REVIEW ARTICLE
Agricultural Reviews, Volume 44 Issue 1: 22-30 (March 2023)

Factors Impacting Rhizobium-legume Symbiotic Nitrogen Fixation with the Physiological and Genetic Responses to Overcome the Adverse Conditions: A Review

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10.18805/ag.RF-257

ABSTRACT

Symbiotic N_2 fixation is essential for the plant's growth because it can fix reactive nitrogen compounds in soil. However, all steps of this process can be hampered by several biotic and abiotic environmental factors. This study mainly focused on discussing the impacts of 12 major factors on this process by reviewing the significant numbers of research works. According to the information from these works, we found some significant physiological and genetic impacts caused by these factors like plasmid deletion, genomic mismanagement, abnormal molecular signals, toxicity, deficiency of minerals, deformation of rhizobial cells, protein denaturation, nucleic acid damage, acetylene reduction and nod factors limitation. Furthermore, expression of heat or acid shock proteins, internal buffering, genes spanning, extracellular immobilization, periplasmic allocation, change of lipopolysaccharides composition, intracellular accumulation of inorganic and organic solutes (Osmolyte) and activation of hydrogenase expression are shown by both micro and macro symbionts as a natural response to adapt to these stress conditions. Though the stress-tolerant strains like HR-3, HR-6, HR-10, HR-12, acta, actP, exoR, lpiA, actR, actS and phrR can be used to sense the external environment and make signals to change gene transcription during the adverse condition, the application of genetic engineering should be expanded more to promote the commercial inoculation by the production of novel stress-tolerant strains or modified genes of rhizobia and legumes.

Key words: Abiotic, Biotic, Rhizobium-legume, Symbiotic nitrogen fixation.

Nitrogen is a critical limiting factor for the growth and production of plants. It is a significant component of chlorophyll, amino acids, ATP and nucleic acids (Werner and Newton, 2005). But this molecule is highly inert for being composed of two nitrogen atoms (N_2) joined by a triple covalent bond. As a result, plants are not able to use the reduced forms of this element. However, it is one of the most abundant elements (About 78% of the atmospheric air) in Earth's atmosphere. However, Symbionts can convert this atmospheric nitrogen into nitrogenous compounds following the fixation process:

1) Physical nitrogen fixation

a) Natural nitrogen fixation

Processed by natural actions such as lightning, burning fire, or magnetic reaction responsible for 10% fixed nitrogen in the soil.

b) Industrial nitrogen fixation

Processed by applying of fertilizer (Ammonia or Nitrate) or organic manure to the soil responsible for 25% fixed Nitrogen in the soil.

2) Biological nitrogen fixation (BNF)

Processed by the living organism that can fix 60% of total global nitrogen in the soil (Wagner, 2011). A specialized group of prokaryotes controls it. About 87 species in 2 genera of archaea, 38 genera of bacteria and 20 genera of cyanobacteria have been identified as diazotrophs that can fix nitrogen (Dixon and Wheel, 1986).

These prokaryotes include several types of BNF that are-

A. Non-symbiotic/Asymbiotic Biological Nitrogen Fixation.
B. Associative Biological Nitrogen Fixation.
C. Symbiotic Biological Nitrogen Fixation.

Symbiotic Biological Nitrogen Fixation is a part of a mutualistic relationship in which approximately 700 genera and about 13,000 species of legumes (Most important nitrogen-fixing symbiotic legumes in agricultural systems are alfalfa, beans, clover, cowpeas, lupines, peanut, soybean and vetches) provide a niche and fixed carbon to six genera

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How to cite this article: Owaresat, J.K., Siam, M.A.H., Dey, D., Jabed, S., Badsha, F., Islam, M.R. and Kabir, M.S. (2023). Factors Impacting Rhizobium-legume Symbiotic Nitrogen Fixation with the Physiological and Genetic Responses to Overcome the Adverse Conditions: A Review. Agricultural Reviews. 44(1): 22-30. doi: 10.18805/ag.RF-257.

