We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Chapter

Promotion of Nitrogen Assimilation by Plant Growth-Promoting Rhizobacteria

Gabriel Monteiro, Glauco Nogueira, Cândido Neto,
Vitor Nascimento and Joze Freitas

Abstract

Nitrogen fertilizers are one of the highest expenses in agricultural systems and usually a limitation to the productions of many agricultural crops worldwide. The intensive use of this element in modern agriculture represents a potential environmental threat, one of the many tools for the sustainable use of this resource without losing productivity is the use of plant growth-promoting rhizobacteria, especially nitrogen-fixing bacteria. However, in considering the competitiveness of the market, studies are still needed to determine the most efficient way to use this resource and if the nitrogen mineral fertilization is indeed substitutable. As a result, this study aims to deepen the scientific knowledge of the plant-microbe interactions by addressing their main characteristics and functionalities for plant growth and development and efficiency in the use of nitrogen. For this we reviewed relevant information from scientific works that address these issues.

Keywords: biochemistry, nitrogen-fixation, growth, nitrogen fertilizers, nitrogen use efficiency

1. Introduction

Nitrogen (N) is a key component of most proteins, secondary metabolites and signaling molecules [1]. It is one of the most important macronutrients for plant development and usually one of the most limiting factor to plant production [2].

The use of N-fertilizers has produced a significant increase in food production in recent decades [3], and its consumption has grown from 11.3 Tg N year^{-1} in 1961 to 107.6 Tg N year^{-1} in 2013 [4]. However, less than 50% of the added N is effectively absorbed by most cultivated plants [5, 6], and even N effectively converted to biomass, eventually returns to the environment [7]. In the soil, N is available to plants in the form of nitrate (NO_3^-), ammonium (NH_4^+) and organic compounds (usually amino acids), being the NO_3, the most abundant [8]. In its ionic form, NO_3^- has a negative charge and high water solubility, being susceptible to leaching and runoff [9]. It can also be volatilized by denitrifying microorganisms [10], and lost to the atmosphere in the form of nitrous oxide (N_2O, a greenhouse gas 296-fold more potent than a unit of CO_2). Leaching of N causes eutrophication of water bodies and contamination of groundwater [11].

N can also be lost to the atmosphere in other forms such as reactive gases (NO_x; NH_3), aggravating the greenhouse effect [12], and is also related to the acidification of soils through the formation of acid rain, and depletion of exchangeable basic soil cations [13].

To minimize the loss of N in the soil, and consequently the total amount of N necessary for a high-quality production, several strategies have been used. One is the use of urease inhibitors such as N-(n-butyl)-thiophosphoric triamide (NBPT) to delay the hydrolysis of urea, thus reducing losses to the atmosphere and microbial transformations [14]. Increased N use efficiency (NUE) has also been the subject of research, through the selection of genotypes with a higher NUE [15] or through biological nitrogen fixation [16]. Biological nitrogen fixation (BNF) occurs through the conversion of atmospheric N_2 to ammonium by free-living or symbiotic diazotrophic bacteria [17]. Plant growth-promoting rhizobacteria (PGPR) can increase the N absorption capacity through BNF [18], phytohormone production [19], stimulate the production and enzymatic assimilation of $NH4+$ [16], as well as the transport and partition of N [20].

The study of plant nutrition related to the use of BNF as an alternative to increase efficiency and sustainability in the use of N in agriculture is essential, given the complex nature of the interactions between soil, plant, and microorganisms. Therefore, the objective of this review is to deepen the scientific knowledge of these interactions, addressing their main characteristics and functionalities for plant growth and development.

2. Mechanisms of biological nitrogen fixation

One of the largest N reservoirs is the atmosphere, second only to the lithosphere in absolute amount of N [21]. N makes up about 78% of the atmosphere [22] and is mainly in the form of molecular N (N_2). The atoms in the N_2 molecule have low-energy orbitals and the bond between the two N molecules is relatively short (1,098 Å) and stable, with a bonding energy of 930 kJ/mol [23]. This set of characteristics gives low reaction potential to the molecule. Alternatively, N_2 can be reduced to NH_3 naturally by microorganisms through the BNF process. The BNF reaction follows the following stoichiometry:

