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NURTURING PULSES: THE VITAL ROLE OF BIO-FERTILIZERS IN ENHANCING PULSE PRODUCTION- A COMPREHENSIVE REVIEW

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ABSTRACT

For many people around the world, pulses constitute a vital source of protein and minerals. However, problems with their production include inadequate soil nitrogen levels, which result in decreased yields. By increasing soil fertility and nutrient availability, bio-fertilizers have become a viable way to increase pulse production. This review paper looks at the types, modes of action, advantages, and disadvantages of bio-fertilizers as they relate to growing pulses. Due to their cost-effectiveness, ease of application, non-toxicity, and environmental friendliness, bio-fertilizers have become a highly effective substitute for chemical fertilizers. Additionally, they serve as a supplement to agrochemicals and enable plants to utilize nutrients that are naturally abundant in soil or the atmosphere. Future prospects and suggestions for optimizing the advantages of bio-fertilizers in pulse cultivation are also covered. Bio-fertilizers present a viable substitute for traditional fertilizers, as they are sourced from naturally occurring sources such fungi, bacteria, and organic debris. Their application minimizes negative environmental effects while promoting plant growth, improving nutrient uptake, and enriching soil fertility.

Keywords: Pulses, Bio-fertilizers, Soil fertility, Nutrient availability, Sustainable agriculture, soil fertility, *Rhizobia and Azotobacter.*

Introduction

The term "bio-fertilizer" refers to materials that contain live microorganisms, such as fungi, bacteria, or algae, either as symbionts that associate with plants or in their free-living state. By stimulating a variety of microbial processes, including nitrogen fixation, phosphate solubilization, and the synthesis of chemicals that promote plant growth, biofertilizers, whether applied to seeds, soil, or plant surfaces, improves soil fertility and plant nutrient uptake. Bio-fertilizers aim to reduce the use of chemical fertilisers and promote soil health in order to support ecologically friendly and sustainable agriculture practices. Unlike most land plants, legumes may work with nitrogen-fixing bacteria to generate symbiotic root nodules that provide the necessary nitrogen for growth (Santos *et al*., 2012). Legumes are constantly exposed to potential harmful bacteria in addition to this advantageous connection; as a result, the capacity to distinguish between pathogens and symbionts is a critical factor in determining a plant's ability to survive in the wild. Here, we provide an overview of recent developments in our knowledge of transcriptional regulation, plant immunity modulation during legume-*Rhizobium* symbiosis, and signaling in root nodule symbiosis. Furthermore, we offer a number of pertinent concerns that need to be answered and shed light on the possibility of designing legume and non-legume plants' ability to fix nitrogen (Raja, 2013). As one of the three main macronutrients needed for plant growth, nitrogen is among the elements that plants depend on the most. Despite being the most plentiful element in the environment (around 79%), most plants cannot directly use the nitrogen gas found in the atmosphere. But plants in the nitrogen-fixation lineage team up with nitrogen-fixing bacteria, particularly those of the genus *rhizobium*. This process, known as symbiotic biological nitrogen fixation (BNF), transforms atmospheric dinitrogen (N_2) into ammonia (NH_3), a form that host plants can use more easily. Pulses are vital parts of a healthy diet and are vital to the world's food security. They support plants in producing siderophores, reducing abiotic stress, solubilizing and widely accessing phosphorus, fixing nitrogen, and utilising biocontrol. *Rhizobium, Azotobacter, Azospirillum, Arbuscular Mycorrhiza* (AM), Plant Growth Promoting Rhizobacteria (PGPR), Blue Green Algae (BGA), Azolla, and Phosphate-Solubilizing Micro-organisms (PSMs) are a few of the significant bio-fertilizers. In addition to this, another intriguing bio-fertilizer is *Piriformospora indica*, an endophytic fungus that may be grown that colonises plant roots and aids in the promotion of plant growth and biomass production (Varma *et al.,* 1999).

