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Analytical Interpretation of the Beneficial Interaction Between Microorganisms and Grasses

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Abstract

Soil microorganisms compose $\frac{1}{4}$ of the biodiversity of our planet and are responsible for important processes such as the decomposition of organic residues and the transformation of the nutrients contained in these residues into nutrients for plants. The microorganisms also aid the grasses implantation, increasing the grasses yield by means of several mechanisms of plant growth promotion. These mechanisms of growth promotion of grasses can be direct, or indirect. In this chapter, we discuss the main mechanisms of growth promotion of grasses by soil microorganisms. It will be explained how the microorganisms in the soil act favoring the growth and development of cultivated grasses. For this, there will be clarified the importance of soil microorganisms in nutrient cycling, the mechanisms of nutrient capture, the production of phytostimulant substances by microorganisms, and the mechanisms of soil pathogen suppression.

Keywords: nutrient cycling, biological nitrogen fixation, interaction between plants, microorganisms

1. Introduction

The microbial population inhabiting the rhizosphere consists of a wide range of organisms, which together interact directly and indirectly with the cultivated plants. Only with regard to the number of bacteria, it is estimated that there are about 2 billion cells per gram of soil [1]. These microorganisms become interesting to the human species, as they interfere in the yield of the cultivated plants, by means of several mechanisms.

A microorganism is considered a plant growth promoter when it is capable of increasing the yield of the crops of interest. To measure this capacity, the interaction of a given microorganism with some plants of interest must first be evaluated under axenic conditions and in comparison

with cultivated plants. It is fundamental that this initial stage is studied in isolation of the interaction of the plant with the organism, thus isolating the interaction of other factors such as climate, environment, and other edaphic or epiedaphic macro or microorganisms to make sure that the effect on the yield of the plants of interest is solely and exclusively due to the inoculated microorganism. Without this initial screening under axenic conditions, it would be impossible to certify and prove that the positive effect observed in the studied plant is due to the microorganism of interest.

Only after the positive effect of the microorganism on the plant has been proven, this interaction will be tested under conditions of greater interference, such as greenhouse, fertilization, or soil conditions with an original field microbial population (nonsterile soil). Under these conditions, the resistance of the interaction to various interference factors will be tested. Once approved in tests conducted under controlled conditions, the microorganisms are tested under field conditions.

Several mechanisms are the mechanisms by which microorganisms act on the yield of plants and can act directly through the production of hormones [2] or nutrient supply, such as nitrogen [3], or indirectly by the suppression of pathogens [4]. The most well-known mechanisms are biological nitrogen fixation (BNF), where symbiotic or associative bacteria can capture atmospheric nitrogen under microaerobic conditions and through the enzyme nitrogenase, to convert it to forms assimilable by plants. Other mechanisms known are involved in the production of phytoestimulatory substances, such as auxin group hormones [5], cytokinins [6], and gibberellins [7].

The constant selection and verification of the effect of plant growth promoting bacteria on species of agronomic interest is necessary for the indication of infective and efficient organisms in the composition of microbial inoculants. Thus, by means of periodic inoculations, it is possible to alter the diversity of the microbial populations interacting with the plants in the rhizosphere, favoring the infection of the roots by efficient and selected microorganisms. With respect to soybean cultivation, for example, in the Brazilian states producing this grain, the reinoculation of the crop induced positive results, compared to the nonreinoculated controls, and in some experiments, increases of up to 23% in yield and up to 25% in the N content of the grains [8]. This contribution favors the economics of mineral fertilizers.

In this chapter, we will discuss the interaction of grasses with soil microorganisms, explain how these microorganisms can benefit the growth and development of grasses, and also elucidate the main forms of interaction between grasses and soil microorganisms.