The BNF process is catalyzed exclusively by an enzyme complex called the nitrogenase complex. The nitrogenase complex is composed of dinitrogenase reductase (Iron-protein) and dinitrogenase (Molybdenum-Iron-protein) (**Figure 1a**). Dinitrogenase-reductase is a dimer of approximately 60 kDa, composed of two identical and symmetrical subunits, which coordinate a redox center 4Fe-4S (**Figure 2**). This enzyme also has sites for the binding of ATP/ADP, one in each subunit, being able to couple the hydrolysis of ATP to fuel the transfer of electrons to the dinitrogenase [26]. Dinitrogenase is a heterotetramer $\alpha_2\beta_2$ with approximately 240 kDa.

Dinitrogenase has two cofactors containing iron (**Figure 1b**), being the group P and the FeMo cofactor [27]. Group P contains a pair of 4Fe-4S centers, which share a sulfur, forming an 8Fe-7S center. The FeMo cofactor is a variation of the iron–sulfur groups, such as the P group, but differs greatly from the other metallic sites within this family. Its structure is composed of [Mo: 7Fe: 9S: C]: Homocitrate [26, 28]. Some microorganisms also have alternative forms of the MoFe cofactor, where Molybdenum (Mo) is replaced by atoms of Vanadium (V) or Iron (Fe) depending on metal availability [29].

In the N_2 reduction reaction (Eq. 1), the reduced dinitrogenase reductase couples the hydrolysis of ATP with the transfer of electrons to the dinitrogenase. The oxidized dinitrogenase reductase detaches from the dinitrogenase, only to be reduced again (by ferredoxins or flavodoxins). Again, there is the coupling between
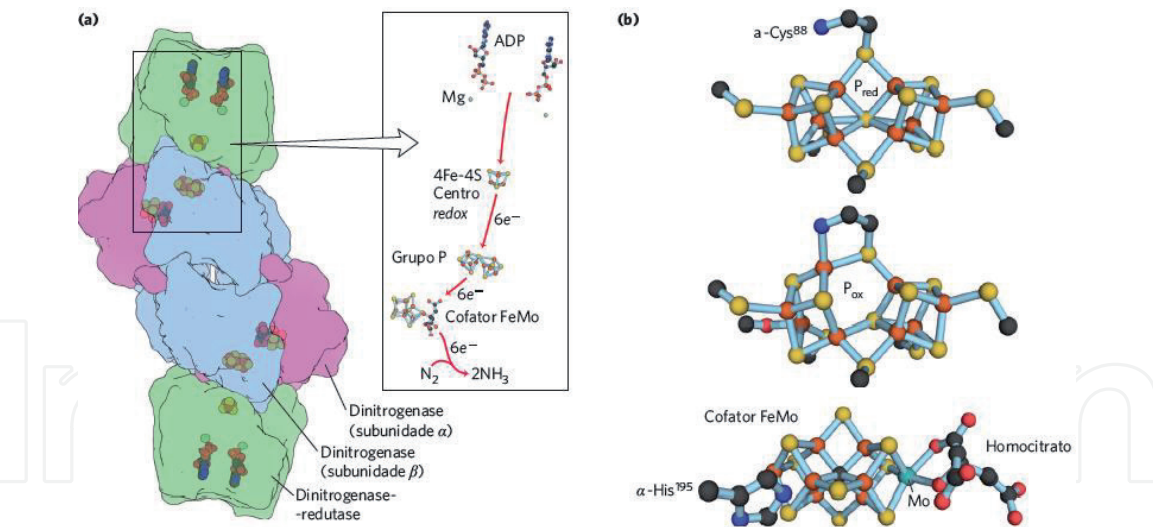
Figure 1.
Enzymes and cofactors of the nitrogenase complex. (a) The enzyme consists of two symmetrical dinitrogenase reductase molecules (in green), each with a 4Fe-4S redox center and binding sites for ATP, and two identical dinitrogenase heterodimers (in purple and blue), each one with a P group and a FeMo cofactor. (b) The cofactors for the electron transfer. A group P is shown here in its reduced (upper part) and oxidized (middle) and the cofactor FeMo is showed at the bottom. Source: Taiz et al. [24].
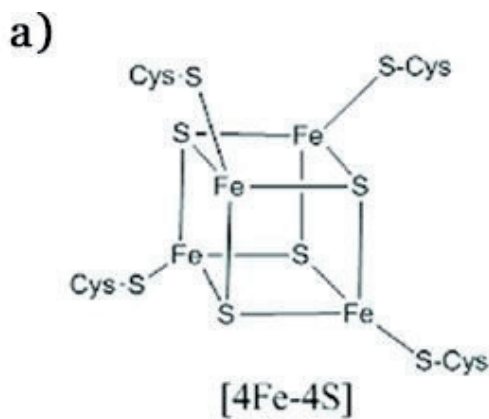

