional regulatory factors, so that the signaling pathways are highly integrated (Trewavas 2000). A major problem in understanding the role of gibberellins is that the scientific information available utilizes many different species and diverse experimental models, making it difficult to extrapolate results across species, genera and family boundaries. To date, 136 different chemical structures have been characterized as naturally occurring gibberellins (<http://www.plant-hormones.info/gainfo.asp>). To this figure should be added an unknown number of glucose-conjugate forms (Schneider 1983) and likely intermediates yet to be identified in biosynthetic and catabolic pathways (Sponsel and Hedden 2004). Of these 136 gibberellins, the 3 β -hydroxylated, C19 gibberellins GA₁, GA₃ and GA₄ (Fig. 1), have been reported by studies with single gene dwarf mutants as being directly effective in promotion of shoot elongation in plants (Crozier et al. 2000). However, it is very likely that other 3 β -hydroxylated C19 gibberellins also function per se as effectors of shoot elongation in a wide range of plant species.

Historically, gibberellins were discovered in culture filtrates of the fungus *Fusarium moniliforme* (*Gibberella fujikuroi* in the sexual form) in 1926 in Japan by Kurosawa (cited in Tamura 1990) and their chemical structure was partially elucidated 10 years later (Yabuta and Sumiki 1938, cited in Tamura 1990). The Western world, however, only became aware of their existence some two decades later when the structure of gibberellic acid was confirmed (GA₃, Fig. 1, Curtis and Cross 1954). Soon afterwards, the first plant gibberellin (GA₁, Fig. 1) was identified by Macmillan and Suter (1958) from *Phaseolus coccineus* seeds.

However, gibberellins are produced not only by higher plants and fungi (MacMillan 2002) but also by bacteria (Atzorn et al. 1988; Bastián et al. 1998; Bottini et al. 1989; Gutiérrez-Mañero et al. 2001; Janzen et al. 1992; MacMillan 2002). In fungi and bacteria there is no known role for gibberellins, rather they seem to be

R. Bottini (✉) · P. Piccoli
Cátedra de Química Orgánica y Biológica, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo,
5505 Chacras de Coria, Argentina
e-mail: rbottini@fca.uncu.edu.ar
Tel.: +54-261-4960004 ext 1228
Fax: 54-261-4960469

F. Cassán
Laboratorio de Fisiología Vegetal, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto,
5800 Río Cuarto, Argentina

P. Piccoli
Area de Investigaciones, Universidad Juan A. Maza,
5500 Mendoza, Argentina

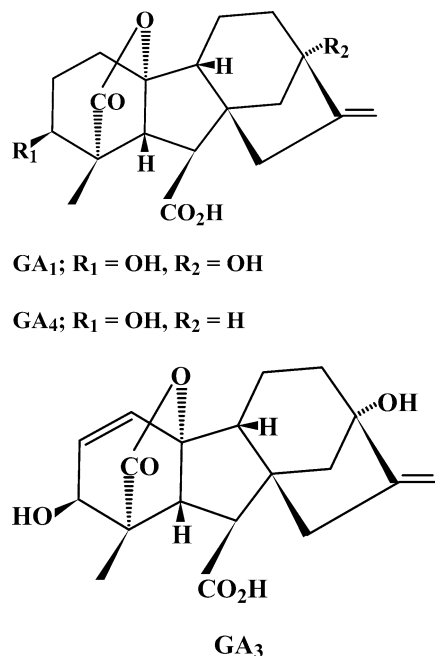


Fig. 1 Chemical structure of GA₁, GA₃ and GA₄, the three gibberellins reported by studies with single gene dwarf mutants as being directly effective in promotion of shoot elongation in plants (Crozier et al. 2000)

secondary metabolites that may play a role as signaling factors towards the host plant.

Bacteria known to improve plant growth and crop yield

In the twentieth century there was a global tendency toward increased use of fertilizers, especially nitrogen, as a way to improve crop productivity. This tendency first developed in industrialized countries, but in the 1960s the “green revolution” extended it to the “third world”. However, the progressive increase in use of mineral fertilizers has posed a severe threat to a wide range of ecological systems. Thus, more recently there has been growing attention focused on more “environmentally friendly” N₂-fixing bacteria as a mode of increasing crop yield (Okon and Labandera-González 1994).