Submitted: 27-08-2022 **Accepted:** 09-02-2023 **Online:** 25-02-2023

of bacteria, collectively called rhizobia in exchange for fixed nitrogen (Noel, 2009).

The principal reaction for dinitrogen reaction is:

$N_2 + 16\ MgATP + 8e- + 8H+ \rightarrow 2NH_3 + H_2 + 16\ MgADP + 16Pi$

This reduction of atmospheric nitrogen is a complex process that is carried out by the activity of several biochemical factors (nitrogenase enzyme, energy, electron flow, leghemoglobin) and morphologically changed unique structures (nodule and bacteroid) of rhizobia and legume. The nitrogenase enzyme is supplied from rhizobia body cells that can catalyze the reduction of several substrates including H^+, N_2 and C_2H_2. However, the enzyme is sensitive to free oxygen in nodules that can inhibit levels of nitrogen fixation. The leghemoglobin supplied by functioning nodules can control the level of oxygen by scavenging this oxygen out from the nodule. The required energy for this reaction (960 $KJmol^{-1}$ N-fixed) in the form of ATP (Hubbell and Kidder, 2009) is supplied from the respiration of carbohydrates from host plants' rhizospheres and the electron is supplied by electron carriers such as the ferredoxin.

The nitrogen compound can also be fixed essentially in the conventional agriculture that depends upon the commercial fertilizer by the Haber-Bosch process in the industry. But this process uses fossil fuels that release 1.10-3.37 t of CO_2/ton of fossil fuel burnt. Though 83% of supplied amount of Synthesized nitrogen (120 Tg) (FAO, 2017) is consumed as nutrients by the crops in agricultural fields, the remaining 17% causes aquatic systems pollution by releasing N_2O or (NO) gases (294 times more GHG effect than carbon dioxide) in the environment (Ladha *et al.* 2016) that indicates 2% consumption of global energy by 2050 may occur due to this chemical synthesis of N fertilizers (Glendining *et al.* 2009). On the other hand, Symbiotic N_2 fixation plays an essential role as an eco-friendly means of sustaining crop productivity and maintaining soil fertility, especially on marginal lands and in smallholder farming systems by providing nitrogen minerals for plants.

However, this symbiotic N_2 fixation is particularly sensitive to various abiotic and biotic stresses such as temperature, light, drought, soil salinity, acidity, pathogen and other nutrient limitations.

Environmental stress imposes a major threat to both symbiotic nitrogen fixation and agriculture influencing the growth, survival and metabolic activities of symbiotic bacteria and plants and their ability to enter into symbiotic interactions and supply N in soil (Werner and Newton, 2005).

The environmental factors affecting the symbiotic N_2 fixation can be divided as following several categories:-

A) Abiotic factors

1. Temperature

Soil temperature is one of the abiotic factors which can hamper the rate of symbiotic nitrogen fixation by affecting the persistence of rhizobial and legume species in soil. Each legume-rhizobia interaction intends to show specificities for optimal performance (Igiehon *et al.* 2019) that correlate with strains and soil types. Hungria *et al.* (1997a) suggested that a difference of 6°C at 5 cm depth can reduce the *Bradyrhizobium sp*. population by more than 10000 cells in g^{-1} soil. Though the optimum temperature range for symbiosis is 25°C to 33°C, it can vary among species (Dwivedi *et al.,* 2015). Eaglesham and Ayanaba (1984) reported that 90% of cowpea rhizobial strains and some legumes (soybean, guar, peanut) in a dry environment can grow well at 35°C -41°C and even some bacteria can survive at 60°C in sandy soils where Matthews and Hayes (1982) and Yuan *et al.* (2020) noted that the inhibition of soybean nodulation can happen below 10°C and even some strains of rhizobia can show strong resilience even at 4°C.