Figure 2.
Structure of 4Fe-4S clusters present in nitrogenase complex. (a) The 4Fe-4S binding site between the Dinitrogenase-reductase and Dinitrogenase contains a cubane-like structure where four iron ions and four sulfide ions are placed at the vertices. The Fe centers are typically coordinated by cysteine residuals. Source: [25].

both enzymes followed by the consumption of ATP to transfer the electrons to the dinitrogenase. This coupling-detaching cycle followed by the electron transfer is repeated until the dinitrogenase is reduced enough to reduce its substrate, which in the case of BNF, is N_2 [30].

Because it deals with large amounts of energy, enough to break the triple bond of the N_2 molecule, the nitrogenase complex is not only inactivated by the presence of oxygen (O_2) but can have its expression reduced [31]. However, diazotrophic bacteria are able to combine N fixation with their aerobic metabolism in different ways to avoid O_2 deactivation [32]. Notably, one of the most advanced means of controlling O_2 concentration is expressed by rhizobia-leguminous symbiosis.

3. Brazil: a success of BNF in legumes

Brazil is one of the best examples in the efficient research and use of the BNF in legumes [33]. The use of FBN is interesting from both an economic and an environmental point of view, since once this process is established, nitrogen fertilizers can

be dispensed with in whole or in part, thus contributing to enable reforestation and minimize possible environmental impacts resulting of use these supplies [34]. It is estimated an annual economy of more than US$ 13 billion with the total or partial substitution of nitrogen fertilizers in legumes in Brazil [33].

Rhizobium-leguminous symbiosis is the most important symbiotic system between microorganisms and plants thanks to the efficiency of the N2 fixation process, and one of the justifications is in the amplitude and geographical distribution of the hosts and the economic impact it causes in agriculture, and one of the main sources of N for the biosphere [35]. The leguminosae family comprises almost 20 thousand species, including tree, herbaceous species used as fodder, producers of raw materials or directly in human food [36].

The most successful case here in Brazil according to Hungria et al. [18], is the symbiosis of Bradyrhizobium spp. with soybean (*Glycine max* (L.) Merrill). The main leguminous species produced in Brazil does not require nitrogen-based fertilization, due to the biological nitrogen fixation which supplies the required N for crop development. To get a sense of how important this symbiotic relationship is in Brazil, in the 2018/2019 harvest, over 35 million hectares were sown. According to the National Supply Company [37], soybean production for this same harvest was 115 million tons, resulting in an average productivity of 3,208 kg ha^{-1}, with almost zero N-fertilizer input. On the other hand, the biological nitrogen fixation with other important legumes, such as beans and peanuts, and non-legumes like sugarcane cannot fully supply the demand for N like in soybeans.

Alfalfa is another plant species with a high potential for biological nitrogen fixation. As a legume, alfalfa is capable of symbiotically associating with rhizobial bacteria, with N inputs to reaching up to 470 kg of N ha-1 [38]. The main symbiotic alfalfa bacteria belong to the genus Ensifer, having Synorhizobium as a synonym, but previously classified as Rhizobium. Sinorhizobium meliloti and Sinorhizobium mediace are the main symbionts reported in several countries [39]. In Brazil, there are three strains of rhizobia that are used in commercial inoculants for alfalfa, which have been validated for more than two decades and with rare tests conducted with the same [39].
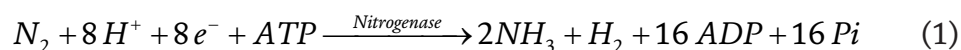
4. Growth promotion by associative and free-living diazotrophic bacteria and stimulating N metabolism