The Significance of Pulses in Crop Production

Pulses include a high percentage of protein (20-25%) along with essential vitamins, minerals, and micronutrients. India is the world's largest producer and consumer of pulses, ranking first in the world rankings. In India, significant growth in area, production and productivity of pulses has been recorded. More visible and significant increasing trends during 2020-21 and 2021-22, whereby the pulses production reached at 23.13 Mt, 25.46 Mt. and 27.30 Mt respectively, is a grand success story in itself. There was no discernible gain in yield potential despite the expansion of the pulse cultivation area (FAO statistics, 2021-22) and inadequate supply of nutrients is suggested to be a major limitation in realizing the genetic yield potential of pulse crops. The use of chemical fertilisers has been the subject of substantial and ongoing efforts to improve pulse yield. But now that it is known that chemical fertilisers have negative impacts on the quality of the soil, water, and air, bio-fertilizers are being considered as viable substitutes. Extensive attempts have been undertaken to improve pulse production by the use of bio-fertilizers in addition to chemical fertilisers. Even though bio-fertilizers were first produced commercially in India in the late 1970s, ongoing efforts are needed to maintain consistent yield responses in the technology of this efficient fertiliser. Therefore, before using bio-fertilizer technology on a broad scale, every facet related to the development of persistent pulses should be thoroughly examined. This includes difficulties as well as the development and application of technology. Legumes develop more quickly when *rhizobia*, bacteria that fix nitrogen, are present. Furthermore, the nitrogen balance in rice agriculture is influenced by both Azolla and blue-green algae (BGA). VAM fungus aid in the uptake of phosphorus in many plants, *Azospirillum* has many benefits, including drought tolerance, disease resistance, and the ability to produce chemicals that promote growth.

Bio-Fertilizer

A bio-fertilizer is essentially any material that has living microorganisms in it. When a seed or plant surface is placed in the soil, the bacteria colonise the rhizosphere and stimulate the plant's ability to absorb nutrients, which in turn leads to growth (Vessey, 2003). A bio-fertilizer is an updated organic fertiliser that has been enhanced by the addition of advantageous microorganisms (Swathi, 2010). According to Hari and Perumal (2010), the term "bio-fertilizer" most frequently refers to specific strains of advantageous soil microorganisms that are produced in a lab and placed in appropriate carriers. The word "bio-fertilizer" can refer, broadly speaking, to any organic resource for plant growth those microbes, plant associations, or plant interactions convert into a form that is readily absorbed by plants (Khosro and Youseff, 2012).

The bio-fertilizers' mechanism of action

It has been proposed that the PGPR species Azospirillum secretes auxins, eltylene, and gibberellins. Certain bacteria associated with plants can also trigger the production of phytohormones. For instance, lodge pole pine roots injected with *Paenibacillus polymyxa* showed increased levels of indole-3-acetic acid (IAA). It was discovered that *Rhizobium* and *Bacillus* could synthesise IAA at varying temperatures, pH values, and carrier materials such as agro-waste. In contrast to other phytohormones, ethylene is the one that stops dicot plants from growing (Ansari *et al.,* 2013). Wherein, in the absence of oxygen, microbes decompose biodegradable materials. Cellulolytic bacteria break down complex organic stuff in the first stage to produce simple molecules like long chain fatty acids and other compounds. The first stage's products are fermented in the second stage, producing simpler intermediates such carbon dioxide, pyruvic acids, and acetic acids, among others. Methanogens react with the products in the third stage, producing a combination of gases called biogas. This can be represented by the following reactions (Ezigbo, 2005).

CH_3COOH	-	$CH_4 + CO_2$
Acetic acid	-	Methane+Carbon dioxide
$2CH_3CH_2OH + CO_2$	-	$CH_4 + 2CH_3COOH$
Ethanol + Carbon dioxide	-	Methane + Acetic acid
$CO_2 + 4H_2$	-	$CH_4 + 2H_2O$
Carbon dioxide + Hydrogen	-	Methane + Water

The distinctions between organic fertilizer and bio-fertilizer

Organic fertiliser was formerly included in the definition of "bio-fertilizer." Technically speaking, there is a significant distinction between them. To differentiate bio-fertilizers from organic fertilisers, Vishal and Abhishek (2012) stated that bio-fertilizers are microbial inoculants made up of live cells of microorganisms such as bacteria, algae, and fungi that can be used alone or in combination to increase crop output. In the rhizosphere of plants, microbial interactions significantly increase biological activity. Conversely, organic fertilisers come from plant sources like green manure or animal sources like animal dung.