2. Soil microbial nitrogen (N) transformations

Soil microorganisms, including bacteria, fungi, and protozoa, are responsible for the decomposition of cultural residues such as leaves, stems, and roots, which release significant elements for plant nutrition from organic residues to the mineral phase absorbed by plants. The transformation of nitrogen (N), sulfur (S), and phosphorus (P) is called nutrient cycling. Some simpler composition residues presenting higher concentration of N and P can be easily decomposed. According to Ref. [9], it is related to the chemical composition of the residues, being facilitated by

the low ratios of C/N, C/P, lignin/N, polyphenols/N, and (lignin + polyphenols)/N, and difficult because of high levels of lignin and polyphenols.

Plants can absorb N either as Ammonium (NH_4^+) or Nitrate (NO_3^-). In order to achieve that, N must be transformed into a mineral nutrient so that plants can absorb it which depends on the C/N ratio of residue added to the soil. When the C/N ratio is greater than 30/1, the decomposition process is slower than usual, with accumulation of plant residues, as microorganisms cannot easily degrade them. Since the microbial population of the soil lacks nutrients, it competes with plants for N, thus causing a temporary immobilization of N. The C/N ratio greater than 70/1 in grass straws makes the decomposition process more difficult to the soil's microorganisms.

Conversely, when the C/N ratio of plant residues is less than 25/1, N is released [10], thus mineralizing this N present in the soil, which consists in the release of nutrients from the plant residues that plants can absorb as NH_4^+ . The legume tissue generally presents a C/N ratio less than 20/1 during the flowering stage. Therefore, after being cut and incorporated into the soil, the legume tissue is a rich source of N to microorganisms which will transform it into a mineral nutrient contributing to the nutrition of grasses and other cultivated plants. As a consequence, part of the mineral N fertilizer can be suppressed in the cultivation of grasses in succession to legumes [11].

Under good drainage conditions, less oxidized forms of N present in the soil, such as ammonium (NH_4^+) and ammonia (NH_3), are transformed into more oxidized forms. Nitrifying bacteria of the genera *Nitrosomonas* sp. transform N into volatile nitrite (NO_2^-). Fortunately, under the same environmental conditions, *Nitrobacter* sp. transforms volatile nitrite (NO_2^-) into nitrate (NO_3^-), which is stable and easily absorbed by grasses and other plant families [10].

Under flood conditions, when the supply of O_2 is absent in the soil, some microorganisms carry enzymes capable of consuming the oxygen from the NO_3^- present in their respiratory chain as an electron acceptor, transforming it into nitrous oxide (N_2O) [12]. N_2O and other volatile N compounds from microbial activity in poorly drained environments return to the atmosphere as gases. The dinitrogen gas (N_2) can be fixed in the soil through biological N fixation by diazotrophic bacteria. This subject will be discussed individually due to its great importance.

3. Beneficial interaction between grasses and bacteria

Soil bacteria are capable of presenting beneficial effects on cultivated grasses. Several mechanisms make bacteria to promote cultivated grasses, providing significant benefits to the plants, mainly regarding nutritional aspects.

3.1. Beneficial interaction between grasses and nitrogen fixing bacteria

About 78% of the Earth's atmosphere gases are composed of N_2 . This gas is neither good nor harmful to mankind. On the other hand, there are in the soil bacteria capable of transforming atmospheric nitrogen (N_2) into nitrogen assimilable by plants (NH_3^+). The enzyme of N-fixing

microorganisms that catalyzes the conversion of N into NH_3^+ is named *nitrogenase*. This enzyme is sensitive to oxygen, requiring molecules of iron (Fe), molybdenum (Mo), and vanadium (V) in its structural components [13], besides being an energetically expensive enzyme, requiring two molecules of ATP for each electron [14].

During the 1970s, Döbereiner's findings discovered that bacteria *Azospirillum* and *Herbaspirillum* could endophytically fix N in cultivated grass tissues [15]. There are currently commercial products based on *Azospirillum*, with bacteria selected for maize, wheat [15], and sugarcane [16]. This environmentally friendly process of N-fixing decreases the consumption of mineral N fertilizers, reducing the cost for small farmers, since the demand of industrial N fertilizers with significant consumption of fossil fuels decreases [17].

