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Recent Advances in Application of Biofertilizers in Pulses: A Review

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ABSTRACT

Globally, India is the largest producer and consumer of pulses. Biofertilizers, a low cost input, help in increasing the crop productivity by way of biological nitrogen fixation, increased availability or uptake of nutrients through solubilization or increased stimulation of plant growth through the synthesis of growth promoting substances such as indole acetic acid (IAA), gibberellins, auxins, ammonia, hydrogen cyanide and siderophore production. Furthermore, biofertilizer inoculation helps to reduce the use of chemical fertilizers. These are environmentally friendly and play a significant role in boosting the pulse crop production. In this review paper, literature regarding different types of biofertilizers and their effect on different pulse crops has been studied. The effect of application of biofertilizer inoculation on biological nitrogen fixation, growth, symbiotic traits, productivity, quality, soil fertility, residual effect and economic benefit has been reviewed and summarized under different headings. Application of biofertilizers increases the plant growth, symbiotic traits (nodule number, nodule weight) and grain yield over no inoculation in different pulse crops without producing any adverse effect on the environment. Moreover, seed inoculation with biofertilizers provides economic benefits over without inoculation. Furthermore, single or dual inoculations of biofertilzers improve the nutrient status of soil and ultimately increase the nutrient uptake by crop along with improvement in protein content. Seed inoculation with biofertilizers also increases organic carbon, available nitrogen, available phosphorus and available potassium and microbial count in the soil after the crop harvest over un-inoculated control. The nitrogen amounts fixed as a result of inoculation leave a net positive N balance in the soil. Thereby, biofertilizers inoculation helps in achieving higher profits while maintaining the agricultural sustainability.

Key words: Biofertilizers, Nodulation, Productivity, Pulses, Symbiosis.

India is the world's largest producer and consumer of pulses. In India during 2019-20, pulses were cultivated on an area of 27.9 million hectares with a production of 23.0 million tones and productivity of 823 kg/ha (INDIASTAT, 2021). India plays a key role in the area (42.6%) and production (28.34%) of pulses globally. Among major pulses grown and produced globally, Indian share is maximum for pigeonpea in the area (73%) and production (67%) followed by chickpea (68% area and 66% production), dry beans (37% area and 18% production), lentil (29% area and 18% production) and dry peas (12% area and 6% production) in total pulses production (FAOSTAT, 2021). The major pulse crops grown and produced in India are chickpea (35% area and 44% production), pigeonpea (15% area and 17% production), urdbean (18% area and 14% production), mungbean (14% area and 8% production), lentil (5% area and 6% production) and fieldpea (1% area and 4% production) in different agro-ecological regions. The major pulse crops grown and produced globally are dry beans (57% area and 48% production), chickpea (17% area and 17% production), pigeonpea (10% area and 10% production), dry peas (9% area and 17% production) and lentil (7% area and 8% production) (Anonymous, 2018). The major pulse producing states in the country are Rajasthan, Madhya Pradesh, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh, which together contribute to about 75% of the total pulses production in the country.

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Nowadays, agrochemicals are extensively applied to obtain higher yields. The intensive application of agrochemicals leads to several agricultural problems. Farmers use more chemical fertilizers than the recommended levels for many crops. Excessive use of chemical nitrogen fertilizer leads to soil acidification, contaminating groundwater and the atmosphere. Successful crop husbandry to a great extent depends upon how successfully one could manage these factors to favour crop production (Kaur *et al.,* 2015).

In the present scenario of the degradation of natural resources, the beneficial effect of pulse crops in improving soil health and sustaining productivity is very important. Pulses have the ability to fix atmospheric nitrogen thereby improving soil fertility and sustaining crop productivity. Moreover, the use of biofertilizers in pulses may help in increasing the productivity of pulses and improving soil

health. Biofertilizers are a cost-effective, eco-friendly and renewable source of plant nutrients to supplement chemical fertilizers in the sustainable agricultural system. Biofertilizers are also ideal input for reducing the cost of cultivation which is a helpful parameter in increasing the farm income.