For many years most efforts emphasized Rhizobiaceae symbiotic associations. In fact, their use now extends worldwide and the benefits from increased nitrogen supply are unanimously recognized. Since the 1970s, however, an increasing interest has developed in “free-living” rhizosphere bacteria (i.e., *Pseudomonas*, *Bacillus*, *Azotobacter*, *Azospirillum*; De-Polli et al. 1977; Döbereiner et al. 1976; Ludden et al. 1978; Okon et al. 1976a,b), including the so-called plant growth promoting rhizobacteria (PGPR, reviewed by Glick et al. 1999). While use of Rhizobiaceae symbiotic bacteria is restricted almost exclusively to the Leguminosae, PGPR are being utilized with cereals like wheat or rice, the primary global source of food. Nevertheless, it has been postulated that the beneficial effects of PGPR microorganisms are not due solely to N₂

fixation (Bashan and Levanony 1990; Okon and Labandera-González 1994, and literature cited therein). The benefits of PGPR use seem to be a consequence of a complex mix of different mechanisms, which may include N₂ fixation by nitrogenase, nitrate reductase activity, siderophore production, and phytohormone synthesis/metabolism and release to the plant (Cassán et al. 2001a,b; Fulchieri et al. 1993). Other mechanisms that have been proposed, such as increasing water and mineral uptake, are more likely to be the result of root growth promotion by any (or all) of the above mentioned mechanisms (Cassán et al. 2003). Nonetheless, although the mechanisms by which PGPR promote increases in crop yield are not fully elucidated, the synthesis of phytohormones, including gibberellins (Bottini et al. 1989), and the absorption of these hormones by the crop plant are considered to be key causal factors (Cassán et al. 2001a,b; Fulchieri et al. 1993; Okon and Labandera-González 1994).

Gibberellin characterization and metabolism in bacteria

The first report of gibberellin characterization in bacteria using physico-chemical methods was by Atzorn et al. (1988), who demonstrated the presence of GA₁, GA₄, GA₉ and GA₂₀ in gnotobiotic cultures of *Rhizobium meliloti*.

In *Azospirillum* sp. several studies have characterized gibberellins by capillary gas chromatography-mass spectrometry (GC-MS), i.e., GA₁, GA₃, GA₉, GA₁₉ and GA₂₀ using gnotobiotic cultures of *A. lipoferum* (Bottini et al. 1989; Piccoli et al. 1996, 1997) and of GA₁ and GA₃ from gnotobiotic cultures of *A. brasilense* (Janzen et al. 1992). The iso-lactone of GA₃ has also been identified in these studies, but its presence may be an artifact produced by the analytical purification or GC conditions. Using biological assays the total amount of gibberellins produced in pure cultures of ca. 10⁸ cfu ml⁻¹ ranged from 20 pg ml⁻¹ to 400 pg ml⁻¹ (Bottini et al. 1989; Piccoli et al. 1996). However, in co-culture with other bacteria (Cacciari et al. 1989; Flouri et al. 1995) or with fungi (Janzen et al. 1992) the total amount of gibberellins produced increased ca. 10-fold.

Apart from *Azospirillum* sp. and *Rhizobium* sp., production of gibberellin-like substances has also been claimed in numerous bacterial genera, although the techniques used (TLC, bioassays, HPLC-UV) are of poor resolution and/or reliability. Using unequivocal physico-chemical methods, such as GC-MS, production of gibberellins has been confirmed in *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* (Bastián et al. 1998) and *Bacillus* sp. (Gutiérrez-Mañero et al. 2001) in addition to *Azospirillum* sp. (see above).

In higher plants gibberellin biosynthesis (Fig. 2; Crozier et al. 2000; Hedden and Phillips 2000) begins with the cyclization of a C₂₀ precursor, geranyl geranyl diphosphate (GGPP). This intermediate is synthesized in plastids starting from isopentenyl diphosphate (IPP) coming either from cytosol-formed mevalonic acid or via the plastid deoxylulose 5-phosphate pathway (Litchenthaler 1999;

Sponsel 2002). In actively growing tissues, cyclization of GGPP yields *ent*-kaurene (*ent*-K) in a two-step synthesis that requires two enzymes: copalyl diphosphate synthase (CPS), which yields copalyl diphosphate (CPP), and *ent*-kaurene synthase (KS), which gives the final product. Subsequently, *ent*-K is converted into “true” gibberellins by a series of oxidative reactions catalyzed by two types of enzymes. The first type are membrane-related cytochrome P450 monooxygenases (*ent*-K oxidase, Kox, and *ent*-kaurenoic acid oxidase, KAox) and lead to formation of the first gibberellin, GA₁₂-aldehyde, which is then converted by a 13-hydroxylase (GA13ox) to GA₅₃-aldehyde or GA₅₃ via GA₁₂. Subsequent metabolism at the C20 stage is accomplished by 2-oxoglutarate-dependent soluble dioxygenases (GA20ox) and 3β-hydroxylases (GA3ox, Fig. 2). A third group of 2-oxoglutarate-dependent dioxygenases, 2β-hydroxylases (GA2ox) hydroxylates the GA molecule at carbon 2, removing its growth-promoting activity (MacMillan 1997; Hedden and Phillips 2000; Sponsel and Hedden 2004).

