

# Chapter 1

## Biological Nitrogen Fixation in Nonlegumes: Introduction



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**Abstract** Sustainable agriculture (SA) is on prime importance in today's scenario. It is achievable via eco-safe application of nitrogen-fixing bacteria (NFB) biofertilizers, where these are applied on nonlegume crops and should not be limited to legume crop. NFB, including rhizobia or free-living rhizobacteria for the development of bioinoculants/biofertilizers/biopesticide, can be utilized for broad range of legume to nonlegumes crops, which contributing toward the sustainable development goal (SDG), "zero hunger". This introductory chapter provides an overview on the importance of its contents, and overall understand the role of nitrogen-fixing bacteria and their application in growth promotion of nonlegume crops, to achieve sustainable development.

**Keywords** Sustainable development · Biofertilizers · Rhizobia · Nitrogen fixation · Nonlegume

### 1.1 Introduction

The human race is on the edge of hunger due to the decline of the world's economy by the recent pandemic of COVID-19 that also caused food scarcity in many developing countries. It is hard to forecast a rise in hunger due to various bottlenecks, like climate change, an ever-growing population, a hike in food prices, etc. The excessive use of chemically produced fertilizers, pesticides, and herbicides is causing negative impacts on human health and agriculture. At this stage, using

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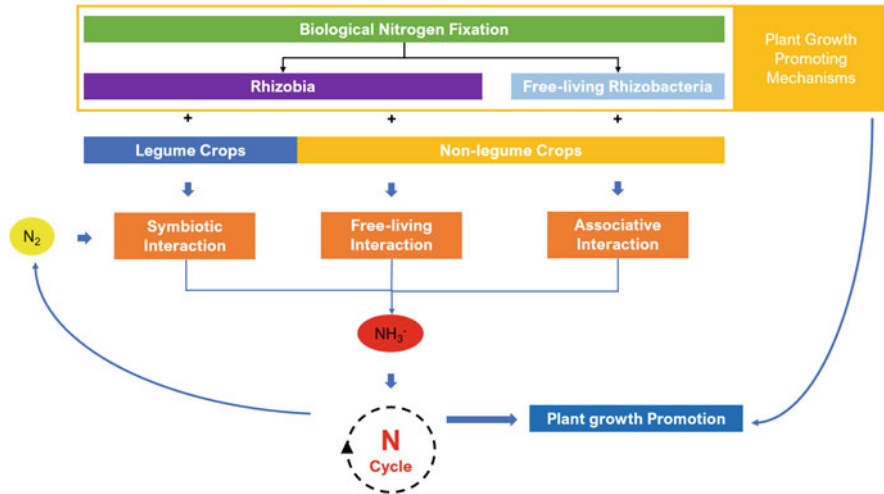
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biofertilizers and mitigation of food security as an eco-friendly alternative is an inevitable and precise way to attain scientific benefits for sustainable agriculture (SA).