World production is dominated by the production of four grasses (FAO Stats 2019), namely: Sugarcane (*Saccharum officinarum* L. 1.95 Gt year-1), Maize (*Zea mays* L. 1.15 Gt year-1), Wheat (*Triticum aestivum* L. 0.76 Gt year-1) and Rice (*Oryza sativa* L. 0.75 Gt year-1). Scientific studies have shown that biological nitrogen fixation is not limited only to legumes, with a potentially important group of diazotrophic bacteria capable of forming associations through root colonization and the internal tissues of grasses [40]. These bacterial-grass associations also have different mechanisms of action than legumes, and in addition to fixing N, increase the absorption and assimilation of N by modulating the architecture and development of the root system through the production of phytohormones, such as indole-3-acetic acid [41] and gibberellins [42]. Cases such as diazotrophic bacteria such as those of the genus Azospirillum sp., Herbaspirillum sp. and Glucanobacter sp. are evidence of the influence of PGPR on N metabolism, inducing physiological and morphological changes that are associated with greater NUE.

Grasses are of great interest for the development of biological nitrogen fixation aiming at greater efficiency, in view of its relatively low NUE [43, 44]. The increase

in NUE is related to several characteristics, Iqbal et al., (2020) working with different cotton genotypes analyzed the biochemical and morphological responses of the accessions according to different concentrations of NO_3^- and the root architecture and efficiency of the enzymes of the assimilation of N were essential for the increase in traction related to NUE. The inoculation of diazotrophic bacteria seems to achieve the same results in different cultures of importance to the global market such as sugarcane (*Saccharum officinarum* L.), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.,) and rice (*Oryza sativa* L.). In sugarcane, the effects vary from the increase in the speed of bud sprouting and the emission of roots in sugarcane stalks used for planting [45], increases in the biomass production of the thatch [46], until the increase in the number of tillers [47]. In maize, inoculation with Azospirillum brasilense increased the transcription of the genes encoding Nitrate reductase (ZmNR), Glutamina sintase (ZmGln1–3), and the intensity of assimilation of the N [48, 49]. In wheat, the inoculation of Azospirillum brasilense was able to modify the N metabolism, resulting in an increase in growth [16].

Modulation of root architecture induced by PGPR is also a morphological trait essential to the increase in NUE. The increase in the area explored by the root triggered by the increase in the volume of the roots caused by these bacteria directly influences the interception of nutrients, among them the N. Besides the influence on the morphological characteristics, the PGPR inoculation has an impact on the metabolism activity of the N. The increase in these characteristics makes these bacteria a potential solution for increasing NUE.

$$N_2 + 8\,H^+ + 8\,e^- + ATP \xrightarrow{\;Nitrogenase\;} 2NH_3 + H_2 + 16\,ADP + 16\,Pi \qquad (1)$$

5. Conclusions

Since the discovery of the Haber-bosch process in the early 20th century, the levels of N added to the biosphere continue to increase each year and its excessive use of this element is a source of numerous environmental problems.

Therefore, biological nitrogen fixation process is an essential tool in the current economic, agricultural, and environmental context of many countries, this can be seen through studies and government data that show a reduction in financial expenses in the order of millions, this technological tool. it is a reality in vegetable crops with high potentials in the agricultural network of emerging powers worldwide, in addition to contributing to the reduction of potentially harmful agents for the worsening of the greenhouse effect.

This technology has been extensively scientifically explored, aiming to expand its possibility in other promising cultures in the agricultural and forestry world, as well as other associative and free-living diazotrophic microorganisms, and their ability to promote plant growth. However, there are still major gaps in knowledge about the diversity and mechanisms of PGPR action and further research is needed to establish the use of these new bacteria as a sustainable agricultural practice.