In fungi, the general pathway is similar to that of higher plants, although the genes and enzymes involved differ. Recently, Tudzynski et al. (2003) completed the cloning of six genes of the gibberellin biosynthesis gene cluster in *Gibberella fujikuroi* and determined the functions of these genes, thus defining the complete gibberellin biosynthetic pathway in this fungus. Notably, all the enzymes involved are membrane-related cytochrome P450 monooxygenases and none are soluble dioxygenases. These enzymes comprise the gibberellin-specific GGPP synthase (GGS2), *ent*-K synthase (CPS/KS), and four cytochrome P450 monooxygenase genes (*P450-1–P450-4*) closely linked in a gene cluster (Mende et al. 1997; Linnemannstöns et al. 1999). *P450-4* encodes Kox, catalyzing the three oxidation steps between *ent*-K and *ent*-KA (Tudzynski et al. 2001), while *P450-1* encodes a highly multifunctional monooxygenase, which catalyzes four steps involving oxidation at two carbon atoms, in the main pathway from *ent*-KA to GA₁₄ via GA₁₂-aldehyde (Rojas et al. 2001). *P450-2* was shown to encode a GA20ox, which converts GA₁₄ to GA₄ by removal of C-20 (Tudzynski et al. 2002). *P450-3*, encodes the 13-hydroxylase that converts GA₇ to the end product, GA₃, while another gene, *orf3*, encodes the desaturase that converts GA₄ to GA₇ (Tudzynski et al. 2003).

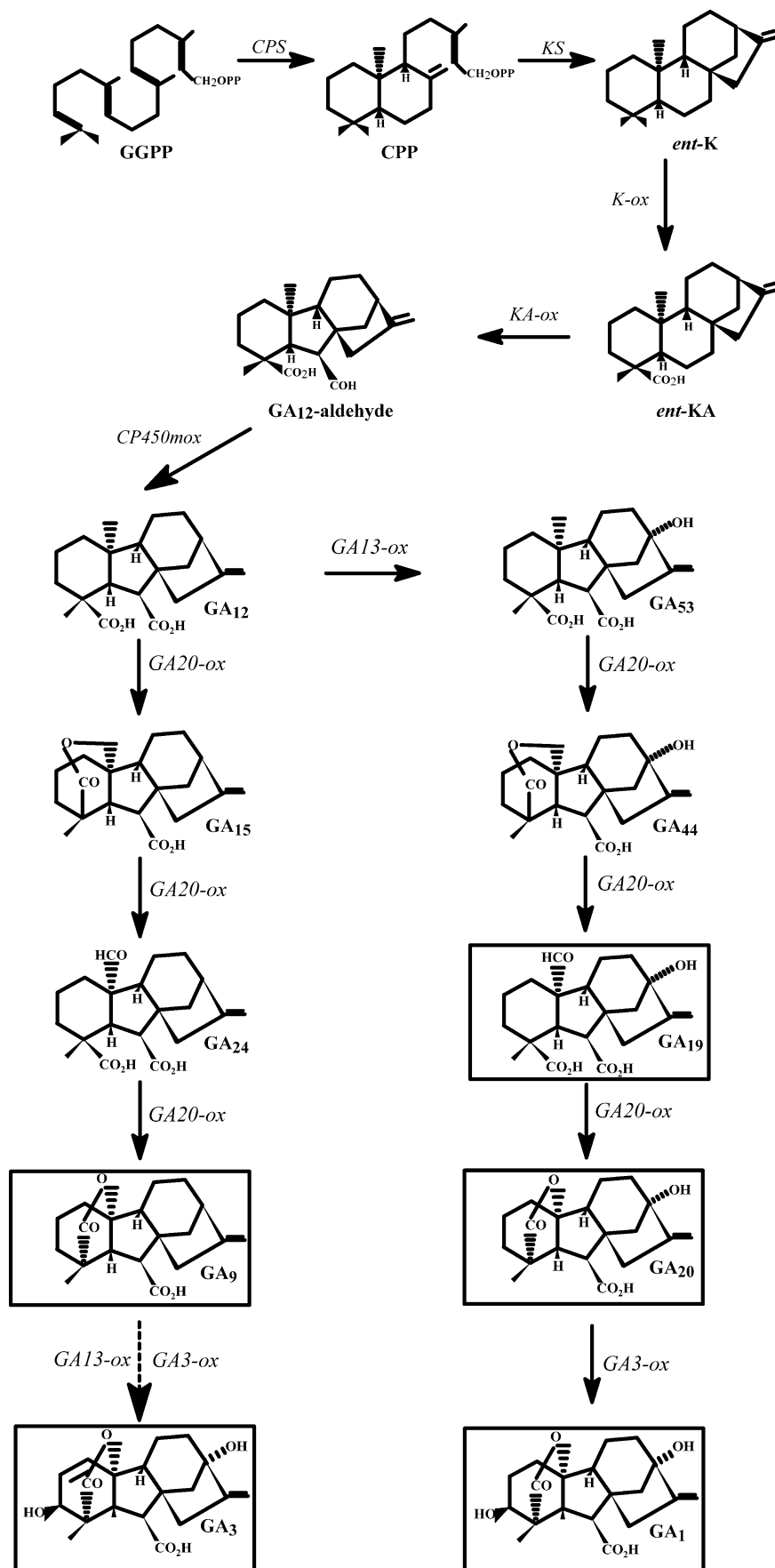
Thus, although information on the enzymes and their genetic control involved in the metabolism of gibberellins in higher plants and fungi is abundant, evidence for bacterial biosynthesis is scarce. Tully et al. (1998) sequenced a cluster of three complete P450 genes (CYP112, CYP114, and CYP117) in *Bradyrhizobium japonicum*, plus a partial P450 gene fragment (CYP115P) previously shown to encode a cytochrome P450. Although the biochemical functions of the products of these genes are uncertain, similarities in structure with other genes suggest an operon involved in terpenoid synthesis bearing some resemblance to plant and *Gibberella* genes for *ent*-KS.