Biofertilizer refers to a preparation containing live microbes which help in enhancing the soil fertility either by fixing atmospheric nitrogen, solubilization of phosphorus or decomposing organic wastes or by augmenting plant growth by producing growth hormones with their biological activities. Biofertilizers play a very significant role in fixing atmospheric nitrogen, both, in association with plant roots and without roots (Liu *et al.,* 2011), solubilizing insoluble soil phosphates (Alori *et al.,* 2017) and producing plant growth substances (Masciarelli *et al.,* 2014; Oteino *et al.*, 2015) in the soil. Annually, pulses fix about 2.95 Tg nitrogen through biological nitrogen fixation (Herridge *et al.,* 2008). Biofertilizers play many important roles in pulses (Singh and Singh, 2018a; Singh and Singh, 2018b). In order to reduce the use of chemical fertilizers, biofertilizers can play a crucial role by increasing the availability of soil nutrients and also sustain crop production.

Types of biofertilizers

There are different types of biofertilizers (Table 1). Some of them fix nitrogen, some solubilize fixed phosphorus by producing organic acids and enzymes and make them available to the crops. Apart from fixing nitrogen, they are also known to release growth promoting substances such as IAA, gibberellins, pantothenic acid, thiamine and niacin which promote root proliferation, plant growth and yield.

Role of biofertilizers in pulse crops

Biological nitrogen fixation

Biological nitrogen fixation (BNF) can act as a renewable and environmentally sustainable source of nitrogen in pulses. Nitrogen is provided through symbiotic fixation of atmospheric N_2 by nitrogenase in rhizobial bacteroids (Hayat *et al*., 2010). The availability and uptake of nitrogen is very important for plant growth and development. Its use can mitigate the need for fertilizer nitrogen. Nitrogen obtained through the process of BNF is used directly by the plant and so is less susceptible to volatilization, denitrification and leaching losses. BNF is a kind of beneficial plant-microbe interaction that provides plants with nitrogen. Significant amount of nitrogen is fixed through the legume-*Rhizobium* symbiosis.

Inoculation with *Rhizobium* increased nitrogen fixation in mungbean crop (Hussain *et al.,* 2012). Similarly, co-inoculation of *Mesorhizobium* sp. and PGPR enhanced the nodulation and N_2-fixation in cowpea (Verma *et al.,* 2013). *Rhizobium* inoculation of dry bean (*Phaseolus vulgaris*) had a significant influence on the amount of fixed nitrogen. Inoculation significantly increased the N fixed in different tissues of the plant such as roots, shoots, pods and whole plants of *Phaseolus vulgaris* grown both in the greenhouse and in the field (Bambara *et al.,* 2010). Seed inoculation with biofertilizer containing *Bradyrhizobium* strains (*B. japonicum* SAY3-7 and *B. elkanii* BLY3-8) and *Streptomyces griseoflavus* P4 promoted nodulation and thereby increased nitrogen fixation in soybean and mungbean (Htwe *et al*., 2019).

Thus, inoculation with biofertilizers improved the N nutrition and resulted in more plant growth and crop grain yield.

Phosphate solubilization and production of growth promoting substances

Application of biofertilizers produces growth promoting substances such as indole acetic acid, gibberellins, auxins *etc*. *Bacillus* spp. strains B-20 and B-24 produced higher IAA equivalents as compared to *Pseudomonas* spp. (Saini and Khanna, 2012). *Mesorhizobium* sp. strain BHURC02, *Pseudomonas fluorescens* BHUPSB06, *Acetobacter chroococcum* and *Bacillus megaterium* BHUPSB14 were found to be positive for NH_3 and IAA production and *Pseudomonas fluorescens* BHUPSB06 was found to be positive for HCN, siderophore production and inhibition of growth of soil-borne phytopathogens (*Fusarium oxysporum* and *Rhizoctonia solani*) (Verma *et al.,* 2012).