Wang *et al.* (2018) noted the impacts of low temperatures that can increase *the* rigidity of cellular membrane and limit the secretion of flavonoids and nod factors (lipochitooligosaccharides) involved in signaling of nodule formation in *Rhizobium leguminosarum bv. Trifolii* and *Brady rhizobium sp.*

However, heat tolerance can be shown by some symbiotic strains during stress conditions. According to Yura *et al.* (2000), the heat shock protein "Chaperone" can be naturally synthesized in both heat-tolerant nodulating rhizobia strains at sudden temperature changes. This thermotolerance can be regulated by overexpression of some molecular chaperons like native GroEL(HSP60), GroES(HSP10), DnaKJ (HSP70), IbpA (sHsPs) and IbpB (sHsPs) proteins that can protect intracellular proteins from misfolding or aggregation, inhibit cell death and preserve the intracellular signalling pathways that are essential for cell survival during stress conditions by correctly folding or refolding proteins that are damaged by the cell stress (Nandal *et al.* 2005).

2. Light

Light intensity has an impact on symbiosis as light can extend the photosynthetic capacity of plants and the required energy and total N content for bacteriodes (Carranca, 2013). The quality and quantity of light effect in vegetation can be influenced by tree canopy or shade (Dubbert *et al.* 2014). This canopy can concentrate a higher rooting volume and senescent leaves, fruits and decomposing fungi (ectomycorrhiza) which can affect soil quality and pasture performance. Trang and Giddens (1980) examined that plants tops and roots with no shade can produce higher N content, total nonstructural carbohydrate (TNC) and nodule mass for getting a higher photosynthetic efficiency than when shaded at 18, 40 and 62% (Fig 1) where Murphy (1986) reported that plants were inoculated with a mutant strain of *Rhizobium trifolii* supplied with a nutrient medium containing 30 ppm N, grown at 26,000 lux for 42 days over 14 hours photoperiod at 15°C-20°C can produce more total N, TNC and nodule mass than the plants grown for further 14 days over 6 hours photoperiod in the same nutrient medium.

3. Acidity

In agricultural production area 25% of the earth's croplands are affected by soil acidity (Graham and Vance, 2000).

The optimum pH for rhizobial growth is between 6.0 and 7.0 (Hungria and Vargas, 2000). However, Brockwell *et al.* (2005) observed that some species of legume like Lucerne *(M. sativa)* are susceptible to acidity while *Lotus tenuis* is relatively acid tolerant. According to Leinonen *et al.* (2019), *R. leguminosarum bv. trifolii, R. tropici, R. meliloti, Mesorhizobium loti, Bradyrhizobium sp*. and *Sinorhizobium meliloti* are highly acid-sensitive (pH 9 or pH 12) whereas *R. loti, R. meliloti* WSM 419, *R. cellulosilyticum, R. taibaishanense* and *Sinorhizobium meliloti* are able to live at pH 4-5.

Soil acidity can directly affect productivity and symbiotic characteristics of rhizobia and legume species and indirectly limit nodulation and root infection in both tropical and temperate soils by inducing toxicity (Al and Mn) and declining nutrients (phosphorus, molybdenum and calcium) supply. Lira *et al.* (2015) reported that higher acidity can disrupt the secretion of flavonoids and nod factors (nodA), the exchange of molecular signals between macro and micro symbiotic partners whereas Farissi *et al.* (2014) noted that alkalinity can affect symbiosis by reducing essential minerals (Fe, Mn) though liming is effective in overcoming soil acidity.

To control ideal intracellular pH, rhizobia can follow some biochemical mechanisms including synthesis of acid shock proteins (ASPs), exclusion and expulsion of protons H^{+}, accumulation of potassium glutamate and polyamines, change of lipopolysaccharides composition membrane permeability, control of internal buffering and prevention of metal ion toxicity (Ormeño-Orrillo *et al.* 2016). Apart from that, legumes (Lentil) can show several protective mechanisms to adapt to acidity by secreting citric, malic, aspartic, gluconic and succinic acids in roots. Furthermore, according to Draghi *et al.* (2016), several genes such as actA, actP, exoR, lpiA, actR, actS and phrR can sense the external environment and make a signal to change gene transcription- essential for rhizobial growth during low pH condition.