For *Azospirillum* sp. it has been demonstrated that the bacterium can metabolize gibberellins in vitro (Piccoli and Bottini 1994a; Piccoli et al. 1996, 1997) as well as in vivo, i.e., in association with a higher plant (Cassán et al. 2001a, b). The finding by Piccoli and Bottini (1994b) that the bacteria produce GA₉ and GA₁₉ in chemically defined culture may indicate the existence of two branches for the biosynthetic pathway in *Azospirillum* sp. (Fig. 2), i.e., early 13-hydroxylation involving the metabolism of GA₁₉ (and its metabolite, GA₂₀) to GA₁, and an early non-hydroxylation branch where GA₉ is (presumably) the precursor of GA₃. In fact, when the chemically defined medium was supplemented with deuterium-labeled GA₂₀, putative deuterium-labeled GA₁ was identified based on the mass spectrum profile (Piccoli and Bottini 1994a). Consistent with this scheme, the feeding of *A. lipoferum* cultures with tritium- and deuterium-labeled gibberellins also indicated that GA₉ produced GA₃, but not GA₁, while GA₁ (but not GA₃) was obtained with GA₂₀ as substrate (Piccoli et al. 1996). The concept of two branches in the gibberellin biosynthetic pathway is also reinforced by the effect of blue light on *A. lipoferum* cultures, i.e., a 2- to 3-fold increase in the amount of GA₃, relative to GA₁ (Piccoli and Bottini 1996).

Other environmental factors, such as N supply (Piccoli and Bottini 1994b), O₂ availability and osmotic potential (Piccoli et al. 1999), can influence both the quantity and type of gibberellin produced by *Azospirillum* cultures. High concentrations of NH₄Cl reduced the amount of GA₃ produced (Piccoli and Bottini 1994b), i.e., like in vascular plants and fungi (Candau et al. 1992), gibberellin synthesis initiates when N availability decreases. The quantity of GA₃ produced was also severely reduced by restricted gas exchange. In the presence of PEG as an osmotic agent ($\Psi_w = -1.21$ MPa), the total amount of GA₃ was reduced only 50% despite a 90% reduction in the number of cells per milliliter of culture medium (Piccoli et al. 1999). This implies a compensatory mechanism in the bacterium's ability to produce GA₃ under drought conditions, which in turn may explain the positive effects of gibberellin produced by endophytic *Azospirillum* sp. noted in water-stressed maize seedlings (Cohen et al. 2001).

Additionally *A. lipoferum* grown in vitro in chemically defined medium hydrolyzed both ether and ester glycosides of GA₂₀ that were isotopically labeled with deuterium (Piccoli et al. 1997). This finding was confirmed in vivo using two mutants of rice deficient in gibberellin synthesis. A reversion of the dwarf phenotype to wild-type (tall) was obtained when these dwarf mutants were fed with deuterio GA₂₀-glycosides, and liberation of the aglycone deuterio GA₂₀ occurred, as did its subsequent 3β-hydroxylation to deuterio GA₁ (Cassán et al. 2001a,b, 2003). When prohexadione-Ca, a specific inhibitor of the 2β-hydroxylation and 3β-hydroxylation steps, was added to the plant system, no reversion of dwarf phenotype was observed in the rice mutant, nor was deuterio GA₁ identified by GC-MS. This suggests that the enzymes that mediate the 3β hydroxylation step in both the rice plant and the bacterium correspond to the family of 2-

Fig. 2 Putative biosynthetic pathway for gibberellins in *Azospirillum* sp. based on well-established steps in vascular plants and fungi, and data available on gibberellin metabolism studies with this bacterium. Gibberellins already characterized by GC-MS as produced by *Azospirillum* sp are boxed. The conversion of GA₉ to GA₃ has been demonstrated in vitro with gnotobiotic cultures of the bacterium, while the metabolism of GA₂₀ to GA₁ has been established both in vitro and in vivo (i.e., in association with *dy* mutants of rice)



oxoglutarate-dependent dioxygenases (Cassán et al. 2001b). Cassán et al. (2003) also found that inoculation with *A. lipoferum* and *A. brasilense* of the *dy* mutant of rice fed with GA₁₂-aldehyde reversed the dwarf phenotype, while addition of Uniconazole-P (an inhibitor of membrane-related cytochrome P450 monooxygenases) repressed the reversion of the dwarf phenotype. Taken together, these results suggest that, as in higher plants, early steps of the gibberellin biosynthetic pathway in the bacterium may be regulated by membrane-related cytochrome P450 monooxygenases, and the late hydroxylative steps by soluble 2-oxoglutarate-dependent dioxygenases.

