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Biological Nitrogen Fixation in Legumes: An Overview

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Abstract

Nitrogen is an essential nutrient for plants and is often a limiting factor in crop growth. Large quantities of fertiliser are often applied to crops which is an energy-consuming, expensive and pollution producing procedure from production to application. Biological nitrogen fixation is a solution to reduce nitrogen-related problems in agriculture. Biological nitrogen fixation, the reduction of dinitrogen (N2) to ammonia, is an essential reaction in the global nitrogen cycle. Many legumes have evolved to establish a symbiosis with nitrogen-fixing soil-bacteria collectively known as Rhizobia. More than 98 species of symbiotic nitrogen-fixing rhizobia exist in 14 taxa in association with legumes.

Keywords: Symbiotic nitrogen fixation, biological N fixation, legumes

1. Introduction

Projections of population growth, particularly in developing nations, and rising life expectancies globally suggest increased demand for food and feed. Agriculture is being intensified to increase production, primarily with monocultures that are heavily dependent on increased chemical inputs, such as pesticides and fertilisers (McArthur and McCord, 2017). To achieve economic returns for farmers, but with stability in long-term production and little negative influence on the environment, we must establish sustainable agriculture practises based on conservationist practises (de Moraes Sa et al. 2017). The most limiting element in ecosystems and a necessary component of all living creatures is nitrogen. Nitrogen is an essential nutrient for plants and is often a limiting factor in crop growth. Consequently, large quantities of fertiliser are often applied to crops. This is an energy-consuming and expensive procedure (Day et al., 2001). Despite the significant contribution of synthetic fertilizers, nitrogen requirements for food production increase from year to year, while the overuse of agrochemicals compromise soil health and agricultural sustainability (Soumare et al., 2020).