Kaur and Sharma (2013) reported that significant amount of IAA was produced by PGPR-3 (70.05 µg/ml) and PGPR-2 (66.79 µg/ml) as compared to PGPR LK 884 (61.58 µg/ml). Furthermore, PGPR-3 (13.45 mg/100 ml) and PGPR-2 (13.15 mg/100 ml) solubilized phosphorus also. Similarly high level of IAA production was recorded in *Pseudomonas* sp., *Bacillus* sp., *Rhizobium* and *Mesorhizobium* sp. by other workers (Verma *et al*., 2012; Verma *et al*., 2013). *Pseudomonas* sp., *Bacillus* sp., *Azotobacter* sp. and *Mesorhizobium ciceri* were reported as good phosphate solubilizers (Verma *et al*., 2013). Isolates of *Pseudomonas* sp. PGPR-2 and PGPR-3 also produced siderophores, HCN, NH_3 and improved seed germination in *kabuli* and *desi* chickpea (Kaur and Sharma, 2013). Similarly, *Pseudomonas aeruginosa* is also capable of siderophore and HCN production in chickpea (Verma *et al.,* 2013). In *Pisum sativum*, use of endophytic *Pseudomonas* strains (L111, L228 and L321) solubilized the insoluble phosphates by producing gluconic acid and also promoted the plant growth (Oteino *et al.,* 2015). In chickpea, seed inoculation with *Mesorhizobium ciceri* synthesized indole-3-acetic-acid (IAA), 1-amino cyclopropane 1-carboxylate (ACC) deaminase, siderophores, exopolysaccharides (EPS), hydrogen cyanide, ammonia and solubilised inorganic phosphate. Moreover, microbial seed inoculation also had the ability to tolerate fungicide by decreased the levels of stressor molecules (proline and malondialdehyde) and antioxidant defense enzymes *viz*. ascorbate peroxidise, guaiacol peroxidise, catalase and peroxidises of plants (Shahid *et al*., 2021). It can be concluded that biofertilizers stimulate the synthesis of growth promoting substances such as indole acetic acid, gibberellins, auxins, NH_3, HCN and siderophore production.

Effect on plant growth, symbiotic traits and productivity

In plants, nitrogen and phosphorus play an important role in growth, development and finally determine the yield of

the crops. Nitrogen is an important nutrient to plant growth and development as it is building blocks of protein. Phosphorus is a fundamental component of the substances that are building blocks of genes and chromosomes. Application of biofertilizers positively affects the plant growth, nodule number, nodule weight and grain yield. Biofertilizers make available nitrogen and phosphorus to the plants, as discussed above and thereby influence the plants.

In chickpea, *Rhizobium* inoculation significantly improved symbiotic traits, number of pods and grain yield over uninoculated control (Singh *et al*., 2011). Seed inoculation with *Mesorhizobium ciceri* significantly increased the length of plants (41%), total dry matter (18%), carotenoid content (9%), leghaemoglobin content (21%), root nitrogen (9%), shoot phosphorus (11%) and pod yield (15%) over control in chickpea (Shahid *et al*., 2021). Similarly, microbial inoculant *Anabaena laxa* biofilm formulation significantly enhanced leghaemoglobin content of nodules, plant growth, yield parameters, grain yield and nutrient uptake as compared to uninoculated control (Bidyarani *et al.,* 2016).

Inoculation of seed with *Mesorhizobium* and plant growth promoting rhizobacteria (PGPR) alone and their

Table 1: Different types of biofertilizers.

