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FOCUS PAPER

Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake

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Abstract

Plant growth-promoting bacteria (PGPB) and NO_3^- availability both affect NO_3^- uptake and root architecture. The presence of external NO_3^- induces the expression of NO_3^- transporter genes and elicits lateral root elongation in the part of the root system exposed to the NO_3^- supply. By contrast, an increase in NO_3^- supply leads to a higher plant N status (low N demand), which represses both the NO_3^- transporters and lateral root development. The effects of PGPB on NO_3^- uptake and root development are similar to those of low NO_3^- availability (concomitant stimulation of NO_3^- uptake rate and lateral root development). The mechanisms responsible for the localized and long-distance regulation of NO_3^- uptake and root development by NO_3^- availability are beginning to be elucidated. By contrast, the signalling and transduction pathways elicited by the rhizobacteria remain totally unknown. This review will compare the effects of NO_3^- availability and PGPB on root morphogenesis and NO_3^- uptake, in order to determine whether interactions exist between the NO_3^--dependent and the PGPB-dependent regulatory pathways.

Key words: N demand, nitrate uptake, plant growth-promoting bacteria, plasticity, rhizosphere, root development.

Introduction

The rhizosphere is the portion of the soil that forms the complex habitat of plant roots, and the composition of which is altered by root activity (Hiltner, 1904). An important component of the rhizosphere is the actively growing microbial population which thrives due to the provision of organic nutrients in root exudates. In turn, the microorganisms that colonize the rhizosphere profoundly affect root and plant biology in relation to nutrition, development and health. Current knowledge of root development and physiology, however, essentially comes from studies that did not take into account the effects of the rhizosphere. On the one hand, the wealth of literature and an understanding of root biology has demonstrated the strength of investigations that avoid microbial interactions in order to decipher the underlying mechanisms involved in nutrient uptake and root development. On the other hand, the influence of the rhizosphere microflora on root biology is so important that it is crucial to evaluate how the basic nutritional and developmental processes of the plant are affected.

Micro-organisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots. Some of these micro-organisms are plant pathogens whereas others trigger beneficial effects. Among the latter, one can distinguish two large categories. First, mycorrhizal fungi and bacteria belonging to the genera *Rhizobium* and *Frankia* are able to establish a symbiotic relationship with the roots of host plants (Harley and Smith, 1983; Broughton and Perret, 1999). Such interactions lead to morphologically distinct structures, namely mycorrhizes and nodules, that are sites with enhanced nutritional functions that neither partner would carry out alone (Hadri *et al*., 1998; Albrecht *et al*., 1999; Martin and Plassard, 2001; Trinchant *et al*., 2001). Second, a subset of bacteria, referred to as plant growth-promoting bacteria (PGPB; Bashan and Holguin, 1998), have beneficial effects on plants without developing such symbiotic associations. While the mechanisms involved in pathogenicity and symbiosis have been extensively studied, the interactions between PGPB and plant roots are not clearly defined (Glick, 1995).

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PGPB are usually classified into two groups according to whether they affect plant growth indirectly or directly, referred to as biocontrol PGPB and PGPB, respectively (Bashan and Holguin, 1998). It is worthwhile to mention that most PGPB are likely to elicit both biocontrol, that prevents deleterious effects of phytopathogenic microorganisms, and plant growth promotion. The mechanisms involved in biocontrol by rhizosphere bacteria have been reviewed elsewhere (Whipps, 2001; Persello-Cartieaux *et al*., 2003) and will not be discussed here. The occurrence of direct effects on plant growth has been demonstrated using gnotobiotic culture systems where a single bacteria strain interacts with the host plant (Lifshitz *et al*., 1987; Bertrand *et al*., 2001). The plant response to PGPB is obviously a very complex phenomenon resulting from the combination of mechanisms which affect several aspects of mineral nutrition and root development (Cleyet-Marel *et al*., 2001). Considering the quantitative importance of nitrogen in plant mineral nutrition and given that nitrogen availability and PGPB are known to trigger changes in both plant nutrition and root development, this review focuses on the possible interactions between the plant responses to these abiotic and biotic factors.

Is the primary effect of PGPB on plants a nutritional or a developmental effect?

Many of the PGPB described to date are free-living diazotrophs that can convert molecular nitrogen into ammonia in a free state by virtue of the nitrogenase enzyme complex (Postgate, 1982). Another feature that has been reported for PGPB is the production of phytohormones, which could affect root development directly. Since nutritional capacity and developmental control are very much dependent on each other and because they both affect the growth rate of the plant, one can ask whether the PGPB primarily act on one or the other.