2. Biological nitrogen fixation

Biological nitrogen fixation is a solution to nitrogen-related problems in agriculture. In this context, the use of microbial inoculants plays a key role, and we may say that we are starting a “microgreen revolution” (Fukami et al., 2018). Symbiotic N2 fixation is the term used to describe the biological conversion of atmospheric N2 to NH3 for use by some plants, many of which are legumes, in the presence of soil bacteria (Rhizobia). The ability of these plants to develop without additional nitrogen fertiliser has obvious benefits for sustainable agriculture. Biological nitrogen fixation, the reduction of dinitrogen (N_2) to ammonia, is an essential reaction in the global nitrogen cycle. Biological N_2 fixation involves the conversion of atmospheric N_2 to NH_3, a reaction catalysed by the enzyme nitrogenase that is found only in certain prokaryotes, including members of the *Rhizobiaceae* that form symbioses with legumes (Day et al., 2001). Indeed, more than 60% of the fixed N on earth results from biological nitrogen fixation. Therefore, optimizing biological nitrogen fixation in agriculture is more and more urgent to help meet the demand of the food production needs for the growing world population. This optimization will require a good knowledge of the nitrogen-fixation (Soumare et al., 2020). About two-thirds of the fixed nitrogen produced on earth is produced through biological nitrogen fixation, which is catalysed by the nitrogenase complex (Rubio and Ludden, 2008). Despite not being present in eukaryotes, N_2-fixation is widely distributed throughout bacteria and archaea, demonstrating the significant diversification among diazotrophs. Most bacterial phylogenetic groupings possess the ability to fix N_2, which is compatible with a wide range of physiologies (Dixon and Kahn, 2004). More than 98 species of symbiotic nitrogen-fixing rhizobia exist in 14 taxa in association with legumes (Berrada and Fikri-Benbrahim, 2014). Many legumes have evolved to establish a symbiosis with nitrogen-fixing soil-bacteria collectively known as rhizobia (including the genera Azorhizobium, Allorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium and Sinorhizobium). Rhizobia invade the roots of compatible legume plants leading to the development of specialized root structures called nodules (Oldroyd et al., 2011). As a result of their unique ability to fix nitrogen through symbiotic relationships, legume crops are important contributors to the global nitrogen cycle and important commercial sources of oil and protein for both humans and animals. Legumes play a significant role in agriculture and also produce a variety of beneficial secondary chemicals, many of which have been demonstrated to have health-promoting properties including resistance to human diseases. Forage legumes are also essential to the productivity of farmed pastures due to their strong N_2 fixation capacity and capacity to grow in soils with low fertility (Jian et al., 2009). Some of the most significant agricultural species in the world are legumes. Legume crops are important economically since they are consumed by millions of people worldwide and have exceptional nutritional qualities. Legume seeds have twice as much protein as most cereals (Jacob et al., 2016). They have the capacity to develop new root organs called nodules, where biological nitrogen fixation becomes possible through a symbiotic relationship with Rhizobia bacterium. Since nitrogen is essential for growth and development, this gives legumes a particular advantage over other plant species. The plant closely controls nodule production, which can be inhibited by a variety of external factors. Factors such as soil composition, water content, temperature and pH can also influence plant and Rhizobia growth, and nodule establishment (Ferguson et al., 2013). Symbiotic N_2 fixation in legumes takes place in nodules on the roots of the host plant. The nitrogenase-producing rhizobia, known as bacteroids, reside in the infected cells of these organs. The bacteroids fix atmospheric N_2 into NH_3 within the specialised, low-oxygen environment of nodule cells, providing the host with a source of nitrogen in exchange for reduced carbon, most likely in the form of dicarboxylic acids like malate (Poole and Allaway, 2000). The interchange of two separate substances, nitrogen (N) and carbon (C), is primarily regulates the unique relationship between the nodulating root and its hosting plant. Reduced-C (carbohydrates) from the plant is used by the bacteria as energy, food, and a catalyst in the N_2 fixation process, while reduced-N is delivered back to the plant by the nodules (White et al., 2007). A symbiosome or peribacteroid membrane of plant origin surrounds the bacteroids inside the infected plant root cells, effectively separating them from the plant cytoplasm and regulating the types and quantities of chemicals that are exchanged between the symbionts. Rhizobia residing inside the nodule are consequently fully reliant on their plant hosts for nutrition. By regulating the flow of metabolites between the two symbionts, the peribacteroid membrane plays a crucial role in the regulation of N_2 fixation and the maintenance of the symbiosis. Although the principal metabolic exchange between the symbiotic partners is "reduced carbon" to the bacteroid for "fixed nitrogen" to the plant, other important nutrient exchange also occurs (Day et al., 2001). Sustainability in agriculture is more crucial for distributing resources as inputs than for producing goods. In order to achieve sustainability, soil biological health and legumes have recently received attention. Particularly in farming systems that rely on legumes, soil rhizobacteria are crucial. This is because the productivity of such systems is frequently constrained by the availability of resources like water and nutrients (Sofi et al., 2018). Rhizobacteria treatment, seed priming, foliar application of various macro and micronutrients, and changing the planting time are a few agronomic techniques that may somewhat mitigate the negative effects of heat and drought stress (Kumari et al., 2021). One of the environmental elements that has the biggest impact on crop output is drought. One of the physiological processes that causes nodulated legumes to first exhibit stress responses during a drought is symbiotic nitrogen fixation (Larrainzar et al., 2007). Despite the potential for cultivating legumes to enhance sustainable agriculture, yield unpredictability continues to be a significant obstacle for their adoption (Prudent et al., 2020). Climate change has increased the frequency of extreme weather patterns globally, which has significantly reduced crop yield and threatened food security. To fulfil the rising world population's demand for food, a higher rate of genetic breakthroughs that increase the productivity of important crops is required. Grain legumes are an essential part of both healthy human diets and animal feed due to their unique nutritional content. Currently, a severe water shortage significantly limits the production of grain legumes (Ye et al., 2018). Legume yields are eventually impacted by reduced leaf area, shoot and root growth, chlorophyll content, stomatal conductance, CO_2 influx, nutrient uptake and translocation, and water-use efficiency (WUE). Grain legume yield loss varies from species to species, and even within a species, from variety to variety, depending on the degree of drought stress and a number of other variables, including phenology, soil textures, and agro-climatic conditions. When a plant is stressed by dryness, stomata closure increases leaf temperature by lowering transpiration rate, which puts the legume plant under further stress. Reactive oxygen species (ROS) generation is the most detrimental effect of drought stress. To adapt to the drought stress, legumes can change their morphology, physiology, and molecular pathways. Drought resistance is greatly influenced by factors such as improved root system architecture, fewer and smaller leaves, stress-induced phytohormone, stomatal closure, antioxidant defense system, solute buildup (such as proline), and changed gene expression. A variety of agronomic, breeding techniques—conventional as well as molecular and biotechnological—are employed to develop a drought-tolerant legume without impacting crop production. Exogenous application of plant-growth regulators (PGRs), osmoprotectants and inoculation by Rhizobacteria and arbuscular mycorrhizal fungi promotes drought tolerance in legumes (Khatun et al., 2021).