Acknowledgements

Appendices and nomenclature

CONAB	National Supply Company
NUE	Nitrogen use efficiency
NBPT	N-(n-butyl)-thiophosphoric triamide
PGPR	Plant growth-promoting rhizobacteria
BNF	Biologic nitrogen fixation

Author details

Gabriel Monteiro[1], Glauco Nogueira[1*], Cândido Neto[2], Vitor Nascimento[3] and Joze Freitas[2]

1 Federal Rural University of Amazon (UFRA), Belém, Pará, Brazil

2 Institute of Agrarian Sciences, Federal Rural University of Amazon (UFRA), Belém, Pará, Brazil

3 Rede BIONORTE/UFPA, Belém, Pará, Brazil

*Address all correspondence to: glauand@yahoo.com.br

IntechOpen

References

[1] Krapp, A. (2015). Plant nitrogen assimilation and its regulation: A complex puzzle with missing pieces. Current Opinion in Plant Biology, *25*, 115-122. https://doi.org/10.1016/j.pbi.2015.05.010

[2] Teixeira, E. I., George, M., Herreman, T., Brown, H., Fletcher, A., Chakwizira, E., de Ruiter, J., Maley, S., & Noble, A. (2014). The impact of water and nitrogen limitation on maize biomass and resource-use efficiencies for radiation, water and nitrogen. Field Crops Research, *168*, 109-118. https://doi.org/10.1016/j.fcr.2014.08.002

[3] Gojon, A. (2017). Nitrogen nutrition in plants: Rapid progress and new challenges. Journal of Experimental Botany, *68*(10), 2457-2462. https://doi.org/10.1093/jxb/erx171

[4] Lu, C., & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. Earth System Science Data, *9*(1), 181-192. https://doi.org/10.5194/essd-9-181-2017

[5] Kant, S. (2018). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. Seminars in Cell and Developmental Biology, *74*, 89-96. https://doi.org/10.1016/j.semcdb.2017.08.034

[6] Plett, D. C., Holtham, L. R., Okamoto, M., & Garnett, T. P. (2018). Nitrate uptake and its regulation in relation to improving nitrogen use efficiency in cereals. Seminars in Cell and Developmental Biology, *74*, 97-104. https://doi.org/10.1016/j.semcdb.2017.08.027

[7] Robertson, G. P., & Vitousek, P. M. (2009). Nitrogen in agriculture: Balancing the cost of an essential resource. Annual Review of Environment and Resources, *34*, 97-125. https://doi.org/10.1146/annurev.environ.032108.105046

[8] Miller, A. J., Fan, X., Orsel, M., Smith, S. J., & Wells, D. M. (2007). Nitrate transport and signalling. Journal of Experimental Botany, *58*(9), 2297-2306. https://doi.org/10.1093/jxb/erm066

[9] Good, A. G., & Beatty, P. H. (2011). Fertilizing nature: A tragedy of excess in the commons. PLoS Biology, *9*(8), 1-9. https://doi.org/10.1371/journal.pbio.1001124

[10] Kuypers, M. M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. Nature Reviews Microbiology, *16*(5), 263-276. https://doi.org/10.1038/nrmicro.2018.9

[11] Sinha, E., Michalak, A. M., & Balaji, V. (2017). Eutrophication will increase during the 21st century as a result of precipitation changes. Science, *357*(6349), 1-5. https://doi.org/10.1126/science.aan2409

[12] Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. Annual Review of Plant Biology, *63*, 153-182. https://doi.org/10.1146/annurev-arplant-042811-105532

[13] Horswill, P., O'Sullivan, O., Phoenix, G. K., Lee, J. A., & Leake, J. R. (2008). Base cation depletion, eutrophication and acidification of species-rich grasslands in response to long-term simulated nitrogen deposition. Environmental Pollution, *155*(2), 336-349. https://doi.org/10.1016/j.envpol.2007.11.006

[14] Silva, A. G. B., Sequeira, C. H., Sermarini, R. A., & Otto, R. (2017). Urease inhibitor NBPT on ammonia

volatilization and crop productivity: A meta-analysis. Agronomy Journal, *109*(1), 1-13. https://doi.org/10.2134/agronj2016.04.0200

[15] Iqbal, A., Qiang, D., Alamzeb, M., Xiangru, W., Huiping, G., Hengheng, Z., Nianchang, P., Xiling, Z., & Meizhen, S. (2020). Untangling the molecular mechanisms and functions of nitrate to improve nitrogen use efficiency. Journal of the Science of Food and Agriculture, *100*(3), 904-914. https://doi.org/10.1002/jsfa.10085