The involvement of gibberellin produced by bacteria in plant growth and yield promotion

Several papers (reviewed by Cassán et al. 2003) have claimed that nodules of different Leguminosae species contain more gibberellin-like substances than do adjacent roots, suggesting that the microorganism modifies hormonal levels in the nodules, either by affecting plant cell metabolism or by affecting gibberellin production by the bacterium. For example, Dobert et al. (1992) demonstrated that *Phaseolus lunatus* plants inoculated with a specific strain of *Bradyrhizobium* sp. showed a marked internode elongation that was not observed in plants inoculated with other compatible bradyrhizobia. Measurement of gibberellin content using deuterated internal standards, and GC-MS analysis, showed that increased levels of GA₁, GA₁₉, GA₂₀, and GA₄₄ in nodules formed by the two bacterial strains that enhanced elongation growth (Dobert et al. 1992). Yanni et al. (2001) noted that indigenous *Rhizobium leguminosarum* bv. trifoli can colonize rice roots in the Egyptian Nile delta where rice has been rotated with *Trifolium alexandrinum* L. since antiquity. The Rhizobium-rice combination promotes root and shoot growth, thereby improving seedling vigor and increasing grain yield. Yanni et al. (2001) also found that pure cultures of these Rhizobium strains produced auxin (IAA) and gibberellin (tentatively identified as GA₇).

Gibberellin production by *Azospirillum* sp. and *Bacillus* sp. has been implicated in the increased ¹⁵N uptake seen after inoculation of wheat roots (Kucey 1988). Application of GA₃ to the roots, in concentrations similar to those produced by the microorganisms, promoted root growth in maize seedlings, and inoculation with different *Azospirillum* strains increased levels of GA₃ in maize roots (Fulchieri et al. 1993); in contrast, non-inoculated seedlings contained predominantly conjugated GA₃. Furthermore, reversal of dwarfism, both genetic and induced by inhibitors of gibberellin biosynthesis, was demonstrated in both rice and maize seedlings that had been inoculated with *Azospirillum* sp., and showed the endophytic presence of the bacteria (Lucangeli and Bottini 1996, 1997). A reversal of the dwarf phenotype was also obtained in these inoculated dwarf rice mutants fed with deuterio GA₂₀-glycosides, and associated with the increased growth was a liberation of the aglycone, deuterio

GA₂₀, and its 3β-hydroxylated metabolite, deuterio GA₁ (Cassán et al. 2001a,b, 2003). It was not possible, however, to determine whether the plant growth response to bacterial inoculation was due to bacterial gibberellin production and deconjugation of gibberellin glycosides by enzymes of the microorganism. It should be noted that the inoculated rice seedlings showed a much lesser, and not significant, response to the applied deuterio GA₂₀-glycoside.

As noted above, gibberellins are known to interact with other hormones. Cohen et al. (2001) tested the ability of *A. lipoferum* to alleviate temporary drought in maize seedlings. In this case, Prohexadione-Ca and fluridone were used to block gibberellin and ABA synthesis, respectively. Inhibition of ABA synthesis was detrimental to the plant mainly because stomatal closure was reduced. However, the most harmful situation was obtained under drought stress, when the synthesis of both hormones had been reduced. Prior inoculation with *A. lipoferum* promoted growth of both roots and shoots under drought, partially reversing the effects of the two biosynthesis inhibitors. Alleviation of water stress symptoms in wheat plants has been reported previously by Creus et al. (1997), with the alleviation effects being attributed, at least in part, to gibberellin production by the bacteria.

The effect of inoculation with *A. diazotrophicus* and of applications of GA₃ at several doses on total carbohydrates, sucrose, glucose and fructose was assessed in shoots of *Sorghum bicolor* (Bastián et al. 1999). Both *A. diazotrophicus* and application of GA₃ were effective in promoting total carbohydrate accumulation, but neither technique yielded an increase in sucrose levels. In contrast, fructose and glucose levels were significantly enhanced by both *A. diazotrophicus* and GA₃ treatments, relative to controls.