It was attractive to explain the positive effect of PGPB on plant growth by the provision of nitrogen fixed by the bacteria to the host plant. This 'biofertilization' hypothesis was all the more attractive since N availability is the main yield-limiting factor in many agricultural situations and the leaching of N fertilizers into groundwater causes environmental problems. Experiments using acetylene reduction measurements and the ^{15}N isotope dilution technique provided conflicting results. For instance, large contributions to the plant N budget from plant-associated bacterial N_2-fixation have been reported with sugar cane (Urquiaga *et al*., 1992; Mirza *et al*., 2001) and mangrove (Bashan *et al*., 1998). Furthermore, gene reporter fusions demonstrated that the *Nif* genes of *Azospirillum* and *Azoarcus* rhizobacteria strains are expressed (enhanced?) when associated with the roots of wheat and rice, respectively (Vande Broek *et al*., 1993; Egener *et al*., 1998, 1999). However the expression of *Nif* genes or active N_2 fixation by the rhizobacteria do not imply that there is a large transfer of newly fixed nitrogen to the plant. On the contrary, evidence that growth promotion by PGPB does not rely on the N_2-fixation process have been obtained for tomato seedlings associated with an *Azospirillum brasilense* mutant strain totally deficient in N_2-fixation capacity (Bashan *et al*., 1989*b*). Overall, most of the inoculation experiments reported in the literature failed to show a significant contribution of N_2 fixation by PGPB to the plant N status (Lethbridge *et al*., 1982; Boddey *et al*., 1986; Okon and Kapulnik, 1986; Bremner *et al*., 1995). This is in complete contrast to symbiotic associations where relatively large amounts of atmospheric N reach the plant as ammonia released by the bacteroids. By contrast, most of the ammonia produced in PGPB by the nitrogenase-catalysed N_2 fixation would be assimilated by the rhizobacteria through the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. Considering the high cost of the N_2-fixation reaction (Phillips, 1980), the bacteria tend to limit the loss of fixed N. The leak of ammonia out of the bacteria cells may be minimized by virtue of the GS/GOGAT pathway operating at a high rate (Steenhoudt and Vanderleyden, 2000). In addition, ammonium transporters found in several PGPB strains are thought to be involved in the reabsorption of NH_4^+ released as a consequence of NH_3 diffusion through the bacterial membrane (Van Dommelen *et al*., 1997). The inoculation of tomato roots with a wild-type strain, but not with *nif* mutant strains, of *Azospirillum brasilense* induced the tomato *LeAmt1.2* gene coding for an ammonium transporter (Becker *et al*., 2002). This suggests that due to its N_2-fixation activity the bacteria excrete NH_4^+ in quantities consistent with the induction of the *LeAmt1.2* gene, i.e. in the micromolar range (higher concentrations reduces *LeAmt1.2* mRNA levels). Quantification of N transfer would be necessary to assess whether the ammonium released by the bacteria is used as an N source by the plant. Alternatively, the authors proposed that NH_4^+ ions play a signalling role in the interactions between PGPB and plants (Becker *et al*., 2002).

The positive effects of PGPB on plant growth are always correlated with remarkable changes in root morphology, namely increased lateral root length and root hair number and length (Tien *et al*., 1979; de Freitas and Germida, 1989; Pacovsky, 1990; Okon and Vanderleyden, 1997; Bertrand *et al*., 2000). It is generally assumed that these developmental responses are triggered by phytohormones produced by the bacteria (Bloemberg and Lugtenberg, 2001; Persello-Cartieaux *et al*., 2003). Among the plant growth regulators, auxin may play a major role supported by the fact that a screen for *Arabidopsis thaliana* mutants insensitive to *Pseudomonas* rhizobacteria resulted in the isolation of two mutants altered in the *Aux1* auxin influx transporter (Persello-Cartieaux *et al*., 2001). One must

keep in mind that the effect of auxin largely depends upon its concentration. Consistent with the hypothesis of an auxin-mediated effect of the *Pseudomonas* strains, inoculation with increasing doses of these rhizobacteria had a positive impact on rooting up to a certain concentration of bacteria above which deleterious effects were observed (Persello-Cartieaux *et al*., 2003). Other phytohormones including cytokinins and gibberellins may be involved in the effect of PGPB on root morphogenesis (Steenhoudt and Vanderleyden, 2000). Furthermore, some PGPB strains have been shown to have an aminocyclopropane carboxylate deaminase (Shah *et al*., 1998), an enzyme that hydrolyses ACC. Such bacteria are likely to divert ACC, the precursor of ethylene, from the plant root, which has the effect of reducing the inhibition of root growth by ethylene (Glick *et al*., 1998). Considering the numerous interactions that exist between the different hormone signalling pathways in plants, it is difficult to assess which of these pathways is the primary target of PGPB. More likely, the rhizobacteria alters not just a single, but several, hormonal pathways, which could account for the different morphological changes observed, for example, lateral root elongation and root hair development.