4. Salinity

Salt stress is usually associated with mineral ion toxicity and nutrient disorder (Na^{+}, Mg^{+2} and Cl^{+}) (Niste *et al.* 2013) where the 40% of the available land surface area is affected by salinity that severely limits productivity potential of legume crops and rhizobia (Zahran, 2001; Brígido *et al.* 2012). The feedback to adapt with saline stress can show variations among rhizobia and legume species. Embalomatis *et al.* (1994) and Lal and Khanna, (1994) reported that the growth of free rhizobia can be inhibited at 100 mM NaCl, some symbiotic rhizobia, such as *Rhizobium leguminosarum* and *Sinorhizobium meliloti* may be tolerant to at 300 to 700 mM NaCl and some rhizobia strains from *Acacia sp., Prosopis sp* and *Leucaena sp.* can also persist on a concentration of 500 to 850 mM NaCl.

Higher salinity can decline bacterial colonization such as *V. faba* can be low in number at 50 to 100 mM NaCl or 100 to 200 mM polyethylene glycol concentration (Zahran and Sprent, 1986). Dardanelli *et al.* (2012) noted that bacteria and plants can experience severe water deficiency for salt damage and can also affect the induction of nod genes (lipochitooligosaccharides). In addition, salt stress can cause a deficiency of carbon and Ca^{+2} that finally reduces the accumulation of sucrose up to 40 to 70% of total nodule sugar content for bacteroids and lead to the deformation of outer membrane structure of rhizobial cell and limit O_2 diffusion (Mohmmadi *et al.* 2012).

Various rhizobial mutant strains' responses to salt tolerance were proved to indicate their evolutionary fitness to the environment (Burghardt, 2020). Dong *et al.* (2017) reported that alleviation of osmotic stress can be regulated by intracellular accumulation of inorganic and organic solutes (Osmolyte) where salt-tolerant strains *R. meliloti, Brady rhizobium sp., R. fredii and Sinorhizobium fredii* can produce K^{+}, glutamate, proline, glycine betaine, proline betaine, trehalose, dipeptide, N-acetyl glutaminyl glutamine amide to

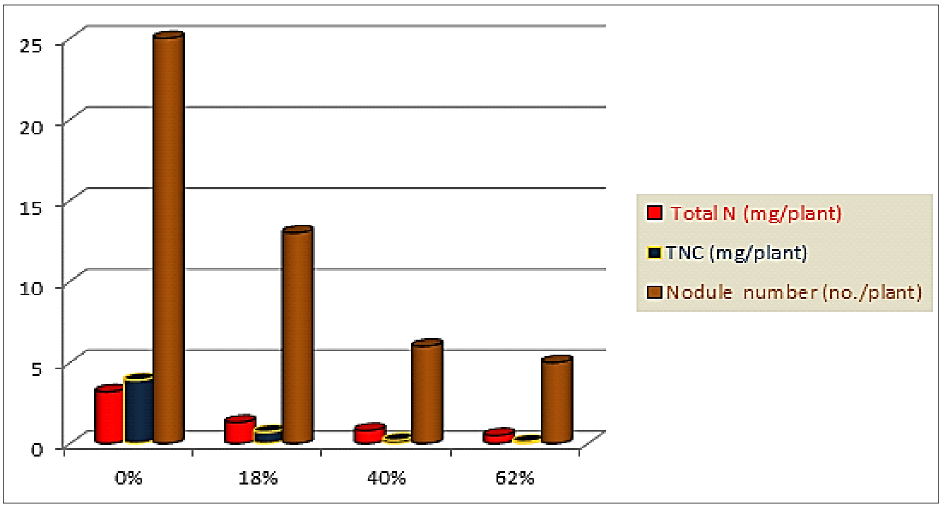

Fig 1: Effect of shading on total N, TNC (Total nonstructural carbohydrate) and nodulation of soybean plant roots (Trang and Giddens, 1980).

produce higher number of nodules, plant dry weight and nitrogen level at high salt stress up to 300 to 400 mM.