Basically, in agriculture, plant life is cultivated in soil, which majorly demands nitrogen, phosphorus, and potassium as sole nutrients to drive their metabolic requirement. Other than these, soil organic matter (SOM) with some trace elements (e.g., Cu, Fe, Mn, Mg, etc.) is required. But, Nitrogen (N) requirement is often considered on the prime importance to the plants. Thereby, utilization of abundant fertilizer in soil, has snatched the soil fertility plus increased fertilizer dosage caused enormous financial burden in agriculture. Therefore, in the current scenario, eco-safe alternative biofertilizers or microbial inoculants are required to attain soil fertility back into course of sustainable agroecosystem. Nitrogen acts as one of the significant indicators for soil fertility, reflects the presence of good soil bacteria. As already known that nitrogen is present in gaseous form in the environment. Nitrogen-fixing bacteria (NFB) known for their ability of nitrogen fixation in legume crops (Soumare et al. 2020) and nonlegume crops (Behera et al. 2021) thereby used as biofertilizers to replace abundant input of chemical fertilizers, as an important approach for sustainable agriculture (Misra et al. 2020). Application of rhizobia to improve growth, yield, nutrient composition, and quality of nonlegume plants has been advocated due to direct and indirect plant growth-promoting activities (García-Fraile et al. 2012). The scope of using rhizobia (symbiont of legume) and free-living bacteria for nitrogen improvement in the soil has been increased with beneficial gears of plant growth promotion (Hayat et al. 2010). Biological nitrogen fixation in soil or rhizosphere has been reflected to increase crop productivity (Gaskins et al. 1985). In fact, rhizobia may associate with nonlegume via rhizosphere colonization and crack entry in xylem tissues exhibited ACC-deaminase activity, production of plant hormones, siderophore, HCN, and mineral (P and K) nutrient solubilization, which also supports growth and productivity of nonlegumes (Martínez-Viveros et al. 2010) other than forming true nodule in the roots and symbiotic fixation of nitrogen. The overall picture of rhizobia, free-living bacteria, and N-cycle can be understood with Fig. 1.1.

This sustains the quest of nonlegume crops with NFB, and judging their ecological roles (successful partnership among plant and bacteria, via production of plant root's exudates and molecular signals by bacteria necessary to engineer N-fixing association with nonlegume plants) in provisioning the benefits to nutrients transformation, soil organic matter mineralization, and carbon dynamics (Barrios 2007).

Application of biofertilizer is an alternative scheme to achieve environment-friendly sustainable crop production system (Seenivasagan and Babalola 2021). Cultivation and N-fertilization in rice is not limited to use rhizobia, because of several limitations, therefore, free-living rhizobacteria, as PGPR contribute significantly in order to achieve better productivity under field conditions (Yanni et al. 1997). Recent development of rhizobia-rice association in context to progress and challenges of developing suitable biofertilizers for rice cultivation has been addressed. Harnessing NFB for the development of bioinoculants/biofertilizers/biopesticide, applicable to broad range of legume to nonlegumes crops like cereals,



**Fig. 1.1** Schematic relationship of rhizobia representing possible role of N cycling and plant growth promotion of nonlegumes. Differentiating this relationship with true symbiosis and free-living interaction of plants with other rhizobacteria

oil seed plants, vegetables, fruits, forages, and other important crops contribute in the sustainable development goal (SDG). On the other hand, a strategy to establish the C cycle coupled to N cycle in consideration to global warming is another route to achieve sustainable development goal.

## 1.2 Ecological Perspectives of Biological Nitrogen Fixation (BNF)

Ecology starts with its primary producers; and on Earth food chain starts with plants. An art or science of growing plant from the soil, requires nutrients, majorly nitrogen, that stands out as most important and more susceptible nutrient to plants as well as soil microbes. To build their proteins, and many components of life fixation of gaseous nitrogen by bacteria in specialized compartments, i.e., root nodules in legume plants have been studied vigorously. The benefits of these bacteria, in the form of nitrogen biofertilizers are inevitable. On the other hand, some free-living and associative bacteria, able to fix nitrogen biologically are in the concern to be utilized as biofertilizers in nonlegume crops. Behind BNF, biochemical genetics of symbiotic and asymbiotic nitrogen fixation has been reviewed in Chap. 2. It enunciates the potentials of symbiotic and free-living nitrogen-fixing bacteria in the transformation of green revolution to ever-green revolution. Application of rhizobia, as PGPR for nonlegume crops, and as a member of nitrogen fertilization has been advocated by many workers (Behera et al. 2021). As this association has received less attention but

with the development of science on molecular (cellular communication) and ecological aspects has augmented its importance in nitrogen fertilization. Plant growth-promoting traits in beneficial bacteria felicitate application in nonlegume as reviewed in Chap. 3. It explores future application of rhizobia as biofertilizer for nonlegume crop, particularly for alleviation of ecological stress. Coupled to this, Chap. 4 is concerned to the biotechnological solutions in the form of certain bacteria and archaea for enhanced nitrogen fertilization and eco-safe crop production. This addresses challenges for production of microbial products and biotechnological approaches as solutions which may be implemented to improve N nutrition in nonlegume crops.