Besides the endophytic grass fixing, *Azospirillum* and *Herbaspirillum* bacteria can also endophytically fix N in other plants, as in several monocotyledons and dicots such as herbs, shrubs, and trees [18]. When not associated with other leguminous plants [15], these free-living nitrogen-fixing bacteria in the soil are considered optional associative N fixers [19].

Other plant growth promoters include *Azospirillum*, producing phytoestimulatory substances, such as the indolyl acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and ethylene [20].

There is also a group of bacteria, named rhizobia, that symbiotically fix the atmospheric N for family *Fabaceae*. Unlike free-living fixers, rhizobia can fix N only when associated within plant root nodules. In the N-fixing symbiosis in leguminous plants belonging to the family *Fabaceae*, the rhizobia receive photo-assimilated carbohydrates and, in exchange, they offer N, which is obtained as N_2 and transformed into NH_3 . The *nitrogenase* complex consists of two proteins: Fe-protein and MoFe-protein [3]. Thus, the metabolic exchanges between rhizobia and plants take place in structures called nodules, where the *nitrogenase* is protected from the atmospheric oxygen, due to the presence of leghemoglobin's heme protein, presented in high concentrations in active nodules, and fixed to oxygen.

Although they do not directly contribute to the grass nutrition, the symbiotic relationship between legumes and these symbiotic N-fixing bacteria in root nodules promotes the contribution of N to the soil, which will contribute to the nutrition of grasses after the crop cycle of the legume through cultural residues decomposition. There are important reports in the literature on the benefits of grasses grown after legumes interacting with symbiotic N-fixing bacteria [5, 21, 22]. Currently, among the 13 symbiotic N-fixing bacteria, including the genera *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, there are the subclasses α -proteobacteria and β -proteobacteria, with two genera belonging to the *Burkholderiales*, and a genus *Pseudomonas* subclass γ -proteobacteria [23].

3.2. The production of phytoestimulatory substances by rhizobia

The previous studies including rhizobia as grass growth promoters were driven by empirical findings in which specific rice plants cultivated in succession to clover produced more when compared to a cultivation without succession under the same soil, climate, fertilization, and management conditions, and this increment was not only related to residual N [21].

It could be proven that rhizobia are able to penetrate the grass tissue through fissures and root insertions [24–26]. In the intraradicular environment, as well as in the rhizosphere, rhizobia

can produce phytoestimulatory substances such as auxins [5, 7], cytokinins [6], and gibberellins [7, 21], which directly favor the yield of cultivated plant species.

It is currently known that rhizobia promote plant growth in interactions with grasses, such as rice [5, 26, 27], barley [28], maize [29], Tanzania grass, and Pensacola [30]. Thus, rhizobia can not only symbiotically fix atmospheric N associated with legumes but they also present a great potential to be exploited as direct promoters of compatible yield increases when inadequately inoculated in succession/rotation cultures systems.

The main phytoestimulatory substances produced by rhizobia are the hormones present in auxins [5, 7], cytokinins [6], and gibberellins [7, 21]. The production of abscisic acid [31], lipochitooligosaccharides (LCO) [28, 32], lumichrome [33], aminocyclopropane carboxylic acid deaminase [34], and riboflavones and vitamins [35] produced by rhizobia have also been reported.

Among all auxin syntheses, the indole-3-acetic acid (IAA) is the most studied and the most produced by bacteria [36]. The IAA is mainly presented in the formation of lateral roots and root hair that increase the plant's nutrients absorption [5]. Different metabolic pathways for IAA biosynthesis have already been identified in bacteria [37], being that two main metabolic pathways, indole-3-acetamide (IAM) and indole-3-pyruvate (IpyA), depend on tryptophan [38]. Probably the IAA biosynthesis pathway in rhizobia is the indole-3-acetonitrile (IAN) [39].