5. Drought

The water stress due to increased rate of transpiration or evaporation (Jaleel *et al*. 2009) can reduce transport of nitrogenous compounds by 26% in soil. The impact of water stress during vegetative growth can be more detrimental to nodulation and nitrogen fixation than the impact during the reproduction stage (Pena-Cabriales and Castellanos, 1993). Besides, the legume (Soybean and Cowpea) transporting high concentrations of N_2 compounds (xylem sap ureides) can be found to be more drought sensitive than those with no or low ureide transport (Sinclair and Serraj, 1995). Turco and Sadowsky (1995) observed that soil water is related to soil pore space where the soil having smaller internal pore spaces is more favorable for the growth of rhizobia. According to Pucciariello *et al*. (2019), soil water has direct impacts on nitrogenase activity, synthesis of leghemoglobin, nodule-specific activity, growth of rhizosphere, root infection by reducing some water activities (diffusion, mass flow and nutrient concentration) below critical tolerance limits. The water deficiency can also cause an indirect effect on host plant growth and root architecture by increasing the nodule's acid abscisic content, accelerating nodules' senescence in soybean activity. Casteriano (2014) reported that when soil moisture is reduced from 5.5 to 3.5%, *R. meliloti* body cell shows undesirable morphological and physiochemical changes including plasmid deletions, genomic rearrangements, lipids peroxidation, protein denaturation and nucleic acid damage in due to the formation of hydroxyl and peroxyl radicals in cell.

However, many species of rhizobia can persist in severe water deficiency by various adaptive strategies including synthesis of chaperones and sugars, stress enzyme 1-aminocyclopropane 1-carboxylic acid, exopolysaccharides, pinitol (o-methylinositol), trehalose, proline and betaine that can improve the nutrient availability, production of siderophores and phytohormones (Furlan *et al*. 2017).

6. Heavy metal

Heavy metals are the most essential inorganic pollutants which can pose an ecotoxicological impact on symbiotic diversity of microorganisms and hosts by limiting nodule formation and effective rhizobium strains number from the soil. The most common heavy metals contaminating the soil are Aluminium (Al), lead (Pb), cadmium (Cd), arsenic (As), zinc (Zn), chromium (Cr), copper (Cu), mercury (Hg) and nickel (Ni) (Li *et al.* 2019). Rhizobial responses to different types of heavy metals are connected with applied concentrations in soil. Stambulska *et al.* (2018) observed that higher amount of Cr^{+6} and Cr^{+3} ions can induce a very strong oxidative stress on symbiotic interactions whereas Cd^{+2} at low concentration (10 µM) can be toxic for metabolic interaction between peanut and *Brady rhizobium sp*. Besides, nod gene expression, nodulation, nodule number and dry weight in cowpea can be reduced by 12.7-17.5 mM Al^{+3} toxicity. Although Cu, Ni, Co and Zn are absorbed as micronutrients, their higher concentrations can be toxic to plants and microorganisms where contamination of Cu^{+2} in soil can decline nodule number in common beans (Laguerre *et al.* 2006).

However, rhizobia can exhibit various resistance responses to ameliorate heavy toxicity in acidic soils. But the strains at high concentrations of heavy metals that are not different in plasmid profiles are ineffective in N_2 fixation because of having lack of genetic diversity such as the Strains of *Rhizobium sp*. and *Bradyrhizobium sp.* are resistant to aluminum (50 mM) stress (Wood *et al.* 1988). According to Pajuelo *et al.* (2011), *Rhizobium sp*. can synthesize huge amounts of siderophores, citric acid, extracellular polysaccharide and lipopolysaccharide to sequester most of the extracellular metal from the body cell. Another resistance system is shown by ATPases and chemiosmotic ion/proton exchangers. An interference in a dmeF gene can play a role in making *R. leguminosarum bv. viciae* susceptible to Ni and Co toxicity. Other mechanisms are extracellular immobilization, periplasmic allocation and biotransformation of toxic products to deal with the metal stress (Cardoso *et al.* 2018).