[16] Silveira, A. P. D. da, Sala, V. M. R., Cardoso, E. J. B. N., Labanca, E. G., & Cipriano, M. A. P. (2016). Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation. Applied Soil Ecology, *107*, 313-319. https://doi.org/10.1016/j.apsoil.2016.07.005

[17] Franche, C., Lindström, K., & Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant and Soil, *321*(1-2), 35-59. https://doi.org/10.1007/s11104-008-9833-8

[18] Hungria, M., Franchini, J. C., Campo, R. J., Crispino, C. C., Moraes, J. Z., Sibaldelli, R. N. R., Mendes, I. C., & Arihara, J. (2006). *Nitrogen nutrition of soybean in Brazil: Contributions of biological N 2 fixation and N fertilizer to grain yield*.

[19] Glick, B. R. (2012). Plant Growth-Promoting Bacteria : Mechanisms and Applications. *2012*.

[20] Mantelin, S., Desbrosses, G., Larcher, M., Tranbarger, T. J., Cleyet-Marel, J. C., & Touraine, B. (2006). Nitrate-dependent control of root architecture and N nutrition are altered by a plant growth-promoting Phyllobacterium sp. Planta, *223*(3), 591-603. https://doi.org/10.1007/s00425-005-0106-y

[21] Stevenson, F. J. (2015). *Origin and Distribution of Nitrogen in Soil* (Issue 22, pp. 1-42). https://doi.org/10.2134/agronmonogr22.c1

[22] Williams, D. R. (2016). Earth Fact Sheet. NASA Fact Sheets, 1. https://nssdc.gsfc.nasa.gov/planetary/factsheet/earthfact.html

[23] Fernandes, M. S., & Pereyra Rossiello, R. O. (1995). Mineral Nitrogen in Plant Physiology and Plant Nutrition. Critical Reviews in Plant Sciences, *14*(2), 111-148. https://doi.org/10.1080/07352689509701924

[24] Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal*. Artmed Editora.

[25] Cammack, R. (2012). Iron–sulfur proteins. The Biochemist, *34*(5), 14-17. https://doi.org/10.1042/BIO03405014

[26] Einsle, O., & Rees, D. C. (2020). Structural Enzymology of Nitrogenase Enzymes. Chemical Reviews, *120*(12), 4969-5004. https://doi.org/10.1021/acs.chemrev.0c00067

[27] Morrison, C. N., Hoy, J. A., Zhang, L., Einsle, O., & Rees, D. C. (2015). Substrate Pathways in the nitrogenase MoFe protein by experimental identification of small molecule binding sites. Biochemistry, *54*(11), 2052-2060. https://doi.org/10.1021/bi501313k

[28] Einsle, O. (2014). Nitrogenase FeMo cofactor: An atomic structure in three simple steps. Journal of Biological Inorganic Chemistry, *19*(6), 737-745. https://doi.org/10.1007/s00775-014-1116-7

[29] Eady, R. R. (1996). Structure-function relationships of alternative nitrogenases. Chemical Reviews, *96*(7), 3013-3030. https://doi.org/10.1021/cr950057h

[30] Rees, D. C., Akif Tezcan, F., Haynes, C. A., Walton, M. Y., Andrade, S.,

Einsle, O., & Howard, J. B. (2005). Structural basis of biological nitrogen fixation. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 363(1829), 971-984. https://doi.org/10.1098/rsta.2004.1539

[31] Gallon, J. R. (1981). The oxygen sensitivity of nitrogenase: a problem for biochemists and micro-organisms. Trends in Biochemical Sciences, 6(C), 19-23. https://doi.org/10.1016/0968-0004(81)90008-6

[32] Goldberg, I., Nadler, V., & Hochman, A. (1987). Mechanism of nitrogenase switch-off by oxygen. Journal of Bacteriology, 169(2), 874-879. https://doi.org/10.1128/jb.169.2.874-879.1987

[33] Auras, N. É., Zilli, J. É., Soares, L. H. de B., & Fontana, J. (2018). Recomendação de uso de estirpes fixadoras de nitrogênio em leguminosas de importância agronômica e florestal. Embrapa Agrobiologia-Documentos (INFOTECA-E).https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1099443/1/recomendacaodeusodeestirpesfixadoras.pdf

[34] Barbieri, A.; Carneiro, M.A.C.; Moreira, F.M.S.; Siqueira, J.O. (1998). Nodulação em leguminosas florestais em viveiros no sul de minas gerais. CERNE, v.4, n.1, p.145-153. https://www.researchgate.net/publication/242531593

[35] Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., & Vöosmarty, C. J. (2004). Nitrogen Cycles: Past, Present, and Future. Biogeochemistry, 70(2), 153-226. https://doi.org/10.1007/s10533-004-0370-0

[36] Cantarella, H. (2007). Nitrogênio. Fertilidade Do Solo, 2, 375-470.