Tryptophan has been found in root exudates. Kravchenko et al. [40] quantified the tryptophan exudation by aseptic tomato and radish roots. Tomato seedlings released 2.8–5.3 ng of tryptophan per plant daily, whereas radish seedlings released 190–390 ng of tryptophan per plant per day. In the same study, the authors conducted experiments in soil pots, where they inoculated both cultures with a *Pseudomonas* plant growth-promoting rhizobacteria and observed that radish root mass increased by 36% in inoculated plants, while proven inefficient in tomatoes. The authors state that the beneficial effect of inoculation on radish plants can be explained by the fact that the rhizobacteria produced the plant growth stimulating hormone IAA.

In an experiment conducted in a growth chamber, Silveira [41] studied the effect of inoculation of five *Rhizobium leguminosarum* bv. *trifolii* strains, and their ability to promote rice growth in cultivar IAC103 in nutrient solution. Considering the accumulation of dry mass, plants inoculated with SEMIA235 and SEMIA250 strains were superior compared to the control treatment. The production of IAA by these strains was low, which can be the key to a great plant stimulation. Barazani and Friedman [42] have also reported that deleterious rhizobacteria produced high levels of IAA. However, with plant growth-promoting rhizobacteria, lower yields could be obtained during the same incubation period.

Biswas et al. [5] conducted laboratory and greenhouse studies to test the ability of rhizobia to promote plant growth in two rice cultivars. The studied rhizobia were assessed for the IAA production using the colorimetric method, which was positive for supernatant cultures for all rhizobia tested, ranging from 1.6 to 2.8 $\mu\text{g mL}^{-1}$. The best responses to inoculations were obtained with *R. leguminosarum* bv. *trifolii* strain E11 and *Rhizobium* sp. strain IRBG74, which presented early stimulation in the plant growth, resulting in an increase in grain and straw yields during the plant's maturity.

Bradyrhizobium japonicum rhizobia isolated from soybean roots, *Azorhizobium caulinodans* isolated from *Sesbania rostrata*, *Rhizobium* NGR234 isolated from *Lablab purpureus*, *Sinorhizobium meliloti* isolated from *Medicago sativa*, *R. leguminosarum* bv. *viceae* Cn6, and *R. leguminosarum* bv. *viceae* strain 30 isolated from *Vicia faba* could infect and colonize sorghum and *Setaria* roots [43]. Considering that this distinct group of rhizobia isolated from different legumes can colonize these two grasses, the authors suggest that the infection of nonlegumes by rhizobia is more likely due to natural conditions than imagined. There was an increase in the growth of inoculated sorghum and *Setaria*, as well as an increase of P in the sorghum. According to the authors, this may have occurred due to the induction of bacteria as phosphate transporters from the plasma membrane of sorghum root cells.

Gibberellins (GAs), phytohormones produced by rhizobia, stimulate stem growth [7, 21], whose effect is mostly observed in grasses, vegetables, and ornamental plants [44]. Important effects of GAs are evident during plant growth, especially on stem elongation, with increased leaf growth and xylem differentiation [45]. At determined GA content, the higher or lower IAA level means the optimal level [46]. Therefore, a certain balance between GA and IAA is essential for the maximum growth rate.

Erum and Bano [7] quantified the production of IAA and GA by rhizobia, using high pressure liquid chromatography (HPLC). The rhizobia, isolated from soil in northern Pakistan, located at 940–3090 m above sea level, produced phytohormones, and the GA production was about 10–30 times higher than the production of IAA. There was a positive and increasing correlation between the GA/IAA ratio produced and this altitude. According to the authors, the IAA and GA concentration gradient may represent a decrease of natural resources, such as radiation intensity, soil moisture, and soil nutrients.