Groups	Examples	Functions	References
N_2 fixing biofertilizers			
Free-living	*Azotobacter, Beijerinkia, Derxia, Nostoc*	Fix atmospheric N_2, mineralize nutrients from soil, sequester Fe, produce growth Promoting substances such as IAA, gibberellins, pantothenic acid, thiamine and niacin which promote root proliferation, plant growth and yield	Tilak *et al.* (2005); Liu *et al.* (2011); Ndakidemi *et al.* (2011); Shahid *et al*. (2021)
Symbiotic	*Rhizobium, Frankia, Anabaena azollae*		
Associative symbiotic	*Azospirillum, Glucono acetobacter diazotrophics*		
P solubilizing biofertilizers			
Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus subtilis, Bacillus circulans, Pseudomonas striata*	Solubilize fixed phosphorous by producing organic acids and enzymes and make them available to the crops and enhance the plant growth by improving biological nitrogen fixation in leguminous plants.	Saini and Khanna (2012); Verma *et al*. (2013); Kaur and Sharma (2013); Das and Pradhan (2016); Alori *et al*. (2017); Kumar *et al*. (2017b); Tian *et al*. (2021)
P mobilizing biofertilizers			
Arbuscular mycorrhiza	*Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., *Sclerocystis* sp.	Facilitate mobilization of soluble phosphorus from distant places in soil and thus increase availability of phosphorus to plants	Ryan and Angus (2003); Jha *et al.* (2012b)
Fungi	*Penicillium* sp., *Aspergillus awamori*		Wang *et al.* (2014); Bahadur *et al*. (2017); Kumar *et al*. (2017a)
Sulphur oxidizers	*Thiobacillus thioxidans*	Formation of sulphuric acid in soil, additions of elemental sulphur augments nutrient mobilization by increasing the level of soluble phosphate, potassium, calcium, manganese, aluminium and magnesium	Bhattacharjee and Dey (2014)
Zinc solubilizers	*Bacillus* sp., *Pseudomonas* sp., *Aspergillus niger*	Solubilizing zinc	Bhattacharjee and Dey (2014)
Potassium and silicate solubilizers	*Erwinia, Pseudomonas* sp., *Bacillus* sp. *Fracturia auerentia*	Solubilizing potassium, silicaand aluminium from insoluble minerals and liberate organic acids	Bhattacharjee and Dey (2014)
Manganese solubilizers	*Pencillium citrinum*	Solubilizing manganese from the low grade manganese ores and from the soil if it is present	Acharya *et al.* (2002); Acharya *et al.* (2004)
Plant growth promoting rhizobacteria (PGPR)	*Pseudomonas fluorescens, Bacillus* sp. Burkholderia, *Azospirillum,Azotobacter, Enterobacter, Rhizobium, Erwinia, Mycobacterium*, *Mesorhizobium, Flavobacterium*	Helps in mineral nutrient solubilizaton and making available for plants, nitrogen fixation, repression of soil-borne pathogens, production of siderophores, hydrogen cyanide, antibiotics, improving plant stress tolerance to drought, salinity and metal toxicity and production of phytohormones like indole acetic acid (IAA), gibberellins *etc*.	Saini and Khanna (2012); Verma *et al*. (2012); Zafar *et al*. (2012); Deshwal and Kumar (2013); Kaur and Sharma (2013); Verma *et al*. (2013); Swarnalakshmi *et al*. (2020); Basu *et al*. (2021)