Bacillus pumilus and *Bacillus licheniformis*, isolated from the rhizosphere of *Alnus glutinosa* L. Gaertn., both have strong growth-promoting activity. Gutiérrez-Mañero et al. (2001) showed that the dwarf phenotype induced in *A. glutinosa* seedlings by Paclobutrazol (an inhibitor of gibberellin biosynthesis) was effectively reversed by applications of extracts from medium incubated with both bacteria and also by exogenous GA₃. GC-MS analysis of extracts of these media showed the presence of GA₁, GA₃, GA₄ and GA₂₀. Probanza et al. (2002) also reported that inoculation with *Bacillus licheniformis* and *B. pumilus* enhanced growth of *Pinus pinea* plants, presumably by bacterial gibberellin production.

In conclusion, the beneficial effect of PGPR on growth and yield (Okon and Labandera-González 1994; Cassán et al. 2003) of many crop plants can likely be explained, at least in part, by: (1) gibberellin production by endophytic bacteria (Bottini et al. 1989; Fulchieri et al. 1993; Janzen et al. 1992; Kucey 1988; Lucangeli and Bottini 1997, Piccoli et al. 1999), (2) deconjugation of gibberellin-glucosyl conjugates exuded by the roots, or in the plant (Piccoli et al. 1997), and (3) 3β-hydroxylation by bacterial enzymes of inactive 3-deoxy gibberellins present in roots,

to active forms such as GA₁, GA₃ and GA₄ (Piccoli and Bottini 1994a; Piccoli et al. 1996; Cassán et al. 2001a,b).