An increase in the root surface area and the volume of soil foraged by the root, which leads to an enhanced nutrient uptake, is the most commonly proposed explanation for the beneficial effects of PGPB on plant growth. The improvement in mineral nutrition would explain the promotion of shoot growth. This rationale is consistent with the observation that plants inoculated with *Azopsirillum* take up N, P, K, and microelements more efficiently from the soil (Okon and Vanderleyden, 1997). However, PGPB may enhance mineral uptake, not only as a consequence of the increase in root surface area but also by stimulating the ion uptake systems. Considering the relationship between the effects of PGPB on root morphology and nutrient uptake, the experimental data published is mainly correlative. The different responses of plants to PGPB include stimulation of root branching and root hair development, stimulation of total nutrient uptake (especially that of nitrogen), and an increase in biomass accumulation. How these different responses are related mechanistically is not known. Considering nitrogen nutrition, *a priori* plants can acquire N under two mineral forms, namely nitrate (NO_3^-) and ammonium (NH_4^+). However, because the concentration of NH_4^+ in the soil is generally much lower than that of NO_3^- (Marschner, 1995) and because the development of most plants is optimal on NO_3^- rather than on NH_4^+ (cf. the ammonium syndrome, Mehrer and Mohr, 1989), the favoured source for N uptake is NO_3^-. On the other hand, NO_3^- availability is known to affect both root branching and NO_3^- uptake itself (Forde, 2002). Because NO_3^- availability and PGPB affect the same developmental and physiological processes in plant roots, the question is whether the responses share a mechanistic basis. Two points will be addressed in this review: (1) Are the responses of root morphogenesis to NO_3^- and to PGPB independent of each other? (2) Do PGPB affect NO_3^- uptake directly or is the gain in plant N only a consequence of the increase in root surface area?

Root morphogenesis as affected by both nitrate availability or PGPB inoculation

As mentioned above, one of the more characteristic effects of PGPB is an increased elongation rate, and perhaps initiation rate, of lateral roots resulting in a more branched root system architecture (Kapulnik *et al*., 1985*a*; Lifshitz *et al*., 1987).

Modifications of root architecture similar to those elicited by PGPB are triggered by the changes in NO_3^- available in the medium (Wiersum, 1958). These plastic responses have been characterized in detail using culture devices with uneven distribution of NO_3^- (vertical layers, split root systems) (Drew *et al*., 1973; Drew, 1975; Friend *et al*., 1990; Burns, 1991). Molecular studies performed in *Arabidopsis thaliana* (Zhang and Forde, 1998; Zhang *et al*., 1999) showed that a dual pathway process operates in plants: a localized control, that senses the presence of exogenous NO_3^-, induces lateral root elongation; a systemic control inhibits root growth in plants with a high N status. The model proposes that with uneven NO_3^- supplies (e.g. a plant which only has a portion of the root system in the presence of NO_3^-), the systemic inhibition of lateral root growth would be released, and lateral root growth enhanced, but only for those roots that develop in the NO_3^--containing zones.

Two genes of the signalling pathway that links external NO_3^- to increased rates of lateral root elongation have been identified, namely the MADS-box transcription factor *ANR1* and the *AXR4* gene involved in the auxin transduction pathway (Zhang *et al*., 1999). By contrast, the systemic pathway remains far more obscure. Neither the sensors of N status in the shoot, nor the signal translocated in the phloem, nor the transduction pathway responding to this signal in the roots have been identified. At the morphological level, high NO_3^- concentrations lead to a less branched root system, corresponding to fewer visible lateral roots (Zhang and Forde, 1998; Zhang *et al*., 1999; Tranbarger *et al*., 2003*a*). Studies performed with nitrate reductase-deficient *Arabidopsis* and tobacco mutants revealed that this response is related to a nitrate pool in the leaf (Scheible *et al*., 1997; Stitt and Feil, 1999; Zhang *et al*., 1999). However, since NO_3^- is not translocated in the phloem, another signal must serve to pass the information about the shoot's N status to the roots. In the case of the systemic control of NO_3^- uptake by the plant's N demand, the phloem-translocated signals are thought to be glutamine or other amino acids (Imsande and Touraine, 1994; Vidmar *et al*., 2000; Nazoa *et al*., 2003).

However, the amino acid pools are unlikely to be involved in the control of root development. This is indicated by the observation that the addition of glutamine or asparagine to the nutrient medium did not reduce the number and length of *Arabidopsis* lateral roots as high NO_3^- concentrations did (Tranbarger *et al*., 2003*a*, *b*). Recently, it has been proposed that hormones like auxin and ABA would act as the long-distance signals responsible for the systemic control of lateral root development (Forde, 2002; Casimiro *et al*., 2003). Also two transcription factor genes, the expression of which, in roots, correlated with the systemic plasticity response of the roots of *Arabidopsis thaliana*, have been identified (Tranbarger *et al*., 2003*a*). These genes are putative candidate targets of the long-distance N status signals arising in the shoot that regulate lateral root development.

The localized and systemic responses of root branching to NO_3^- appear to be exerted at different steps of lateral root development. The external presence of NO_3^- stimulates lateral root elongation by increasing the meristematic activity in lateral root tips (Zhang *et al*., 1999), while the accumulation of NO_3^- in shoot leads to a reduction in the number of lateral roots by stunting their development at some stage just before or after emergence (Zhang *et al*., 1999; Tranbarger *et al*., 2003*a*). Compared with what is known for NO_3^-, the manner in which lateral root development is affected by PGPB is much less characterized. In the literature, positive effects of PGPB have been mentioned for both the length or elongation rate and the number of lateral roots (Kapulnik *et al*., 1985*b*; Frommel *et al*., 1991; Sarig *et al*., 1992). Preliminary studies performed with a new strain of *Phyllobacterium* isolated from the rhizosphere of oilseed rape (Bertrand *et al*., 2001) indicated that these rhizobacteria have a marked effect on the length rather than the number of lateral roots (Larcher, *et al*., 2003). With the present state of knowledge, the possibility that the different PGPB strains may affect lateral root development at different stages cannot be ruled out. In addition, the morphological descriptions currently available are not detailed enough to determine whether NO_3^- and PGPB can affect the same step of lateral root development or not.