Nitrogen-fixing bacteria (NFB) as effective microorganisms (EM) have great contribution to the green revolution (Lynch 2007). Chapter 5 is opened with the past of NFB and their application for field crop, rice, and its productivity enhancement. Further, understanding of genetic engineering for transferring nitrogen-fixing genes in rice plants has been advocated; however, with due to some limitations of extremely complex process of BNF which regulated by absence of oxygen has been criticized, therefore, demanded to develop some newer technologies. It is an exclusive account to understand application of QTL regions for BNF in rice, as a result of advance molecular biology. Further, authors suggest more research to be carried out to re-discover rhizospheric colonization mechanisms in NFB at molecular level. The diverse genera of bacteria, archaea, etc. have also understood as potential agents for BNF (Raymond et al. 2004). Similarly, reviewed in following Chap. 6; BNF in nonlegume has been proved as an important approach for sustainable crop growth and productivity enhancement under agroecological practices.

### 1.3 Playbacks of Nitrogen-Fixing Bacteria (NFB)

Application of rhizobia to improve growth, yield, nutrient composition, and quality of nonlegume plants has been augmented (Santoyo et al. 2021). Direct and indirect PGP traits of bacteria have been documented by a majority of workers (Orozco-Mosqueda et al. 2021). Biocontrol mechanisms of NFB has increased their importance to promote growth and increase the yield of nonlegumes (Nosheen et al. 2021). All these benefits are summarized in Chap. 7 with current research advancement on rhizobia and nonlegume interaction with cereals, as a holistic approach has been covered in Chap. 8. The increase in productivity of cereals demonstrates as a central theme of this chapter, which explores the beneficial roles of diazotrophs in biological nitrogen fixation and plant growth promotion. With the advent of PGP mechanisms of rhizobia population, and research in support, have claimed the application of rhizobia for nonlegume crops (Behera et al. 2021).

Therefore, there is a scope of using rhizobia (symbiont of legume) free-living and associative bacteria for nitrogen elevation in the soil, well suit for plant growth promotion (Nosheen et al. 2021). Chapter 9 states, nitrogen fertilizers are essential for producing high crop yields and are used extensively by farmers, besides its

abundant use has decreased the soil fertility (Rahman et al. 2021). Thus, pollution-free alternative to synthetic fertilizer in the form of diazotroph, those in play to fix atmospheric nitrogen and release in the form of  $\text{NH}_3$  (Pankievicz et al. 2021). On the background of previous chapters, this chapter entrusts establishment of diazotroph as NFB, with a showcase of hurdles and success of creating microbial consortia as nitrogenous biofertilizers.

Exclusive benefits of nitrogen fixation in soil or rhizosphere often reflect in plant growth promotion and yield improvement (Zvinavashe et al. 2021). Analogous to this concept, production improvement of commercial crops such as Cacao and Coffee, using NFB has been reviewed in the Chap. 10. This highlights on NFB as eco-safe alternative over chemical fertilizers, as a successful outcome of diazotrophs with nonlegume plants. Recommendations to explore management of N-sources from the environment either via intercropping or inoculating diazotrophic rhizobacteria have been proposed.