Different Types of Bio-fertilizers

Microbial inoculants including nitrogen-fixing, phosphate- solubilizing, and potassium-mobilizing bacteria are examples of helpful microorganisms found in bio-fertilizers. These microbes use a variety of processes, such as nitrogen fixation, phosphate solubilization, and organic matter breakdown, to enhance soil health and nutrient availability, Khosro and Yousef, (2012)

Table 1: Biofertilizers are divided into groups based on their nature and function Singh *et al*. (2014a, b)

Groups	Example
N_2-fixing bio-fertlizers	
Free-living	*Azotobacter, Beijerinckia, Clostridium, Klebsiella, Anabaena, Nostoc*
Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
Associative symbiotic	*Azospirillum*
P solubilizing bio-fertilizers	
Bacteria	*Bacillus megaterium var. phosphaticum, Bacillus subtilis, Bacillus circulans, Pseudomonas striata*
Fungi	*Penicillium spp., Aspergillus awamori*
P mobilizing bio-fertilizers	
Arbuscular mycorrhiza	*Glomus spp., Gigaspora spp., Acaulospora spp., Scutellospora spp., Sclerocystis spp.*
Ectomycorrhiza	*Laccaria spp., Pisolithus spp., Boletus spp., Amanita spp.*
Ericoid mycorrhiza	*Pezizella*
Orchid mycorrhiza	*Rhizoctonia solani*
Bio-fertilizers for micronutrients	
Silicates and Zn Solubilizers	*Bacillus spp.*
Plant growth-promoting rhizobacteria (PGPR)	
Pseudomonas	*Pseudomonas fluorescens*

Nitrogen-Fixing Bacteria in Legume

Rhizobium Symbiosis:

Both leguminous and non-leguminous plant root zones include *rhizobia*, or soil bacteria. But only with leguminous plants do they form a symbiotic partnership, infecting their roots and producing nodules. Examples of non-leguminous plants that *Rhizobia* can nodulate are *Trema* and *Parasponia* spp. The nodulated legumes, which vary in amount depending on the crop, significantly contribute to the 50-200 kg N ha^{-1} of fixed nitrogen in the biosphere. When it comes to replacing lost soil nutrients in areas where farmers cannot afford expensive inputs-especially in hazardous environments- bio-fertilizers, especially *Rhizobium*, may provide a useful intermediary. The annual average amount of N fixed on land is 135 million metric tonnes. About 30 million hectares of land are used for the cultivation of pulses in India (Rosen and Horgan, 2009). Pulsed legumes include chickpea, red-gram, pea, lentil, and black-gram, among others. Certain legumes allow it to colonise their roots, forming tumor-like growths known as root nodules that serve as factories for the synthesis of ammonia. In a symbiotic relationship with legumes and some non-legume plants like *Parasponia*, *Rhizobium* can fix atmospheric N (Saikia and Jain, 2007). In combination with or in symbiosis with plants, biological N fixation (BNF) takes place. For example, *Rhizobium trifoli* is used for berseem, *Rhizobium melilotti* for leucerne, *Rhizobium phaseoli* for green and black gram, *Rhizobium japonicum* for soyabean, *Rhizobium leguminoserum* for peas and lentils, and *Rhizobium lupini* for chickpeas. *Rhizobium* is a symbiotic bio-fertilizer that can be used for legume crops and trees (e.g., leucerne). The capacity of these inoculants to fix atmospheric N-in a symbiotic relationship with plants by developing nodules in their roots (stem nodules in *Sesabania rostrata*) is well recognized. However, *Rhizobium's* selectivity limits how many legumes can benefit from this symbiosis. An estimated 44 million metric tonnes of nitrogen are fixed annually by leguminous and other crops. Depending on the type of crop, the inoculants used, the soil conditions, and the field settings, crop responses to *Rhizobium* inoculation have been highly diverse. The biggest combustion of combined N occurs in terrestrial settings through the symbiotic fixation of N by rhizobia (Philippot and Germon 2005).