As mentioned above, the effect of PGPB on root morphogenesis is usually attributed to the release of phytohormones by the rhizobacteria, auxin being the most cited of these plant growth factors. Since a link between the signalling pathway involved in the localized response to NO_3^- and the auxin pathway has been reported (Zhang *et al*., 1999), there is speculation whether NO_3^- and PGPB effects on root morphogenesis share common steps or not. As far as is known, no experimental data are available to date to address this question. Nevertheless, because NO_3^- is often present in the soil, especially in agricultural conditions, but at varying concentrations, a more interesting consideration than the possible interactions between PGPB and the localized NO_3^- response pathway are the interactions between PGPB and the systemic NO_3^- response pathway. Again, no physiological, cellular or molecular data are available to respond unambiguously. However, a recent study performed with *Arabidopsis* with the *Phyllobacterium* strain isolated by Bertrand *et al*. (2001) suggests that high N status and PGPB affect lateral root development independently from each other: the rhizobacteria promoted lateral root growth whatever the NO_3^- concentration in the nutrient medium; conversely, increasing the NO_3^- concentration repressed lateral root growth whether the plants were inoculated or not (unpublished results).

A priori, one could envisage that PGPB could interfere with the NO_3^- effect on lateral root development via changes either in the rhizosphere NO_3^- concentration or the plant's N status. Some PGPB have been shown to have a nitrate reductase activity (Bothe *et al*., 1992; Larcher *et al*., 2003) suggesting that they can use NO_3^- as an N source. No evidence exists, but it is possible that NO_3^- used by the PGPB decreases the NO_3^- concentration at the root cell surface. By contrast, plants inoculated with PGPB generally have a higher N content than the uninoculated plants (Saudibet *et al*., 2002; authors' unpublished results), although this is not always observed (Dobbelaere *et al*., 2002). Decreasing NO_3^- concentration at the root surface *per se* is expected to have no effect on lateral root growth as long as NO_3^- concentration remains sufficient for *ANR1* induction. An increase in N status inhibits, not stimulates, lateral root development. Therefore, if changes in the NO_3^- rhizosphere concentration or the plant's N status due to PGPB are ever high enough to trigger significant effects on lateral root development, these effects would be negative, not positive as regularly observed.

Do PGPB affect the NO_3^- uptake systems?

It is generally assumed that PGPB trigger an increase in root surface area which results in an increased mineral uptake and, in turn, enhances shoot biomass accumulation. An enhanced plant 'nutrient uptake' by PGPB is widely described (Okon, 1985; Okon and Kapulnik, 1986; Bertrand *et al*., 2000), however, what uptake means must be clarified. Uptake refers to a function, namely the transport of a nutrient through the plasma membrane of root cells, i.e. the net flux from the growth medium to the plant that should theoretically be expressed per unit absorbing surface. Because of the difficulty in measuring this surface, it is usually expressed per unit of root weight. Therefore, if the increase in the amount of nutrient taken up from the medium in PGPB-inoculated plants were simply due to an increase in root surface, the effect of PGPB would be entirely dependent on root development, and not on the uptake function. NO_3^- uptake is known to be tightly regulated by the plant's N demand (Imsande and

Touraine, 1994; Touraine *et al*., 1994), changes in which normally result in opposite variations of the rates of root growth and NO_3^- uptake. This has been shown with lettuce plants grown in a split root device (Burns, 1991). The supply of an N-free nutrient solution to 90% of the root system first led to an increase of NO_3^- uptake by the 10% of the roots that remained supplied with NO_3^-. However, subsequently the NO_3^- uptake rate decreased while the proportion of root biomass in the NO_3^--containing part increased. It would thus be expected that the increase of root surface area in PGPB-inoculated plants be accompanied by a decrease of NO_3^- uptake rate, in order to maintain the total amount of NO_3^- taken up by the plant at the same level as in non-inoculated plants. Conversely, for the NO_3^- uptake rate to remain constant while the root surface increases, it would be required that the N demand regulation does not operate. This would imply that either the regulation of NO_3^- uptake in PGPB-inoculated plants differs from that in non-inoculated plants, or that N nutrition limits plant growth so that NO_3^- uptake is not down-regulated by the plant's N status. Considering the variety of culture conditions used in the different studies published, plant N nutrition is unlikely to be limited when plant growth promotion by rhizobacteria is observed. Furthermore, it was observed that *Arabidopsis thaliana* grown on non-limiting mineral nutrient medium inoculated with a *Phyllobacterium* strain had a positive effect on plant growth, while increasing the concentration of NO_3^- in the medium resulted in either no effects or even negative effects on plant growth (unpublished data).