7. Minerals

Though heavy metals cause extensive adverse effects on symbiotic N_2 fixation, some metals with specific concentrations can act as nutrients for improving rhizobium-legume symbiosis process. Iron (Fe) is considered an essential micronutrient for increasing shoot growth, abundance of cytochromes, nodule mass, bacteroid in soil by activating several key enzymes like nitrogenase complex, electron carrier ferredoxin and hydrogenases. It can provide a "heme component" for leghemoglobin to control respiration demand (Tang *et al*. 1990). Copper (Cu) is required for N_2 fixation in rhizobia playing a role in a protein that is expressed coordinately with nif genes and can affect efficacy of bacteroid function. High Calcium (Ca^{+2}) concentration (3.0-10 mM) at pH 4.5-5.2 can increase nodulation, attachment of rhizobia to root hairs, nitrogenase activity and nod gene expression of common bean (*Phaseolus vulgaris*) and rhizobial cell wall integrity and membrane transport systems as 5-10 times as doing low Ca^{+2} concentration (0.13 mM) at pH 4.5. Potassium (K) can apparently lessen the effects of water shortage on symbiotic N_2 fixation of *V. faba* and *P. vulgaris* stress (Karanja and Wood, 1988). Sulfur (S) is an essential element for growth and physiological functioning of legume plants. Boron (B) can impact on rhizobium-legume cell-surface interaction, infection of threads and nodule development in pea. Manganese (Mn) can play a role in synthesising polyamines and detoxifying active oxygen species, which has an overall impact on legume plant growth and development (Hohenberg and Munns, 1984). Nickel (Ni) is essential for root nodule growth and hydrogenase activation that can control oxidation of hydrogen where the latter provides ATP required for N reduction to ammonia. Cobalt (Co) in N_2 fixation is essentially attributed to its role as a cofactor of cobalamin (Vitamin b6) which can act as a coenzyme involved in N_2 fixation and nodule growth (O'Hara *et al*. 1989).

8. H_2 evolution

The extent of hydrogen evolution during nitrogen reduction is a major factor influencing the extent of nitrogen fixation by wasting ATP. Notaris *et al.* (2021) recently reported that the magnitude of energy loss in terms of efficiency of electron transfer to nitrogen *via* nitrogenase in the excised nodules indicates that hydrogen production can severely decline nitrogen fixation in many legumes. For instance, with most symbionts including soybeans, only 40-60% of electron flow can be transferred to nitrogen whereas the remainder can be lost through hydrogen evolution. Some non-leguminous symbionts, such as red alder (*Alnus rubra)* and few legumes Asparagus bean (*Vigna sinensis*) apparently can evolve this mechanism of minimizing net hydrogen production to increase the efficiency of electrons.

According to Rainbird *et al*. (1983), the greater H_2 evolution causes a change that can result in suboptimal function including decreased synthesis of leghemoglobin, drastic drop in nitrogenase activity and inefficient allocation of electrons to N_2 reduction.

9. Phytohormone

Phytohormone (indole-3-acetic acid (IAA), cytokinins, gibberellins and abscisic acid) has a positive impact on symbiotic N_2 fixation. Indole-3-acetic acid (IAA) is the most advanced phytohormone that can enhance root growth, nodule formation and plant development being involved in cell division, differentiation and vascular beam. Cytokinin can also cause plant cell division, root development and the formation of root hairs (Frankenberger and Arshad, 1995).

Though, several of the isolated rhizosphere bacteria can produce IAA and cytokinins *via* indole-3-pyruvic acid and indole-3-aldehyde acetic pathway, the environmental (acidic pH, osmotic stress and carbon limitation) and genetic stressors (auxin biosynthetic genes and expression mode) can influence the biosynthesis of AIA (Spaepen and Vanderleyden, 2011).