References

- Atzorn R, Crozier A, Wheeler C, Sandberg G (1988) Production of gibberellins and indole 3-acetic acid by *Rhizobium phaseoli* in relation to nodulation of *Phaseolus vulgaris* roots. *Planta* 175:532–538
- Bashan Y, Levanony H (1990) Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. *Can J Microbiol* 36:591–608
- Bastián F, Cohen A, Piccoli P, Luna V, Baraldi R, Bottini R (1998) Production of indole-3-acetic acid and gibberellins A₁ and A₃ by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. *Plant Growth Regul* 24:7–11
- Bastián F, Rapparini F, Baraldi R, Piccoli P, Bottini R (1999) Inoculation with *Acetobacter diazotrophicus* increases glucose and fructose content in shoots of *Sorghum bicolor* (L.) Moench. *Symbiosis* 27:147–156
- Bottini R, Luna V (1993) Bud dormancy in deciduous fruit trees. *Curr Top Plant Physiol* 1:147–159
- Bottini R, Fulchieri M, Pearce D, Pharis RP (1989) Identification of Gibberellins A₁, A₃, and isoA₃ in cultures of *Azospirillum lipoferum*. *Plant Physiol* 90:45–47
- Cacciari I, Lippi D, Pietrosanti T (1989) Phytohormone-like substances produced by single and mixed diazotrophic cultures of *Azospirillum* sp. and *Arthrobacter*. *Plant Soil* 115:151–153
- Candau R, Avalos J, Cerdá-Olmedo E (1992) Regulation of gibberellin biosynthesis in *Gibberella fujikuroi*. *Plant Physiol* 100:1184–1188
- Cassán F (2003) Activación de giberelinas in vivo por bacterias endofíticas a través de la deconjugación de glucosil conjugados y la 3β-hidroxilación. PhD Thesis, Universidad Nacional de Río Cuarto
- Cassán F, Bottini R, Schneider G, Piccoli P (2001a) *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA₂₀ and metabolize the resultant aglycones to GA₁ in seedlings of rice dwarf mutants. *Plant Physiol* 125:2053–2058
- Cassán F, Lucangeli C, Bottini R, Piccoli P (2001b) *Azospirillum* spp. Metabolize [17,17-²H₂]Gibberellin A₂₀ to [17,17-²H₂] Gibberellin A₁ in vivo in *dy* rice mutant seedlings. *Plant Cell Physiol* 42:763–767
- Cassán FD, Piccoli P, Bottini R (2003) Promoción del crecimiento vegetal por *Azospirillum* sp. a través de la producción de giberelinas. Un modelo alternativo para incrementar la producción agrícola. In: Albanesi A, Kunst C, Anriquez A, Luna S, Ledesma R (eds) *Microbiología Agrícola. Un aporte de la investigación en Argentina para la sociedad*. Universidad Nacional de Santiago del Estero, Santiago, pp 1–16
- Cohen A, Travaglia C, Reinoso H, Piccoli P, Bottini R (2001) *Azospirillum* inoculation and inhibition of gibberellin and ABA synthesis in maize seedlings under drought. *Proc Plant Growth Regul Soc Am* 28:88–93
- Creus C, Sueldo R, Barassi C (1997) Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. *Plant Physiol Biochem* 35:939–944
- Crozier A, Kamiya Y, Bishop G, Yokota T (2000) Biosynthesis of hormones and elicitor molecules. In: Buchanan BB, Gruissem W, Jones RL (eds) *Biochemistry and molecular biology of plants*. American Society of Plant Physiology, Rockville, pp 850–929
- Curtis PJ, Cross BE (1954) Gibberellic acid. A new metabolite from the culture filtrates of *Gibberella fujikuroi*. *Chem Ind* 1066
- Davies PJ (1995) The plant hormones: their nature, occurrence and functions. In: Davies PJ (ed) *Plant hormones. Physiology, biochemistry and molecular biology*. Kluwer, Dordrecht, pp 1–12
- De-Polli H, Matsui E, Döbereiner J, Salatti E (1977) Confirmation of nitrogen fixation in two typical grasses by ¹⁵N₂ incorporation. *Soil Biol Biochem* 9:119–123
- Dobert RC, Rood SB, Blevins DG (1992) Gibberellins and the legume-Rhizobium symbiosis. I. Endogenous gibberellins of lima bean (*Phaseolus lunatus* L.) stems and nodules. *Plant Physiol* 98:221–224
- Döbereiner J, Mariel IE, Nery M (1976) Ecological distribution of *Spirillum lipoferum* Beijerinck. *Can J Microbiol* 22:1464–1473
- Flouri F, Sini K, Balis C (1995) Interactions between *Azospirillum* and *Phialophora radiculicola*. *NATO ASI Ser G* 37:231–237
- Fulchieri M, Lucangeli C, Bottini R (1993) Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots. *Plant Cell Physiol* 34:1305–1309
- Glick BR, Patten CL, Holquin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College, London
- Gutiérrez-Mañero F, Ramos-Solano B, Probanza A, Mehouchi J, Tadeo FR, 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
- Hedden P, Phillips A (2000) Gibberellin metabolism: new insights revealed genes. *Trends Plant Sci* 5:523–530
- Janzen R, Rood S, Dormar J, McGill W (1992) *Azospirillum brasilense* produces gibberellins in pure culture and chemically-medium and in co-culture on straw. *Soil Biol Biochem* 24:1061–1064
- King RW, Evans LT (2003) Gibberellins and flowering of grasses and cereals: prising open the lid of the “Florigen” black box. *Annu Rev Plant Physiol Plant Mol Biol* 54:307–328
- Kucey RMN (1988) Plant growth-altering effects of *Azospirillum brasilense* and *Bacillus C-11-25* on two wheat cultivars. *J Appl Bacteriol* 64:187–196
- Linnemannstons P, Voß T, Hedden P, Gaskin P, Tudzynski B (1999) Deletions in the gibberellin biosynthesis gene cluster of *Gibberella fujikuroi* by restriction enzyme-mediated integration and conventional transformation-mediated mutagenesis. *Appl Environ Microbiol* 65:2558–2564
- Litchenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol* 50:47–65
- Lucangeli C, Bottini R (1996) Reversion of dwarfism in *dwarf-1* maize (*Zea mays* L.) and *dwarf-x* rice (*Oryza sativa* L.) mutants by endophytic *Azospirillum* spp. *Biocell* 20:223–228
- Lucangeli C, Bottini R (1997) Effects of *Azospirillum* spp. on endogenous gibberellin content and growth of maize (*Zea mays* L.) treated with uniconazole. *Symbiosis* 23:63–72
- Ludden PW, Okon Y, Burris RH (1978) The nitrogenase system of *Spirillum lipoferum*. *Biochem J* 173:1001–1003
- MacMillan J (1997) Biosynthesis of the gibberellin plant hormones. *Nat Prod Rep* 14:221–243
- MacMillan J (2002) Occurrence of gibberellins in vascular plants, fungi and bacteria. *J Plant Growth Regul* 20:387–442
- Macmillan J, Suter PJ (1958) The occurrence of gibberellin A₁ in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). *Naturwissenschaften* 45:46
- Mende K, Homann V, Tudzynski B (1997) The geranylgeranyl diphosphate synthase gene of *Gibberella fujikuroi*: isolation and expression. *Mol Gen Genet* 255:96–105
- Okon Y, Labandera-González C (1994) Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biol Biochem* 26:1591–1601
- Okon Y, Albrecht SL, Burris RH (1976a) Factors affecting growth and nitrogen fixation of *Spirillum lipoferum*. *J Bacteriol* 127:1248–1254
- Okon Y, Albrecht SL, Burris RH (1976b) Carbon and ammonia metabolism of *Spirillum lipoferum*. *J Bacteriol* 128:592–597
- Pharis RP, King RW (1985) Gibberellins and reproductive development in seed plants. *Annu Rev Plant Physiol* 36:517–568