If the increase in root surface alone cannot explain the gain in the plant's N status, then the possible effects of PGPB on NO_3^- uptake has to be considered. The data published on this question are very scarce. *Azospirillum brasilense* and *A. irakense* strains stimulated overall plant growth, including root development and grain yield of spring wheat and maize (Dobbelaere *et al*., 2002), but neither of the rhizobacteria changed the N concentration in plants or grains. The authors concluded that the inoculation did not enhance the uptake of minerals into the plant. By contrast, another study (Saudibet *et al*., 2002) led to the conclusion that NO_3^- uptake by the roots of spring wheat is stimulated by another *A. brasilense* strain. This conclusion was based on the observations that both the shoot:root ratio and the plant NO_3^- and N contents were higher in the inoculated plants so that the N accumulated:root biomass ratio was higher in inoculated plants. The effect of PGPB on NO_3^- uptake was investigated in oilseed rape inoculated with an *Achromobacter* strain using an electrophysiological approach (Bertrand *et al*., 2000). As frequently observed with PGPB, this strain led to a higher increase of shoot biomass than root biomass, resulting in a higher shoot:root ratio. This implies that the effect of PGPB on root branching and the resulting increase in root surface is not sufficient to explain the increase in total NO_3^- uptake since this latter increased more than the root surface did. Two different working hypotheses can be proposed: (1) PGPB could induce an increase in NO_3^- uptake rate, leading to stimulated shoot growth rate driven by the improved N nutrition; (2) PGPB could stimulate shoot growth rate by an alternative mechanism, leading to an increased NO_3^- uptake rate driven by the nutritional demand. In both hypotheses, whether it is a cause or a consequence of shoot growth stimulation, the NO_3^- uptake rate itself is expected to be higher in the roots of inoculated plants. Using ion-specific microelectrodes, it has been shown that the uptake rates of NO_3^- in the seminal root of oilseed rape increased in response to the inoculation with *Achromobacter* (Bertrand *et al*., 2000). It is remarkable that the uptake rate of the other ion tested, K^+, and the efflux of H^+ also increased, as described by others (Bashan *et al*., 1989*a*; Bashan, 1990). These findings suggest that the rhizobacteria affect mineral ion uptake globally due to a stimulation of the proton pump ATPase activity. Alternatively the *Achromobacter* strain could enhance the activity of several ion transporters, either directly by affecting the processes involved in their regulation in roots or indirectly via an increased nutritional demand as envisaged in hypothesis (2). Such changes in the plant's nutritional demand are known to trigger changes in mineral uptake rate. For instance, changing the growth rate of a plant, and hence the N demand, by modifying one environmental factor while the supply of NO_3^- to roots was kept constant, resulted in changes of NO_3^- uptake rate (Gastal and Saugier, 1989; Touraine *et al*., 1994).

Beside the possible direct effect of PGPB on NO_3^- uptake and the indirect effect via the increased root surface area due to the stimulation of lateral root development, the effect on root hair development has to be considered. Several PGPB strains have been shown to increase root hair size and number (de Freitas and Germida, 1989; Okon and Vanderleyden, 1997; Bertrand *et al*., 2000), and these tubular extensions of root epidermal cells can be involved in mineral uptake capacity in two ways. First, root hairs represent a large surface available for ion uptake and, second, they are believed to play an important role in nutrient uptake. The role of root hairs in mineral absorption, however, is usually largely overestimated. Indeed, anatomical observations and fluorescent dye experiments have shown that the epidermal cell layer forms a symplastic domain separated from the rest of the root symplasm due to a very low density of plasmodesmata (Morot-Gaudry and Touraine, 2000). Due to this anatomical feature, the main site where mineral ions enter the root symplasm for further transport to the other plant organs would be the outer cortical cell layers, not the epidermis. This assumption is supported for NO_3^- by the results of a compartmental analysis performed in barley. This study indicated that the roots contain two different NO_3^- pools, a small metabolic pool which does not supply NO_3^- for

xylem secretion, and a larger pool exchanging with the medium at one end and with the xylem sap at the other end (Siddiqi *et al*., 1991). The role of cortical cells in NO_3^- uptake is further supported by the finding that the high affinity NO_3^- transporter *NRT2.1* in *Arabidopsis* is expressed at higher levels in these cells than in the epidermal cells (Nazoa *et al*., 2003).

Conclusion

Despite their ability to fix atmospheric nitrogen, PGPB are unlikely to provide large amounts of this nitrogen to the plants. However, they have a great impact on nitrogen nutrition by increasing NO_3^- uptake capacity, indirectly as a consequence of stimulated lateral root development and possibly directly by stimulating NO_3^- transport systems. The question of the ability of PGPB to stimulate NO_3^- uptake, however, remains to be addressed directly. Although NO_3^- transporters are likely to be post-translationally regulated, the results of numerous expression studies suggest that NO_3^- uptake is primarily regulated at the transcriptional level (Forde, 2000; Vidmar *et al*., 2000; Touraine *et al*., 2001; Glass *et al*., 2002; Nazoa *et al*., 2003). The molecular tools available in *Arabidopsis* should, therefore, help to determine whether PGPB have an effect on NO_3^- transporters *per se*, directly or indirectly, or if the increased N efficiency is only due to the increase of root surface area elicited by the PGPB.