combination significantly enhanced shoot and root dry matter (Singh *et al*., 2017b), symbiotic traits, chlorophyll content and grain yield of chickpea than uninoculated control (Sharma *et al.,* 2013). Similarly, combined and alone inoculation of *Rhizobium*, phospho-solubilizing bacteria and arbuscular mycorrhizae (AM) significantly improved growth parameters, yield attributes and grain yield of chickpea over uninoculated control (Praminik and Bera, 2012). In another study also, PGPR alone or in combination with bioinoculants (*Mesorhizobium* and PSB) reduced plant mortality and increased grain yield of chickpea. Combined inoculation of chickpea seed with PSB+*Rhizobium* gave significantly higher number of nodules, fresh and dry weight of nodules, grain, straw and biological yields compared to uninoculated and PSB or *Rhizobium* inoculation alone (Singh *et al.,* 2014). Similarly, significant increases in nodule number, dry matter and nutrient content of chickpea were recorded in co-inoculation of *Mesorhizobium*sp. BHURC02 and *Pseudomonas fluorescens* BHUPSB06 followed by co-inoculation of *Mesorhizobium* sp., *Azotobacter chroococcum* and *Bacillus megatrium* BHUPSB14 over uninoculated control (Verma *et al.,* 2012). Co-inoculation of *Mesorhizobium* sp. and PGPR (*Pseudomonas aeruginosa*) was found significantly better for nodulation (62 and 86%), plant growth [dry weight of root (44 and 57%) and shoot (26 and 45%)] over uninoculated control in pot and field condition, respectively and grain yield (32%) and straw yield (41%) of chickpea over uninoculated control in field condition (Verma *et al.,* 2013). The recommended dose of fertilizers (RDF) with 1 g ammonium molybdate/kg seed+*Rhizobium* +PSB, RDF with 2 g ammonium molybdate+1 g $FeSO_4$/kg seed+*Rhizobium* and PSB recorded the highest number of nodules/plant, pods/plant and grain yield of chickpea (Gangwar and Dubey, 2012). The inoculation of seed with biofertilizers such as *Rhizobium* and PSB increases the yield because the soil bacteria and fungi possess the ability to bring insoluble phosphate in soil into soluble forms by secreting various organic acids namely formic, acetic, propionic, lactic *etc*. These acids lower the pH and bring about the dissolution of bound form of phosphate. Hence, supplementation of micronutrients along with *Rhizobium* and PSB inoculation in chickpea may increase biological nitrogen fixation and phosphorus availability and thereby its productivity. In the controlled environment and in the field trials, single, dual and triple inoculations of chickpea seed with *Rhizobium*, N_2-fixing *Bacillus subtilis* (OSU-142) and phosphorus solubilizing *Bacillus megaterium* (M-3) significantly increased plant height, shoot, root and nodule dry weight, pod number, grain yield and total biomass yield compared with the control, equal to or higher than N, P and NP treatments. In the field integrated use of 12.5 kg N/ha along with Rhizobium inoculation was better for nodulation than the use of *Rhizobium* alone in mungbean (Singh *et al.,* 2021a). Seed inoculation with *Mesorhizobium* also enhanced grain yield and nitrogen use efficiency of chickpea (Doaei *et al.,* 2020). Seed inoculation with *Mesorhizobium* sp. *ciceri* (LGR-33) alone or in combination with PGPRs [RB-1 (*Pseudomonas argentinensis*) and RB-2 (*Bacillus aryabhatta*)] improved the growth, symbiotic properties, yield attributes and yield of chickpea due to its P solubilizing property (Singh *et al*., 2021b).

In lentil, Saini and Khanna (2012) reported that inoculation of seed with *Rhizobium* alone and its combination with different strains of PGPR enhanced the symbiotic parameters and grain yield of lentil to the tune of 13.6% (*Rhizobium*+P-1), 14.6% (*Rhizobium*+KB-133) and 14.9% (*Rhizobium*+B-40) over the control. Treatment of integrated use of seed inoculation with PSB+PGPR along with recommended fertilizers+25 kg $ZnSO_4$/ ha^{-1}+1.0 g ammonium molybdate/ kg seed provided the highest grain yield of lentil (34.0% higher over control) (Singh *et al*., 2019). It also gave the highest and significantly more nodule occupancy at 45 and 90 days after sowing (DAS) than *Rhizobium* sp. alone inoculation and also recorded a significant increase in grain yield and straw yield over *Rhizobium* sp. and PSB alone inoculations. The symbiotic traits and productivity (Singh *et al*., 2018), gross returns, net returns and BC ratio in lentil (Singh *et al*., 2017a) increased significantly with *Rhizobium* inoculation over uninoculated control, which further increased with the dual inoculation of *Rhizobium*+PGPR.