Table 2 : Nitrogen (N) contribution of N-fixing legumes of Indian soils

Crops	N-fixed (Kg/ha/year)
Alfalfa(*Medicago sativa*)	100-300
Clover (*Trifolium spp.*)	100-150
Chickpea (*Cicer arietnum*)	20-63
Cow pea (*Vigna sinensis*)	50-85
Green gram (*Vigna radiate*)	50-55
Groundnut (*Arachis hypogaea*)	112-152
Pea (*Pisum sativum*)	46
Soyabean (*Glycine max*)	49-130

Source: Subaa Rao (1988)

Beijerinck first isolated and grew a microbe he named Bacillus radicola from the roots of legumes in 1888. It was later given the new name *Rhizobium*. Legume plants fix and use nitrogen through a symbiotic interaction with *Rhizobium* in the form of nodules on their roots. The host plants provide a home for the bacteria in this mutualistic relationship and provide energy for the fixation of atmospheric N_2. The plant obtains nitrogen in exchange, but in a stable form like protein. The term "cross inoculation group" (CGI) describes a group of leguminous plants that, upon inoculation with rhizobia derived from nodules of any member of that legume group, will grow nodules Chang and Yang (2009).

Table 3 : Rhizobia and the legumes they nodulate in cross-inoculation groupings (Adopted from Alexander, 1978).

Rhizobia	Legume cross inoculation groups	Crops
Pea rhizobia (*R. leguminosarum*)	Pea group	Peas (*Pisum spp.*); vetches (*Vicia spp.*); lentils (*Lens culinaris*); faba bean (*Vicia faba*)
Bean rhizobia (*R. phaseoli*)	Bean group	Beans (*Phaseolus vulgaris*); scarlet runner bean (*P. coccineus*)
Clover rhizobia (*R. trifolii*)	Clover group	Clovers (*Trifolium spp.*)
Alfalfa rhizobia (*R. meliloti*)	Alfalfa group	Alfalfa (*Medicago spp.*); sweet clovers (*Melilotus spp.*); fenugreek (*Trigonella spp.*)
Chickpea rhizobia (*Rhizobium sp.*)	Chickpea group	Chickpea (*Cicer arietinum*)
Soybean rhizobia (*Bradyrhizobium japonicum*)	Soybean group	Soybeans (*Glycine max*)
Leucaena rhizobia (*Rhizobium sp.*)	Leucaena group	Leucaenas (*Leucaena leucocephala*; *L. shannoni; L. lanceolata; L. pulverulenta); Sesbania grandiflora; Calliandracalothyrsus; Gliricidiasepium; Acacia farnesiana*)

Phosphorus-Solubilizing Microorganisms (PSM):

The majority of Indian soils have a low to medium P status, and a significant portion of applied P is fixed into inorganic phosphates that are only sporadically soluble, which increases the effectiveness of phosphate fertilisers. PSM can occasionally generate plant growth hormones (IAA, GA, etc.). Numerous studies have looked at the solubility of several insoluble inorganic phosphate compounds, including rock phosphate, hydroxyapatite, tri-calcium phosphate, and dicalcium phosphate. *Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium*, and *Erwinia* are some of the bacterial genera that possess this ability. Because of its low levels of mobility and solubility as well as its propensity to become fixed in soil, P, which is naturally occurring in soil, is largely unavailable to crops when added to inorganic fertilisers. The PSB are living organisms that can aid plants in enhancing their uptake of phosphate in a variety of ways. The more prevalent soil bacteria are those from the genera *Bacillus, Pseudomonas*, and Fungi. The primary microbiological mechanism via which insoluble P compounds are released is the generation of organic acids, which is followed by the medium becoming more acidic. Microorganisms found in many bio-fertilizers have the ability to solubilize phosphorus in the soil, increasing its availability to pulse crops. The development and flowering of pulses depend on phosphorus, which bio-fertilizers help to use effectively Bhattacharyya and Jha, 2012).

Plant growth promoting bio-fertilizer (PGPB): *Pseudomonas* sp. and other similar bacteria are examples of *plant* growth rhizobacteria; they function by generating hormones and anti-metabolites that encourage root growth and the breakdown of organic matter, which aids in soil mineralization and increases nutrient availability and crop yield (Khosro and Yousef, 2012; Bhattacharyya and Jha, 2012).

Potassium solubilizing bio-fertilizer (KSB): *Aspergillus niger* and *Bacillus sp.* are two examples. The majority of the potassium found in soil is found in silicate minerals that are inaccessible to plants. Only when these minerals are gradually worn or dissolved are they made accessible. By generating organic acids that lead to the breakdown of silicates and aid in the removal of metal ions, potassium solubilizing microorganisms help to solubilize silicates and make them available to plants. Broad range bio-fertilizers are those that solubilize potassium.