3. Some notes on biological nitrogen fixation is selected legume crops

Soybean (*Glycine max* [L.] Merr) seeds contain a high proportion of protein, about 40% based on dry weight, therefore, they require a large amount of nitrogen to produce high yields. About 8 kg N is required for 100 kg of soybean seed production. Soybean can use atmospheric dinitrogen (N_2) by nitrogen fixation of root nodules associated with soil bacteria Rhizobia. Soybean plants can absorb combined nitrogen such as nitrate for their nutrition either from soil mineralized N or fertilizer N. It is well known that heavy supply of nitrogen fertilizer often causes the inhibition of nodulation and nitrogen fixation. Therefore, only a little or no nitrogen fertilizer is practically applied for soybean production. However, soybean plants only depend on the nitrogen fixation shows poor growth and low seed yield, because of the early decline in photosynthesis by decreased supply of nitrogen during the pod filling stage. Both soil N and symbiotic N are required for the optimum soybean production (Ohyama et al., 2011). Historical gains in soybean seed yields are primarily due to increases in seed biomass, which demonstrates an improvement in seed partitioning efficiency (Koester et al., 2014). The increase in partitioning efficiency and seed biomass requires larger N demand (Balboa et al., 2018), primarily met by biological N_2 fixation and soil N mineralization (Ciampitti and Salvagiotti, 2018). The amount of atmospheric N_2 fixed by a soybean crop varies widely. Typical values are about 100–175 kg N ha^{-1}, which represents about 50% of crop needs (Unkovich and Pate, 2000). This partial contribution of N_2 fixation to meet soybean N needs has encouraged research on nitrogen fertilization of soybean for years. In general, nitrogen fertilization of soybean at sowing failed to increase seed yield (Mendes et al. 2003). This general lack of response to nitrogen added at sowing is related to the well-known inhibitory effect of high levels of soil nitrates on both nodule formation and nitrogenase activity in nodules already formed (Gutiérrez-Boem et al., 2004). Reduction in N_2 fixation when soil nitrate availability increases is due to direct effects of nitrate on nodule metabolism (Zhang and Smith, 2002). As a result of this inhibition, nitrogen fertilization at sowing usually induces a substitution of fixed N_2 by added N without affecting total N assimilation. An alternative approach to nitrogen fertilization of soybean is to apply nitrogen late in the growing season (Gutiérrez-Boem et al., 2004). In soybean, N derived from the atmosphere via BNF can range from 0 to 98% of the total N uptake, representing 0 to 337 kg N ha^{-1}, depending on rhizobia activity. However, N removal from the system (i.e., by seed N) is determined by different factors that affect seed yield and N harvest index (NHI; seed N uptake to total N uptake) (Salvagiotti et al., 2008). A review study summarizing 108 scientific papers published from 1966 to 2006 documented an average N derived from the atmosphere contribution of 50 to 60% (Salvagiotti et al., 2008). However, the question, whether N2 fixation alone can supply N for a high-yielding soybean while maintaining a neutral partial N balance (fixed N in aboveground biomass minus N removed in seeds), remains unanswered. Although the scientific literature has extensively discussed the relationships between soybean seed yield, nitrogen uptake, biological N_2 fixation, and response to N fertilisation, a thorough summary and interpretation of these interactions with a focus on high yield environments is lacking. The field studies that assessed these factors and were published in refereed journals between 1966 and 2006 totaled 637 data sets (site-year-treatment combinations). On an average, 50–60% of soybean N demand was met by biological N_2 fixation. In most situations the amount of N fixed was not sufficient to replace N export from the field in harvested seed. The partial N balance (fixed N in above-ground biomass − N in seeds) was negative in 80% of all data sets, with a mean net soil N mining of −40 kg N ha^{-1}. However, when an average estimated below-ground N contribution of 24% of total plant N was included, the average N balance was close to neutral (−4 kg N ha^{-1}). The gap between crop N uptake and N supplied by biological N_2 fixation tended to increase at higher seed yields for which the associated crop N demand is higher. Soybean yield was more likely to respond to N fertilization in high-yield (>4.5 Mg ha^{-1}) environments. A negative exponential relationship was observed between N fertilizer rate and N_2 fixation when N was applied on the surface or incorporated