In pigeonpea, application of 80 kg/ha P_2O_5 and 60 kg/ ha sulphur with PSB inoculation significantly improved growth and yield attributes and grain yield. Combined inoculation of *Rhizobium*, PSB and PGPR significantly increased grain yield of pigeonpea (Goud and Kale, 2010). Similar beneficial effect of PSB on grain yield of pigeonpea was also reported by Sathe *et al*. (2011). Inoculation of pigeonpea seed with *Rhizobium* alongwith 50% RDF significantly increased branches, pods/plant and grain yield (Reddy *et al.,* 2011). *Rhizobium* strain and the dual inoculation with either *Pseudomonas putida*, *Pseudomonas fluorescens* or *Bacillus cereus* resulted in a significant increase in plant growth, nodulation and enzyme activity over *Rhizobium* inoculated and uninoculated control plants of pigeonpea (Tilak *et al.,* 2006).

Inoculation of greengram seed with *Rhizobium* and PSB alone and their combination recorded significantly higher nodules/plant, yield attributing characters and grain yield than control (Kumawat *et al.,* 2010). Patel *et al.* (2013) also recorded significantly higher grain yield of greengram inoculated with PSB than uninoculated and it also gave the highest net returns. The seed yield increased with the inoculation of biofertilizers, however, combined inoculation of *Rhizobium* and PGPR produced significantly higher seed yield over single inoculations and control in summer mungbean (Kaur and Sharma, 2016). Single or dual inoculations of bacterial strains (*Pseudomonas fluorescens* BAM-4, *Burkholderia cepcia* BAM-6 and *Burkholderia cepcia* (BAM-12) enhanced the growth of greengram plants in comparison with uninoculated control (Jha *et al*., 2012a). The seed inoculation of cowpea with *Rhizobium* and PSB

alone and their combination increased grain yield significantly (Khan *et al.,* 2013). Seed inoculation with biofertilizer containing *Brady rhizobium* strains (B. *japonicum* SAY3-7 and B. *elkanii* BLY3-8) and *Streptomyces griseoflavus* improved plant growth, nodulation and seed yield of soybean and mungbean (Htwe *et al*., 2019).

It can be concluded that seed inoculation with biofertilizer treatments influence the nodulation, growth and grain yield significantly over no inoculation. Furthermore, dual or triple inoculation of *Rhizobium*, PSB and PGPR improves these parameters over single or dual inoculations only.

Nutrient uptake and nutritional quality

Biofertilizers improve the quality of produce. Co-inoculation of *Mesorhizobium* sp. and *Pseudomonas aeruginosa* showed significant uptake of nitrogen (65%) and phosphorus (58.9%) by grain of chickpea over control. The co-inoculation of *Mesorhizobium* sp. and *Pseudomonas aeruginosa* enhanced the acquisition of phosphorus and iron in chickpea (Verma *et al.,* 2013). Inoculation of chickpea seed with *Rhizobium* and PSB alongwith RDF and ammonium molybdate improved the uptake of N and P by the crop and also increased the protein content in the chickpea seed (Gangwar and Dubey, 2012). Combined inoculation of chickpea seed with PSB+*Rhizobium* recorded significantly higher nutrient uptake compared to uninoculated and PSB or *Rhizobium* inoculation alone (Singh *et al.,* 2014).