Although cytokinins are produced by rhizobia [6], they have been little studied as it is difficult to detect and quantify them. Cytokines stimulate cell division (cytokinesis), being produced in the plant's root and transported through the xylem to the plant. The levels of auxin and cytokinins are inversely correlated in the plant [47]. Other phytoestimators produced by rhizobia, the lipochitooligosaccharides (LCO), also known as Nod factors, are responsible for the morphogenetic changes in legume roots during nodulation [48]. They have also stimulated the germination of maize, rice, beet, and cotton, under laboratory, greenhouse, and field conditions [32].

Although the key role of LCO produced by rhizobia in nodule formation is clear, other morphogenetic activities in plants were attributed to LCOs, including the stimulation of genes in the cell division cycle and stimulation of mitotic divisions in protoplasm cultures of legumes and nonlegumes [49].

Miransari and Smith [28] tested the effect of LCO extracted from *B. japonicum* and gibberellin on barley seed germination. In the treatment with 10^{-5} M of gibberellin, there was 18% increase in the seedling germination compared to the control treatment, whereas in the treatment with 10^{-6} M of LCO, the increase represented 44%.

Some rhizobia can lower the level of ethylene excreted by the plants by forming the aminocyclopropane carboxylic (ACC) acid deaminase, an enzyme that breaks ACC, a precursor of ethylene [50]. This enzyme was found in rhizospheric bacteria of the genus *Pseudomonas*,

Alcaligenes, *Rhodococcus*, and *Rhizobium* [34, 51]. In addition, some bacteria of *Rhizobium japonicum* (*B japonicum*) species synthesize phytotoxic antibiotics, aminoethoxyvinylglycine, and rhizobitoxin, which inhibit the formation of ethylene in plants [52]. The ethylene is a plant growth inhibitor, therefore bacteria that regulate its production can indirectly stimulate the plants and may be associated with the cell development, cell extension and the postponement of the fall of leaves and fruits [52].

S. meliloti can produce lumichrome [33]. The application of lumichrome in nanomolecular concentrations promoted the growth of legumes and grasses [43]. According to Ref. [48], the lumichrome stimulated the photosynthetic index of maize on the first and second day after application. Gouws [53] reported an increase in the root biomass of *Lotus japonicus* and tomato when treated with lumichrome. According to the author, the treatment with lumichrome caused complex changes in the gene expression of *L. japonicus* and tomato, being mainly affected the genes associated with the transcription regulation and ribonucleic acid (RNA) signaling, synthesis, degradation, proteins modification, and plant stress responses. The mechanism by which lumichrome promotes the plants growth still needs to be clarified.

3.3. Other phytostimulating-producing bacteria

Other microorganisms, such as *Azospirillum* spp. [54, 55], *Acetobacter diazotrophicus* [56], *Herbaspirillum seropedicae* [56], *Klebsiella pneumoniae* [55], *Pseudomonas syringae* [57], and *Paenibacillus polymyxa* [58], also produce phytostimulatory substances and are also related to the stimulation of grasses and other nonleguminous species.

3.4. Siderophore-producing bacteria

Siderophores are iron-chelating compounds, nutrients that limit the microbial population growth in the soil's environment. Fe must be present as Fe^{2+} , and many microorganisms such as bacteria and fungi have developed mechanisms to chelate Fe^{3+} through the production of siderophores before being transformed into Fe^{2+} .

Siderophores are Fe sequestrants of high affinity and low molecular weight. Among the siderophores known, pyoverdine and enterobactin are secreted by microorganisms in response to the low availability of Fe_3^+ in solution [59]. The siderophores are iron-chelating compounds, entering the cell without reducing Fe^{3+} [60]. Thus, siderophores can capture Fe_3^+ produced under iron-deficiency conditions by fungi and bacteria in order to incorporate this mineral into the cell metabolism [61].