The effect of PGPB on root morphogenesis does not appear to be antagonistic to the effect exerted on root architecture by NO_3^-. To investigate whether the two underlying regulatory pathways are totally independent or if they share some common elements will require more detailed knowledge of the individual signalling pathways. In addition, the rapid progress of knowledge on the hormonal transduction pathways in plants will provide tools to determine which steps of these transduction pathways are targets of the PGPB.

Finally, the response processes of root development and mineral uptake to NO_3^- availability and PGPB inoculation are probably independent of each other. If this is true, the effects of these abiotic and biotic factors must be additive, i.e. the total root length would be the highest in inoculated plants grown with low N fertilization. This conclusion is consistent with the observation that the effects of inoculation are the highest in fields with low N fertilizer supply (Okon, 1985; Dobbelaere *et al*., 2002).

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References

Albrecht C, Geurts R, Bisseling T. 1999. Legume nodulation and mycorrhizae formation: two extremes in host specificity meet. *European Molecular Biology Organisation Journal* **18,** 281–288.

Bashan Y. 1990. Short exposure to *Azospirillum brasilense* Cd inoculation enhanced proton efflux of intact wheat roots. *Canadian Journal of Microbiology* **36,** 419–425.

Bashan Y, Levanony H, Mitiku G. 1989*a*. Changes in proton efflux of intact wheat roots induced by *Azospirillum brasilense* Cd. *Canadian Journal of Microbiology* **35,** 691–697.

Bashan Y, Holguin G. 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* **30,** 1225–1228.

Bashan Y, Singh CS, Levanony H. 1989*b*. Contribution of *Azospirillum brasilense* Cd to growth of tomato seedlings is not through nitrogen fixation. *Canadian Journal of Botany* **67,** 2429–2434.

Bashan Y, Puente ME, Myrold DD, Toledo G. 1998. *In vitro* transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. *FEMS Microbiology Ecology* **26,** 165–170.

Becker D, Stanke R, Fendrik I, Frommer WB, Vanderleyden J, Kaiser WM, Hedrich R. 2002. Expression of the NH_4^+-transporter gene *LEAMT1;2* is induced in tomato roots upon association with N_2-fixing bacteria. *Planta* **215,** 424–429.

Bertrand H, Nalin R, Bally R, Cleyet-Marel J-C. 2001. Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus*). *Biology and Fertility of Soils* **33,** 152–156.

Bertrand H, Plassard C, Pinochet X, Touraine B, Normand P, Cleyet-Marel JC. 2000. Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium (*Achromobacter* sp.). *Canadian Journal of Microbiology* **46,** 229–236.

Bloemberg GV, Lugtenberg BJJ. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobia. *Current Opinion in Plant Biology* **4,** 343–350.

Boddey RM, Baldani VLD, Baldani JI, Döbereiner J. 1986. Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field-grown wheat. *Plant and Soil* **95,** 109–121.

Bothe H, Körsgen H, Lehmacher T, Hundeshagen B. 1992. Differential effects of *Azospirillum*, auxin and combined nitrogen on the growth of the roots of wheat. *Symbiosis* **13,** 167–179.

Bremner E, Jenzen H, Gilbertson C. 1995. Evidence against associative N_2 fixation as a significant source of N in long-term wheat plots. *Plant and Soil* **175,** 13–19.

Broughton WJ, Perret X. 1999. Genealogy of legume–*Rhizobium* symbioses. *Current Opinion in Plant Biology* **2,** 305–311.

Burns I. 1991. Short and long-term effects of a change in the spatial distribution of nitrate in the root zone on N uptake, growth and root development of young lettuce plants. *Plant, Cell and Environment* **14,** 21–33.

Casimiro A, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G, Bennett MJ. 2003. Dissecting *Arabidopsis* lateral root development. *Trends in Plant Science* **8,** 165–171.

Cleyet-Marel J-C, Larcher M, Bertrand H, Rapior S, Pinochet X. 2001. Plant growth enhancement by rhizobacteria. In: Morot-Gaudry J-F, ed. *Nitrogen assimilation by plants. Physiological, biochemical and molecular aspects.* Enfield (NH, USA), Plymouth (UK): Science Publishers, 184–197.

De Freitas JR, Germida JJ. 1990. Plant growth-promoting rhizobacteria for winter wheat. *Canadian Journal of Microbiology* **36,** 265–272.

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Okon Y, Vanderleyden J. 2002. Effect of inoculation with wild type *Azospirillum brasilense* and *A. irakense* strains on development and nitrogen uptake of spring wheat and grain maize. *Biology and Fertility of Soils* **36,** 284–297.

Drew MC. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytologist* **75,** 479–490.

Drew MC, Saker LR, Ashley TW. 1973. Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on the growth of axes and laterals. *Journal of Experimental Botany* **24,** 1189–1202.

Egener T, Hurek T, Reinhold-Hurek B. 1998. Use of green fluorescent protein to detect expression of *nif* genes of *Azoarcus* sp. BH72, a grass-associated diazotroph, on rice roots. *Molecular Plant–Microbe Interactions* **11,** 71–75.