Seed inoculation with *Rhizobium*+PSB recorded significantly higher nutrient uptake (N and P_2O_5) over *Rhizobium* alone and uninoculation in pigeonpea (Goud and Kale, 2010). As *Rhizobium*+PSB inoculation increased the availability of N and P in the soil, it resulted in higher N and P uptake and improved the content (N and P nutrient) in the seed. This is mainly due to the reason that *Rhizobium* inoculation fixes nitrogen through nodules of the plant and PSB solubilizes native phosphorous, bringing more phosphorus to the soil solution. Combined inoculation of *Rhizobium*+PSB+PGPR recorded the highest and significantly more N, P, K and S uptake than the other treatments due to their additive effects (Prasad *et al*., 2002). Uptake of N, P, K and S also increased with PSB inoculation in pigeonpea (Sathe *et al.,* 2011). N, P and K content in the grains and straw were significantly enhanced where recommended nitrogen was applied in combination with seed inoculation, basal application of $ZnSO_4$ and seed treatment with 1 g ammonium molybdate than their single applications in lentil (Singh *et al*., 2019).

Dual inoculation of *Glomus fasciculatum* and *Rhizobium* enhanced the chlorophyll, nitrogen and phosphorus contents of pigeonpea (Bhattacharjee and Sharma, 2012). Inoculation of pulses with *Rhizobium* increases the nodulation, thereby causing more nitrogen fixation and making it available for the plants. Moreover, it also increases rhizospheric microflora *viz*. acid producers and phosphate solubilizers, causing more availability of phosphorus. In soybean also, dual inoculation of *Bradyrhizobium* and PGPR significantly increased the population of *Bradyrhizobium* and PGPR in soil after harvest (Virk *et al*., 2017a).

The improvement in nodulation with inoculation using phosphorus-solubilising organisms may have resulted in higher nitrogen fixation and hence higher nitrogen availability to plants, whereas solubilisation of fixed phosphorus by PSB probably resulted in increased content and uptake of phosphorus by plants. Marko *et al.* (2013) reported significantly higher nutrient uptake by blackgram under dual and individual inoculation of *Rhizobium* and PSB than control. Bahadur and Tiwari (2014) recorded significantly higher N and P uptake and quality parameters like sulphur content, protein content and methionine content in mungbean under dual inoculation with *Rhizobium*+PSB than control. Seed inoculation with biofertilizer containing *Bradyrhizobium* strains (*B. japonicum* SAY3-7 and *B. elkanii* BLY3-8) and *Streptomyces griseoflavus* P4 enhanced nitrogen, phosphorus and potassium uptake in soybean and mungbean (Htwe *et al*., 2019).

The increased leaf N uptake might have increased the amino acid synthesis and thereby could have improved the grain protein content *via* their translocation to grains. Further, the inoculation with P biofertilizer might have increased P availability and its subsequent utilization for the formation of carbon skeletons and the synthesis of amino acids and adenosine triphosphate (ATP). That, in turn, might have led to an enhanced synthesis of protein during grain development.

Therefore, single or dual or triple inoculation of biofertilzer(s) improve the nutrient uptake, nutrient content and protein content, thereby the quality of the grain.

Soil fertility

All the living diazotrophs fix atmospheric nitrogen in the soil and lower the C:N ratio, which in turn enhances mineralization of N. Several biofertilizers containing microorganisms secrete growth promoting substances like IAA, gibberellic acid (GA), cytokinin *etc*. Single or dual inoculations of bacterial strains (*Pseudomonas fluorescens* BAM-4, *Burkholderia cepcia* BAM-6 and *Burkholderia cepcia* (BAM-12) were able to solubilize the insoluble inorganic phosphorus in mungbean (Jha *et al*., 2012a).

Seed inoculation with biofertilizers significantly influences the available N and P in the soil. Seed inoculation with *Rhizobium*+PSB+PGPR and *Rhizobium* alone recorded significantly higher available N in the soil over seed inoculation with PSB alone and uninoculated control (Goud and Kale, 2010). Biofertilizers enhance soil fertility by solubilizing unavailable sources of elemental nitrogen and bound phosphate into available forms in order to facilitate the plant to absorb them (Singh and Yadav, 2008). Khan *et al.* (2013) reported that seed inoculation of cowpea with *Rhizobium* and PSB significantly increased organic carbon, available nitrogen, available phosphorus and available potassium than uninoculated control. In chickpea, microbial inoculant *Anabaena laxa* biofilm formulation increased nitrogen fixation and soil available nitrogen (Bidyarani *et al.,* 2016).