[37] CONAB. (2019). *Boletim da Safra de Grãos*. Acompanhamento Da Safra 2018/19 Brasileira de Grãos - 12° Levantamento. https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos

[38] Ormeño-Orrillo, E., Hungria, M., & Martinez-Romero, E. (2013). Dinitrogen-fixing prokaryotes. In *The Prokaryotes: Prokaryotic Physiology and Biochemistry* (Vol. 9783642301414, pp. 427-451). Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-642-30141-4_72

[39] de Soares, L.H. B, Michel, D. C., & Zilli, J. É. (2020). Fixação biológica do nitrogênio. In Ministério da Agricultura, Pecuária e Abastecimento (Ed.), *Alfafa: do cultivo aos múltiplos usos* (p. 173). MAPA/AECS.

[40] Rosenblueth, M., Ormeño-Orrillo, E., López-López, A., Rogel, M. A., Reyes-Hernández, B. J., Martínez-Romero, J. C., Reddy, P. M., & Martínez-Romero, E. (2018). Nitrogen fixation in cereals. *Frontiers in Microbiology*, 9(AUG), 1-13. https://doi.org/10.3389/fmicb.2018.01794

[41] Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, 31(4), 425-448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

[42] Bottini, R., Cassán, F., & Piccoli, P. (2004). Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Applied Microbiology and Biotechnology, 65(5), 497-503. https://doi.org/10.1007/s00253-004-1696-1

[43] Herrera, J. M., Rubio, G., Häner, L. L., Delgado, J. A., Lucho-Constantino, C. A., Islas-Valdez, S., & Pellet, D.

(2016). Emerging and established technologies to increase nitrogen use efficiency of cereals. Agronomy, *6*(2), 11-18. https://doi.org/10.3390/agronomy6020025

[44] Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. Nature, *418*(6898), 671-677. https://doi.org/10.1038/nature01014

[45] Landell M.G. de A, Campana, M. P., Figueiredo, P., Xavier, M. A., Anjos, I. A. dos, Dinardo-Miranda, L. L., Scarpari, M. S., Garcia, J. C., Bidóia, M. A. P., & Silva, D. N. da. (2012). Sistema de multiplicação de cana-de-açúcar com uso de mudas pré-brotadas (MPB), oriundas de gemas individualizadas. *Ribeirão Preto: Instituto Agronômico de Campinas*, *17*.

[46] Gírio LA da S, Dias, F. L. F., Reis, V. M., Urquiaga, S., Schultz, N., Bolonhezi, D., & Mutton, M. A. (2015). Plant growth-promoting bacteria and nitrogen fertilization effect on the initial growth of sugarcane from pre-sprouted seedlings. Pesquisa Agropecuaria Brasileira, *50*(1), 33-43. https://doi.org/10.1590/s0100-204x2015000100004

[47] Oliveira, A. R. De, & Simões, W. L. (2016). Cultivares de cana-de-açúcar inoculadas com bactérias. Energia Na Agricultura, *31*, 154-161.

[48] da Fonseca Breda, F. A., da Silva, T. F. R., dos Santos, S. G., Alves, G. C., & Reis, V. M. (2019). Modulation of nitrogen metabolism of maize plants inoculated with Azospirillum brasilense and Herbaspirillum seropedicae. Archives of Microbiology, *201*(4), 547-558. https://doi.org/10.1007/s00203-018-1594-z

[49] Pereira-Defilippi, L., Pereira, E. M., Silva, F. M., & Moro, G. V. (2017). Expressed sequence tags related to nitrogen metabolism in maize inoculated with Azospirillum brasilense. Genetics and Molecular Research, *16*(2), 1-14. https://doi.org/10.4238/gmr16029682