Potassium mobilizing bio-fertilizer (KMB): *Bacillus sp.* is an example of a bio-fertilizer that mobilises potassium. They function by dispersing the insoluble forms of potassium found in soil, known as silicates. It has been discovered that certain phosphate-soluble bio-fertilizers, like *Bacillus* and *Aspergillus species,* may both mobilise K and solubilize P.

Sulphur oxidizing bio-fertilizer (SOB): *Thiobacillus* species are an example of a microbe that oxidises S. These function by oxidising S to sulphates, which plants can absorb.

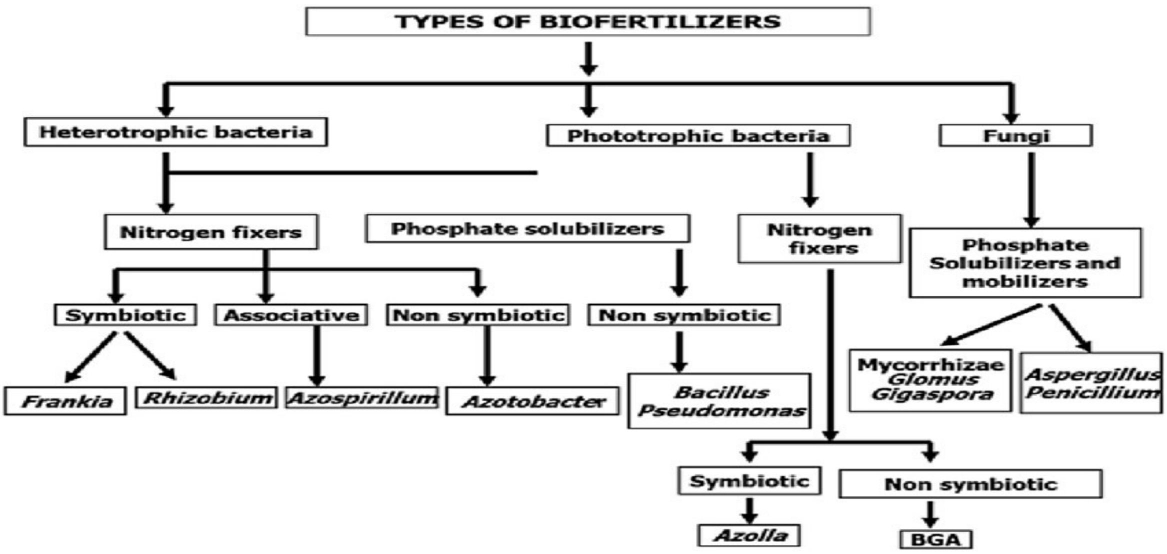

Fig. 1: Types of bio-fertilizer

The drawbacks of bio-fertilizer

When compared to inorganic fertilisers, the primary drawback of bio-fertilizer is its lower nutrient content. This could cause plants cultivated with the bio-fertilizer to exhibit deficiencies. However, by enriching the fertiliser with materials like phosphate rock, bone meal, or wood ash-all of which are high in K and phosphorus-this issue can be effectively addressed. Additionally, the problem can be solved by using nutrient-rich wastes, such as wood ash, which is rich in K, and palm wastes, which are rich in potassium, to make bio-fertilizer. According to Mahimairaja *et al.* (2008), adding phosphorus to wastes improves the balance of the bio-fertilizer and lowers nitrogen losses. Once more, the method bio-fertilizer is stored greatly influences how effective it is. Even though using bio-fertilizer has numerous benefits, there are situations where it does not provide the desired outcomes. This could be due to pre-application exposure to extreme heat or other unfavourable conditions. The best approach to store bio-fertilizer is either at room temperature or in a cold storage area away from heat sources and direct sunlight. Low density polythene bags, which are used to package bio-fertilizer, should have a thickness of roughly 50-75 microns (Mishra and Dadluck, 2010). Additional barriers to the application of bio-fertilizer technology include those related to the environment, human resources, ignorance, lack of availability of appropriate strains, lack of availability of appropriate carriers, and so on (Ritika and Uptal, 2014).