Further, attaining answer to the quest of sustainability of nonlegume crop, and judging with the theme of the book, plant growth-promoting bacteria (PGPBs) and nitrogen-fixing bacteria (NFBs) have been studied for their ecological roles, imparting benefits to nutrients transformation, soil organic matter mineralization, and carbon dynamics (Prasad et al. 2021). In this context, Chap. 11 identifies avenue of development of microbial inoculants for various crops like cereals, oil seed plants, vegetables, fruits, forages, and other important crops. In continuation, Chap. 12 unravels the benefits of using rhizobia in cereal crops, imparts stress regulation via enzymatic ethylene regulation by ACC deaminase, production of plant hormones, siderophore for iron management, and mineral (P and K) nutrient management via solubilization, mobilization of other nutrients. Not forming true nodule in the roots, it exclusively enters in cereal's root through crack entry and colonizes in the intercellular spaces such as in the xylem tissues.

In an overview, rhizobia have been emerged not only a true symbiont of legume crops but also an associative bacterium for nonlegume crops where, in the nonhabitual niche, they perform like a contender and function as plant growth-promoting rhizobacteria (PGPR).

## 1.4 Biofertilizer: A Step Toward Sustainable Development Goals

As stated earlier, food production via sustainable agriculture (SA) is a fundamental concept for curbing food security. It directly connects to the sustainable development goals (SDG) as a “blueprint to achieve a better and more sustainable future for all.” The outcome target of SDG, particularly sustainable food production systems and resilient agricultural practices, is achievable using biofertilizers, contributing to SA. Biofertilization toward SA is a driving force to counter Goal 2—“Zero Hunger” of the SDG. It involves promoting SA technologies in support to increase wheat, rice, and other nonlegume's crop productivity. To address the challenge of global

food security, it is required to be headed toward sustainable approach of using biofertilizers.

In line with this aspect, Chap. 13 embodied research evidences of biological nitrogen fixation in nonlegumes and contribution to sustainable development goal (SDG). It attempts to understand the rationale of using NFB, their ecological relationship with nonlegume, in-brief, besides major discussion focused on present challenges, future vision, and mission. Uninterruptedly, Chap. 14, explores the role of NFB, as potential producers of secondary metabolites modulate ecological behavior with nonlegume crops. It tries to identify mechanisms, involves in successful partnership among plant and bacteria, via production of plant root's exudates and molecular signals by bacteria necessary to engineer N-fixing association with nonlegume plants. A successful association between microbe and plants can increase nutritional ability of crop, as a result in improving the nutrient use efficiency, exclusively in the context to nitrogen use efficiency (Huang et al. 2022).

Application of biofertilizer is an alternative scheme to raise environment-friendly sustainable crop production system (Seenivasagan and Babalola 2021). Equally important as wheat, rice requires ample amount of nitrogen during cultivation and, therefore, application of NFB-based biofertilizer has gained prominence. Recent development of rhizobia-rice association in context to progress and challenges of developing suitable biofertilizers for rice cultivation has been addressed in Chap. 15.

N-fertilization in rice is not limited to use of rhizobia, because of several limitations; free-living rhizobacteria, as PGPR can contribute its production under field conditions, which is advocated in the Chap. 16. The past and present findings on the eco-physiological and agronomic aspects of free-living and endophytic N fixation in nonlegume crops with emphasis on rice have been reviewed. This is not limited to sustainable crop production of rice, besides embodied a clear commentary on the significant progress made on molecular-microbial aspects by development of meta-DNA/RNA analysis, indicating functioning N systems in the soil and plant. Uncovering metabolic aspects of NFB as microbial community, it identifies contributions to promote sustainable development. On the other hand, a strategy to establish the C cycle coupled to N cycle in consideration to global warming has been covered. Chapter 17 is concluding remarks on NFB and their role for sustainable growth of nonlegumes.

## 1.5 Conclusions

Future direction of development of biofertilizers to achieve sustainable agriculture and ever green revolutions is not limited to producing specialized biofertilizers for legume and nonlegume crops. With the recent science interventions of rhizobia-nonlegume interaction, free-living and associative rhizobacteria interaction with nonlegumes has created a notion to produce broad spectrum biological nitrogen-fixing biofertilizers for wide range of crops. This may emerge as an era of shaping future of human race as far as scarcity of food, safety, and security are concerned.

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