Because of their great ability to compete for the cell metabolism, microorganisms producing siderophores are capable of suppressing the growth and development of pathogenic microorganisms that inhabit the rhizosphere, thus indirectly contributing to the health of cultivated species of plants, such as grasses. Some rhizobacteria of the genera *Pseudomonas* can produce iron-chelating compounds, present in low concentrations in the rhizosphere, and thus suppressing the presence of pathogens near the roots [62].

3.5. Phosphate solubilizing bacteria

Together with N and K, P is one of the required macronutrients for the cultivation of grasses, whose content concentration is always lower than N and K. However, it is commonly necessary to use a great amount of phosphate fertilizers in agricultural crops, because in spite of the soils contain a large amount of P their availability to the plants is very little as P tends to form very low solubility compounds in the soil [63].

Phosphorus is an essential element to grasses, since it is necessary and irreplaceable for the composition of ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), responsible for the transmission of the genetic code to the plants, protein production, and other essential compounds for the plant structure and seedling production. Grasses absorb soil P as H_2PO_4^- and HPO_4^{2-} , just like other plant species; thus, insoluble phosphates like tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) make this nutrient unavailable to the plant. Some of the soil bacteria are important in the process of dissolving these insoluble solutions, facilitating the access to this essential nutrient.

Inorganic phosphate-solubilizing microorganisms excrete inorganic acids and protons associated to these acids, which directly dissolve the insoluble phosphate, or chelate the cations with the phosphate anion [64]. Among phosphate-solubilizing bacteria, *Burkholderia* [65], *Bacillus*, and *Penicillium* strains [66] have been reported.

4. Mycorrhizae

Mycorrhizal fungi are associated with roots of plants and play an important role in the soil phosphorus cycling as extensions of the root system, increasing the absorbing area of the root and the absorption rate of phosphorus. The mycorrhizal association does not substitute phosphate fertilization, but efficiently increases the use of phosphorus or an added compound through fertilization [67]. Grasses such as maize, sorghum, wheat, rice, and cultivated forage grasses may have their roots naturally colonized by mycorrhizal fungi [68].

5. Fungi of the genus *Trichoderma* and the biological control of diseases

Fungi of the genus *Trichoderma* are biological control agents that act against phytopathogenic edaphic fungi, however, colonizing plant roots to stimulate plant growth and protect them against infections. Root colonization often increases root development, crop productivity, resistance to abiotic stresses, and improved nutrient use [69].

Fungi present different mechanisms to controlling and suppressing the soil's phytopathogens, such as mycoparasitism, antibiosis and antagonism [70]. In addition, *Trichoderma* strains are active in the production of fungal cell walls, enzymatic degradation, including pectinases, cellulases, and chitinases, involved in the biological control [71].

Compared to the chemical control, the use of biological substances to control soil diseases is beneficial, since it does not induce resistance from the target organism, effectively controlling

it in a long term. There are currently commercial products based on genus *Trichoderma* strains, properly registered in the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA) that are indicated for the controlling of diseases caused by phytopathogenic agents, such as *Rhizoctonia*, *Fusarium*, and *Sclerotinia* [72]. Harman [4] and Machado et al. have described the benefits of *Trichoderma* inoculation on grass yield, observing an increase in maize and black oat yields, respectively.

6. Final considerations

Soil microorganisms are able to influence the establishment and yield of grasses by means of several mechanisms. The nutrients cycling by soil microorganisms, the biological nitrogen fixation by associative bacteria, phytohormones production by soil bacteria, and the acquisition of phosphorus by mycorrhizal fungal hyphae networks are just some examples of direct mechanisms of beneficial interaction between soil microorganisms and cultivated grasses. As examples of indirect mechanisms, we can mention the suppression of pathogens by mechanisms of predation or competition, as we also discussed. Given this wide range of mechanisms presented by microorganisms for the benefit of cultivated grasses and consequently of the human benefit, it is imperative that these mechanisms are well studied to be inserted in systems of conservationist agriculture, which must obtain the maximum agronomic yield of the crops, allied to the rational use of natural resources.

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