Egener T, Hurek T, Reinhold-Hurek B. 1999. Endophytic expression of *nif* genes of *Azoarcus* sp. strain BH72 in rice roots. *Molecular Plant–Microbe Interactions* **12,** 813–819.

Forde BG. 2000. Nitrate transporters in plants: structure, function and regulation. *Biochimica et Biophysica Acta* **1465,** 219–235.

Forde BG. 2002. Local and long-range signaling pathways regulating plant responses to nitrate. *Annual Review of Plant Physiology and Plant Molecular Biology* **53,** 203–224.

Friend AL, Eide MR, Hinckley TM. 1990. Nitrogen stress alters root proliferation in Douglas fir seedlings. *Canadian Journal of Forest Research* **20,** 1524–1529.

Frommel MI, Nowak J, Lazarovits G. 1991. Growth enhancement and developmental modifications of *in vitro*-grown potato. *Solanum tuberosum* spp. *tuberosum*) as affected by a non-fluorescent *Pseudomonas* sp. *Plant Physiology* **96,** 928–936.

Gastal F, Saugier B. 1989. Relationships between nitrogen uptake and carbon assimilation in whole plants of tall fescue. *Plant, Cell and Environment* **12,** 407–418.

Glass ADM, Britto DT, Kaiser BN, *et al*. 2002. The regulation of nitrate and ammonium transport systems in plants. *Journal of Experimental Botany* **53,** 855–864.

Glick BR. 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41,** 109–117.

Glick BR, Penrose DM, Li J. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology* **190,** 63–68.

Hadri A-E, Spaink HP, Bisseling, Brewin NJ. 1998. Diversity of root nodulation and rhizobial infection processes. In: Spaink HP, Kondorosi A, Hooykaas PJJ, eds. *The Rhizobiaceae*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 347–360.

Harley JL, Smith SE. 1983. *Mycorrhizal symbiosis*. London: Academic Press.

Hiltner L. 1904. Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbackteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache. *Arbeiten Deutscher Landwirtschafts Gesellschaft* **98,** 59–78.

Imsande J, Touraine B. 1994. N demand and the regulation of nitrate uptake. *Plant Physiology* **105,** 3–7.

Kapulnik Y, Okon Y, Henis Y. 1985*a*. Changes in root morphology of wheat caused by *Azospirillum* inoculation. *Canadian Journal of Microbiology* **31,** 881–887.

Kapulnik Y, Gafni R, Okon Y. 1985*b*. Effect of *Azospirillum* spp. inoculation on root development and NO_3^- uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponic system. *Canadian Journal of Botany* **63,** 187–193.

Larcher M, Muller B, Mantelin S, Rapior S, Cleyet-Marel J-C. 2003. Early modifications of *Brassica napus* root system architecture induced by a plant growth-promoting *Phyllobacterium* strain. *New Phytologist* **160,** 119–125.

Lethbridge G, Davidson MS, Sparling GP. 1982. Critical evaluation of the acetylene reduction test for estimating the activity of nitrogen-fixing bacteria associated with the roots of wheat and barley. *Soil Biology and Biochemistry* **14,** 27–35.

Lifshitz R, Kloepper JW, Kozlowski M, Simonson C, Carlson J, Tipping EM, Zaleska I. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Canadian Journal of Microbiology* **33,** 390–395.

Marschner H. 1995. *Mineral nutrition in higher plants*. London: Academic Press.

Martin F, Plassard C. 2001. Nitrogen assimilation by ectomycorrhizal symbiosis. In: Morot-Gaudry J-F, ed. *Nitrogen assimilation by plants. Physiological, biochemical and molecular aspects.* Enfield (NH, USA), Plymouth (UK): Science Publishers, 169–183.

Mehrer I, Mohr H. 1989. Ammonium toxicity: description of the syndrome in *Sinapis alba* and the search for its causation. *Physiologia Plantarum* **77,** 545–554.

Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA. 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro*. *Plant and Soil* **237,** 47–54.

Morot-Gaudry JF, Touraine B. 2000. Sources of nitrogen, nitrogen cycle, root structure and nitrogen assimilation. In: Morot-Gaudry J-F, ed. *Nitrogen assimilation by plants. Physiological, biochemical and molecular aspects.* Enfield (NH, USA), Plymouth (UK): Science Publishers, 5–14.

Nazoa P, Vidmar JJ, Tranbarger TJ, Mouline K, Damiani I, Tillard P, Zhuo D, Glass ADM, Touraine B. 2003. Regulation of the nitrate transporter gene *AtNRT2.1* in *Arabidopsis thaliana*: responses to nitrate, amino acids and developmental stage. *Plant Molecular Biology* **52,** 689–703.

Okon Y. 1985. *Azospirillum* as a potential inoculant for agriculture. *Trends in Biotechnology* **3,** 223–228.

Okon Y, Kapulnik Y. 1986. Development and function of *Azospirillum*-inoculated roots. *Plant and Soil* **90,** 3–16.

Okon Y, Vanderleyden J. 1997. Root-associated *Azospirillum* species can stimulate plants. *ASM News* **63,** 366–370.