10. CO_2

Symbiotic N_2 fixation under drought is associated with CO_2 concentration where this photosynthetic element can increase nitrogenase activity as well as the respiration (Huang *et al*. 1975). Murphy, (1986) experimented that the effect by higher enrichment of atmospheric CO_2 (1000 µl $l^{-1)}$ on N_2 fixation of white clover (blanca, S100), red dover, pea and lucerne (grown at 25°C temperature, 25,000 lux intensity for 42 days over 14hours photoperiod) can increase N_2 fixation rate (C_2H_2) more highly compared to the rate in low CO_2 enrichment (300 µl l^{-1}) of same medium (Fig 2). Besides, some legumes (Soybean) can also get more control on higher N_2 fixation rates by declining ARA (acetylene reduction activity) inhibition rate more and increasing TNC concentration more under elevated [CO_2] compared to that under both ambient and enriched [CO_2] treatments due to no accumulation of ureides in leaf and nodule under elevated [CO_2] in response to Alac (allantoic acid) application in plant tissues [CO_2] treatment (Serraj, 2003). Moreover, Legume plants under elevated [CO_2] can change from being drought-sensitive to being very drought-tolerant during water deficiency, indicating that sufficient carbon can help legumes overcome low water stress.

11. Soil nitrate (NO_3^-)

Soil nitrate (derived from indigenous sources like soil mineralization, irrigation and atmospheric deposition) can negatively affect the nodulation and inoculation response for *A.auriculiformis, A. mangium* and *A. mearnsii* (Lucinski *et al.* 2002; Turk *et al.* 1993). Mohammadi *et al.* (2012) reported that the extent of soil-N impacts on N_2 fixation is surely determined by plant growth stage, dose of NO_3^-, drought, types of bacteria and legume species. For instance, application of fertilizer-N (25 mg of N per kg of soil) during

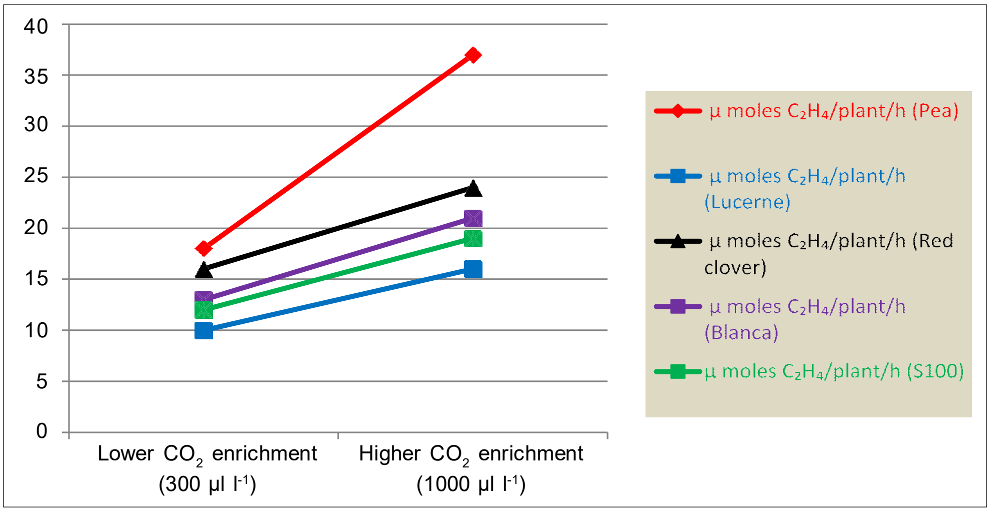

Fig 2: Impact of different CO_2 enrichment N_2 (C_2H_2) fixation in grain and herbage legumes grown for 42 days at 25°C and 25000 lux (14 hours photoperiod) (Murphy, 1986).

sowing can be less detrimental to N_2 fixation by *P. vulgaris* than during vegetative growth (Muller and Pereira, 1995). Apart from that Salvagiotti *et al.* (2008) experimented that when no N fertilizer is applied, maximum amount of N_2 fixation can be 337 kg ha^{-1} and when 100 and 300 kg ha^{-1} of fertilizer-N is applied, the rate of N_2 fixation can be expected 129 and 17 kg N ha^{-1} respectively.