Lack of a specific strain resulting in the unavailability of a compatible strain: One of the main obstacles to the development of bio-fertilizer is this. Based on the fact that some strains can endure in the carrier of the inoculants as well as the broth.

Lack of an appropriate carrier: It is challenging to keep the bio-fertilizer's shelf life intact if an appropriate carrier material is not available. The order is peat, lignite, charcoal, farmyard manure, soil, and rice bran, depending on appropriateness.

Farmers' ignorance of the following: The benefits of bio-fertilizers in boosting crop yields are not well known to farmers. They are ignorant of the harm that constant usage of inorganic fertiliser has to the ecosystem.

Insufficient human capital and unskilled personnel: This presents an additional issue. This is a result of inadequate and unskilled staff farmers not receiving appropriate application instruction.

Environmental constraints: The utilisation of bio-fertilizers is impacted by soil properties such as salt, acidity, dryness, and water logging (Ritika and Uptal, 2014).

Benefits of using bio-fertilizer as opposed to chemical fertilizers

Enhanced Nutrient Absorption: Bio-fertilizers help pulse crops absorb more nutrients by fostering root development in a favourable environment. Better absorption of vital nutrients results from this, which benefits pulse plants by making them healthier and more fruitful.

Improved Soil Structure and Microbial Activity: Applying bio-fertilizers improves the microbial diversity and soil structure. This in turn creates an environment that is favourable for the strong growth of pulse crops by encouraging the cycling of nutrients, retaining more water, and decreasing soil-borne illnesses.

Reduced Dependency on Chemical Fertilizers: By using bio-fertilizers, the need of synthetic fertilisers is decreased, which is consistent with sustainable agricultural methods. This helps to create stronger and more resilient agro ecosystems while also reducing the negative effects of chemical fertilisers on the environment.

Another importance's:

- Bio-fertilizers are known to make a number of positive contributions in agriculture.
- Supplement fertilizer supplies for meeting the nutrient needs of crops.
- Add 20-200 kg N/ha (by fixation) under optimum conditions and solubilise/mobilise 30-50 kg P_2O_5/ha.
- They liberate growth promoting substances and vitamins and help to maintain soil fertility.
- They suppress the incidence of pathogens and control diseases.
- Increase the crop yield by 10-50%. N_2 fixers reduce depletion of soil nutrients and provide sustainability to the farming system.
- Cheaper, pollution free and based on renewable energy sources.

Environmental Limitations for Application of Bio-fertilizer

1. Unavailability of suitable carrier Resource constraint
2. Market level constraints and lack of awareness of farmers
3. Lack of quality assurance and limited resource generation for bio-fertilizers production
4. Seasonal and un assured requirement
5. Soil and climatic factors and inadequate experienced staff
6. Native microbial population, faulty inoculation techniques and mutation during fermentation

Caution in the use of bio-fertilizers

The following precautions must be followed when using bio-fertilizer, according to Hari and Perumal (2010): never apply fungicides directly on bio-fertilizers, never mix bio-fertilizers with nitrogen fertilisers, and store bio-fertilizers at room temperature-never below 0°C or above 35°C. Additionally, don't store used solutions overnight.

Conclusion

The growth of industries manufacturing dangerous chemicals that can upset the ecological balance and pose a threat to human health has been facilitated by our reliance on chemical pesticides and fertilisers. Indeed, because of the detrimental impact that eating food cultivated with chemical fertilisers can have on one's body, the focus is currently changing from eating food grown with organic fertilisers. The issue of feeding the world's growing population can be resolved with the use of bio-fertilizers. In particular, the use of pellets for direct soil application and methylcellulose for seed coating are two novel application approaches that should be supported. Increased mineral N-starter doses decrease nodulation, which lowers *rhizobium* response, although phosphate deficiency can also act as an inhibitor. In order to improve pulse production, guarantee food security, and encourage environmental stewardship, bio-fertilizers emerge as a viable and environmentally responsible option. Bio-fertilizers enhance the resilience of pulse crops by fixing nitrogen, soluble phosphorus, and promoting better soil health. This results in a more productive and sustainable agricultural environment. Incorporating bio-fertilizers into pulse growth methods is essential for attaining long-term food security and agricultural sustainability, as the demand for pulses worldwide keeps growing.

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