Pacovsky RS. 1990. Development and growth effects in the *Sorghum–Azospirillum* association. *Journal of Applied Bacteriology* **68,** 555–563.

Persello-Cartieaux F, David P, Sarrobert C, Thibaud M-C, Achouak W, Robaglia C, Nussaume L. 2001. Utilization of mutants to analyze the interaction between *Arabidopsis thaliana* and its naturally root-associated *Pseudomonas*. *Planta* **212,** 190–198.

Persello-Cartieaux F, Nussaume L, Robaglia C. 2003. Tales from the underground: molecular plant–rhizobia interactions. *Plant, Cell and Environment* **26,** 189–199.

Phillips DA. 1980. Efficiency of symbiotic nitrogen fixation in legumes. *Annual Review of Plant Physiology* **31,** 29–49.

Postgate JR. 1982. Biological nitrogen fixation: fundamentals. *Philosophical Transactions of the Royal Society of London, Series B* **296,** 375–385.

Sarig S, Okon Y, Blum A. 1992. Effect of *Azospirillum brasilense* inoculation on growth dynamics and hydraulic conductivity of *Sorghum bicolor* roots. *Journal of Plant Nutrition* **15,** 805–819.

Saudibet MI, Fatta N, Barneix AJ. 2002. The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant and Soil* **245,** 215–222.

Scheible WR, Lauerer M, Schulze ED, Caboche M, Stitt M. 1997. Accumulation of nitrate in the shoot acts as a signal to

regulate shoot–root allocation in tobacco. *The Plant Journal* **11,** 671–691.

Shah S, Li J, Moffatt BA, Glick BR. 1998. Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria. *Canadian Journal of Microbiology* **44,** 833–843.

Siddiqi MY, Glass ADM, Ruth TJ. 1991. Studies of the uptake of nitrate in barley. III. Compartmentation of NO_3^-. *Journal of Experimental Botany* **42,** 1455–1463.

Steenhoudt O, Vanderleyden J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews* **24,** 487–506.

Stitt M, Feil R. 1999. Lateral root frequency decreases when nitrate accumulates in tobacco transformants with low nitrate reductase activity: consequences for the regulation of biomass partitioning between shoots and root. *Plant and Soil* **215,** 143–153.

Tien TM, Gaskins MH, Hubbell DH. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum*). *Applied and Environmental Microbiology* **37,** 1016–1024.

Touraine B, Clarkson DT, Muller B. 1994. Regulation of nitrate uptake at the whole plant level. In: Roy J, Garnier E, eds. *A whole plant perspective on carbon–nitrogen interactions*. The Hague, The Netherlands: SPB Academic Publishing, 11–30.

Touraine B, Daniel-Vedele F, Forde BG. 2001. Nitrate uptake and its regulation. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin, Heidelberg: INRA-Editions and Springer-Verlag, 1–36.

Tranbarger TJ, Al-Ghazi Y, Muller B, Teyssendier de la Serve B, Doumas P, Touraine B. 2003*a*. Transcription factor genes with expression correlated to nitrate-related root plasticity of *Arabidopsis thaliana*. *Plant, Cell and Environment* **26,** 459–469.

Tranbarger TJ, Al-Ghazi Y, Muller B, Teyssendier de la Serve B, Doumas P, Touraine B. 2003*b*. A macro-array-based screening approach to identify transcriptional factors involved in the nitrogen-related root plasticity response of *Arabidopsis thaliana*. *Agronomie* **23,** 519–529.

Trinchant J-C, Drevon J-J, Rigaud J. 2001. Symbiotic nitrogen fixation. In: Morot-Gaudry J-F, ed. *Nitrogen assimilation by plants. Physiological, biochemical and molecular aspects.* Enfield (NH, USA), Plymouth (UK): Science Publishers, 121–134.

Urquiaga S, Cruz KHS, Boddey RM. 1992. Contribution of nitrogen fixation to sugarcane: nitrogen-15 and nitrogen balance estimates. *Soil Science Society of America Journal* **56,** 105–114.

Van Dommelen A, Van Bastelaere E, Keijers V, Vanderleyden J. 1997. *Genetics* of *Azospirillum brasilense* with respect to ammonium transport, sugar uptake, and chemotaxis. *Plant and Soil* **194,** 155–160.

Vande Broek A, Michiels J, Van Gool A, Vanderleyden J. 1993. Spatial-temporal colonization patterns of *Azospirillum brasilense* on the wheat root surface and expression of the bacterial *nifH* gene during association. *Molecular Plant–Microbe Interactions* **6,** 592–600.

Vidmar JJ, Zhuo D, Siddiqi MY, Schoerring JK, Touraine B, Glass ADM. 2000. Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley. *Plant Physiology* **123,** 307–318.

Whipps JW. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* **52,** 487–511.

Wiersum LK. 1958. Density of root branching as affected by substrate and separate ions. *Acta Botanica Neerlandica* **7,** 174–190.

Zhang H, Forde BG. 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279,** 407–409.

Zhang H, Jennings A, Barlow PW, Forde BG. 1999. Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences, USA* **96,** 6529–6534.