Seed inoculation of *Rhizobium* and PSB increased the microbial count in soil after the harvest of mungbean (Bahadur and Tiwari, 2014). An increase in the population of total bacteria by *Rhizobium* inoculation may be due to higher availability of available nitrogen and carbon, which act as food for these microbes. PSB inoculation increased the phosphorus availability by solubilizing the fixed P in the soil, which provides the energy to the microbes for their better proliferation. Therefore, seed inoculation with biofertilizers improve organic carbon, available nitrogen, available phosphorus and available potassium and microbial count over uninoculated control and thus improve soil health.

Residual effect

Seed inoculation with biofertilizers such as *Rhizobium* leaves a net positive N balance in the soil due to biological nitrogen fixation. Under satisfactory N-fixation conditions, the N fixed as a result of inoculation leave a net positive N balance in the soil. It is a general observation that under such conditions, symbiotic nitrogen-fixing systems satisfy a major portion of their N requirement from atmospheric nitrogen and additional fixed N, as expected, will be contributed to soil reserves for the succeeding crops. The quantity of N fixed depends on the species of pulses, location, yield level and also the effectiveness of the specific *Rhizobium* strain.

Dual biofertilizers (*Rhizobium*+PSB) resulted in maximum N-balance in the soil (230.5 kg/ha) after pigeonpea (Ahirwar *et al.,* 2016). The biological N-fixation was also found the highest in dual biofertilizers (*Rhizobium*+PSB) treatment (84.5 kg/ha) (Ahirwal *et al.,* 2016).

Economic benefit

Economic evaluation of crop responses to biofertilizers application is an important aspect to assess the profitability of their use. Such an assessment is carried out by studying the gross returns, net returns and the benefit:cost ratio. *Rhizobium*+PSB inoculation of pigeonpea seed recorded higher net returns and B:C ratio over control (Singh *et al*., 2013). The cost of *Rhizobium*+PSB and *Rhizobium* is very low in comparison to the added output. Similarly, combined inoculation of *Bradyrhizobium* and PGPR recorded the highest gross returns, net returns and benefit: cost ratio in soybean (Virk *et al*., 2017b). Reddy *et al.* (2011) reported that 50% RDF+seed inoculation with *Rhizobium* recorded the highest net returns followed by 50% RDF+dual inoculation with *Rhizobium* and PSB. Patel *et al.* (2013) recorded higher net returns of greengram inoculated with PSB than uninoculated control. The net returns and B:C ratio of combined inoculation of *Rhizobium* and PGPR was maximum in summer mungbean (Kaur and Sharma, 2016) and in chickpea (Singh *et al*., 2021b). Treatment of integrated use of seed inoculation with PSB+PGPR along with recommended fertilizers+25 kg $ZnSO_4$/ ha+1.0 g ammonium molybdate/kg seed provided the highest gross returns (34.0%) and net returns (54.8% higher over control) in lentil (Singh *et al*., 2019). Seed inoculation with biofertilizers resulted in marked yield increase and thus higher net returns over uninoculation.

CONCLUSION

This review focused on the potential role of biofertilizers in pulses with respect to growth, nitrogen fixation, nutrient uptake, soil fertility and grain yield of pulses. The results from different studies showed that biofertilizer inoculation had positive effects on all parameters measured, suggesting the important role of biofertilizers in increasing pulse production. Therefore, use of biofertilizers(s) inoculants is the best option in improving pulses yield, reducing dependence on chemical fertilizers and also for sustainable soil health and the environment.

Conflict of interest

All authors declared that there is no conflict of interest.

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