- Piccoli P, Bottini R (1994a) Metabolism of 17,17- $^{2}\text{H}_2$ gibberellin A_{20} to 17,17- $^{2}\text{H}_2$ gibberellin A_1 by *Azospirillum lipoferum* cultures. *AgriScientia* XI:13–15
- Piccoli P, Bottini R (1994b) Effects of C/N relationships, N content, pH, and time of culture on growth and gibberellin production of *Azospirillum lipoferum* cultures. *Symbiosis* 17:229–236
- Piccoli P, Bottini R (1996) Light enhancement of gibberellin production by *Azospirillum lipoferum* cultures. *Biocell* 20:200–207
- Piccoli P, Masciarelli O, Bottini R (1996) Metabolism of 17,17- $^{2}\text{H}_2$ -Gibberellins A_4 , A_9 , and A_{20} by *Azospirillum lipoferum* in chemically-defined culture medium. *Symbiosis* 21:167–178
- Piccoli P, Lucangeli D, Schneider G, Bottini R (1997) Hydrolysis of [17,17- $^{2}\text{H}_2$]Gibberellin A_{20} -Glucoside and [17,17- $^{2}\text{H}_2$]Gibberellin A_{20} -glucosyl ester by *Azospirillum lipoferum* cultured in a nitrogen-free biotin-based chemically-defined medium. *Plant Growth Regul* 23:179–182
- Piccoli P, Masciarelli O, Bottini R (1999) Gibberellin Production by *Azospirillum lipoferum* cultured in chemically-defined medium as affected by oxygen availability and water status. *Symbiosis* 27:135–146
- Probanza A, García JAL, Palomino MR, Ramos B, Manero FJG (2002) *Pinus pinea* L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). *Appl Soil Ecol* 20:75–84
- Reinoso H, Dauría C, Luna V, Pharis R, Bottini R (2002) Dormancy in peach (*Prunus persica* L.) flower buds VI. Effects of gibberellins and an acylcyclohexanedione (Cimectacarb) on bud morphogenesis in field experiments with orchard trees and on cuttings. *Can J Bot* 80:656–663
- Rojas MC, Hedden P, Gaskin P, Tudzynski B (2001) The *P450-1* gene of *Gibberella fujikuroi* encodes a multifunctional enzyme in gibberellin biosynthesis. *Proc Natl Acad Sci USA* 98:5838–5843
- Schneider G (1983) Gibberellin conjugates. In: Crozier A (ed) *The biochemistry and physiology of gibberellins*, vol 1. Praeger, New York, pp 389–456
- Sponsel VM (2002) The deoxy xylulose phosphate pathway for the biosynthesis of plastidic isoprenoids: early days in our understanding of the early stages of gibberellin biosynthesis. *J Plant Growth Regul* 20:332–345
- Sponsel VM (2003) Gibberellins. In: Henry HL, Norman AW (eds) *Encyclopedia of hormones*, vol 2. Academic, pp 29–40
- Sponsel VM, Hedden P (2004) Gibberellin biosynthesis and catabolism. In: Davies PJ (ed) *Plant hormones: biosynthesis, signal transduction, action!* Kluwer, Dordrecht
- Tamura S (1990) Historical aspects of gibberellins. In: Takahashi N, Phinney BO, MacMillan J (eds) *Gibberellins*. Springer, Berlin Heidelberg New York, pp 1–8
- Tanimoto E (1987) Gibberellin-dependent root elongation in *Lactuca sativa*: recovery from growth retardant-suppressed elongation with thickening by low concentration of GA_3 . *Plant Cell Physiol* 28:963–973
- Trewavas A (2000) Signal perception and transduction. In: Buchanan BB, Gruissem W, Jones RL (eds) *Biochemistry and molecular biology of plants*. American Society of Plant Physiology, Rockville, pp 930–987
- Tudzynski B, Hedden P, Carrera E, Gaskin P (2001) The *P450-4* Gene of *Gibberella fujikuroi* encodes *ent*-kaurene oxidase in the gibberellin biosynthesis pathway. *Appl Environ Microbiol* 67:3514–3522
- Tudzynski B, Rojas MC, Gaskin P, Hedden P (2002) The gibberellin 20-oxidase of *Gibberella fujikuroi* is a multifunctional mono-oxygenase. *J Biol Chem* 277:21246–21253
- Tudzynski B, Mihlan M, Rojas MC, Linnemannstons P, Gaskin P, Hedden P (2003) Characterization of the final two genes of the gibberellin biosynthesis gene cluster of *Gibberella fujikuroi*: des and P450-3 encode GA4 desaturase and the 13-hydroxylase, respectively. *J Biol Chem* 278:28635–28643
- Tully RE, van Berkum P, Lovins KW, Keister DL (1998) Identification and sequencing of a cytochrome P450 gene cluster from *Bradyrhizobium japonicum*. *Biochim Biophys Acta* 1398:243–255
- Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A, Corich V, Giacomini A, de Bruijn F, Rademaker J, Maya-Flores J, Ostrom P, Vega-Hernández M, Hollingsworth RI, Martínez-Molina E, Mateos P, Velázquez E, Wopereis J, Triplett E, Umali-García M, Anarna JA, Rolfe BG, Ladha JK, Hill J, Mujoo R, Ng PK, Dazzo FB (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. trifolii with rice roots. *Funct Plant Biol* 28:845–870