However, N_2 fixation in legumes under stress conditions might be regulated by feedback involving N metabolism. When NO_3^- ion levels are sufficiently high (0 to 20 cm) and nodulation is suppressed on the primary root, the nodulation and significant nitrogenase activity (C_2H_2 reduction) can occur on adventitious roots or lateral roots of *Glycine max*, *V. faba* and *P. sativum*. In addition, several species of rhizobia can overcome the impact of nitrates by activation of hydrogenase expression (Luciñski *et al.* 2002).

B. Biotic factor

Pathogens

Pests and diseases can potentially be responsible for substantial loss of nitrogen fixation and crop yield. Some nematodes *(Pratylenchus penetrans*) can interfere with the soybean-rhizobia symbiosis and decrease nodule number (Elhady *et al.* 2020) and an infection by soybean mosaic virus can adversely affect nodulation in soybean (Andreola *et al.* 2019).

However, bacteria can form nodule structures in the legume root with the coexistence of a certain arbuscular mycorrhizal (AM) known as tripartite symbiotic system. According to Antunes (2006a), this fungi can induce root infection and form nodules by which it can increase nodule dry matter, P_{atm} and uptake of N, Zn, Cu. Xiao *et al.* (2010) reported that AM fungi and *Rhizobium* sp. can colonize the root together but the two endophytes can compete for host photosynthesis during carbohydrate deficiency where AM fungi can usually present a competitive advantage for carbohydrates over *Rhizobium* sp. The pot study with common bean, %AM colonization in the -AM treatment measured 70 DAE in both -Rh and +Rh treatments showed that inoculation with AM significantly can increase %AM colonization in both studies, but the increase was greater in the +Rh treatment. This indicates that this dual inoculation has a synergistic effect regarding colonization by both AM and Rh.

CONCLUSION

Nitrogen is an important element for synthesizing proteins, nucleic acids and other cellular parts of the living organism. This mineral can be naturally enriched in soil by the biological nitrogen fixation process where the rate of this process intensely depends upon the optimum range of the environmental abiotic and biotic factors. But, today global warming and environmental pollutions are changing the optimum range of these factors in world environment. So, several eco-friendly strategies or management should be followed for fixing nitrogen minerals naturally in croplands. Here, the applications of several managements such as field-fumigation, no-till management, intercropping, multiple cropping, crop rotation, use of crop residue (soybean meal, rice stubbles, wheat straw), compost, animal manure and plant manure have been recommended to induce root infection and nodulation by developing soil structure, soil nutrients and diversity of bacterial strains for N_2 fixation.

Though the naturally stress-tolerant species of rhizobia and legumes can adapt to adverse conditions and the selection of natural strains for inoculation in host roots is being followed in most crop fields, genetic engineering should broadly be used as a promising tool to decode the heritable traits of superior rhizobia and legume strains for promoting commercial inoculation in today's agriculture. Moreover, deep study is needed to know about the mechanisms of H_2O evolution, Phytohormone, tripartite symbiotic process and molecular DnaKJ system more on symbiotic biological N_2 fixation. Finally, a well-planned socioeconomic policy and sufficient training for personnel through awareness programs should be conducted that would make the farmers more cautious about the detrimental effects of synthesized fertilizer on our environment and also make them more interested in applying biological management in crop fields.

Data availability

The authors confirm that the data supporting the findings of this study are documented within this article.

Author contribution

In this paper, the authors have collectively evaluated the findings of previous original research papers on nitrogen fixation.

Funding

The authors declare that they got no funds from anywhere.

Conflict of interest

The authors declare no conflict of interest.

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