in the topmost soil layers. For attaining a yield response to N fertilisation in high-yielding conditions, deep placement of slow-release fertiliser below the nodulation zone or late N administrations during reproductive stages may be attractive alternatives. The results of several N fertilisation tests are frequently complicated by biological N_2 fixation that has not been properly optimised or by other management choices that may have prevented yields mediated by biological N_2 fixation from approaching the yield potential ceiling. More studies will be needed to fully understand the extent to which the N requirements of soybean grown at potential yields levels can be met by optimizing biological N_2 fixation alone as opposed to supplementing biological N_2 fixation with applied N. Such optimization will require evaluating new inoculant technologies, greater temporal precision in crop and soil management, and most importantly, detailed measurements of the contributions of soil N, biological N_2 fixation, and the efficiency of fertilizer N uptake throughout the crop cycle. Such information is required to develop more reliable guidelines for managing both biological N_2 fixation and fertilizer N in high-yielding environments, and also to improve soybean simulation models (Salvagiotti et al., 2008). Applying fertilizer-N has been proposed as an aid for increasing available N in the soil. Studies of nodulated soybeans showed significant yield response to frequent N additions when the N_2 fixation apparatus could not meet N demand. However, yield response of soybean to fertilizer N has been inconsistent at economically acceptable levels (Barker and Sawyer, 2005). An important research question is whether fertilizer N can alleviate N limitations without compromising the N_2 fixation capacity of the crop and doing so in a cost effective manner. Those studies reporting no increase in grain yield assumed that the crop simply substitutes the N it ordinarily would have derived from BNF with N from fertilizer, or that more N translocation from vegetative reserves occurs when applied N lowers the rate of N_2 fixation. Theoretically, late N applications at reproductive stages (R3 to R5) should theoretically increase yields in high-yielding environments, but empirically measured responses in grain yield to fertilizer-N applied at late R-stage are frequently observed (Gutiérrez-Boem et al., 2004). An early-season N deficiency may hinder early crop growth and, consequently, the development of an effective nodulation system, whereas early application of even small amounts of N frequently results in temporary inhibition of nodule establishment and subsequent activity (Hungria et al., 2005a). Overall, inconsistent results from N fertilisation studies make it unclear if N fertilisation is necessary to supplement the N supply from BNF in order to produce soybean yields that are close to their yield potential levels (Salvagiotti et al., 2008). Cowpea (*Vigna unguiculata* L. Walp.) is a versatile legume with several uses. It is rich in protein for cattle fodder and contains high-quality protein for human use. It enhances the soil by recycling nutrients through nitrogen fixation in collaboration with nodulating microorganisms (Omomowo and Babalola, 2021). Cowpea can develop successful tripartite symbiotic associations with nitrogen-fixing bacteria (*Sinorhizobium meliloti*) and arbuscular mycorrhizal fungi, albeit appropriate arbuscular mycorrhizal fungi species and rhizobial strains that promote cowpea growth must be chosen (Kavadia et al., 2021). *Pisum sativum* L. is a *Fabaceae* family legume plant that grows in winter season. It is grown in over 95 countries largely for its protein-rich seeds, which are used for food and feed (Gondalia et al., 2022). The pea is a temperate legume that is grown as a vegetable and a pulse crop (Javaid et al., 2022). One of the most significant self-pollinating legume crops is the garden pea (*Pisum sativum* L.). Peas are a valuable crop that can be used for food, feed, and industrial purposes all around the world. Garden peas are an annual winter-season crop that is grown from winter to early summer in different parts of the world. It is an important component of low-input cropping systems because of its symbiosis with nitrogen-fixing bacteria. Dry pea (*Pisum sativum* L.) is a good source of low-digestible carbohydrates, protein, and micronutrients in terms of nutrition (Thavarajah et al., 2022). *Pisum sativum* is a leguminous crop that may be grown every soil. It's utilized in animal feed as a fodder or dry seed supplement, and, more recently, as a non-traditional oilseed (Addo et al., 2022).

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