

Chapter

Characterization of *Rhizobium* and Plant Growth Promoting Rhizobacteria from French Bean Rhizosphere and Their Effect on French Bean Productivity

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Abstract

French bean (*Phaseolus vulgaris* L.) is used profusely by the common people as an alternative diet of protein. The sparse nodulation in French bean mainly may be due to lack of threshold level of specific rhizobial cells in soil at the time of sowing. The isolates streaked on YEMA with BTB changed to yellow color showing the production of acid which is the characteristic of *Rhizobium*. Utilization of different carbon sources is an efficient tool to characterize the isolates. Plant growth promoting rhizobacteria is the beneficial rhizobacteria inoculation of which increases growth and yield of French bean through different direct and indirect mechanisms. Inoculation of French beans with rhizobial and rhizobacterial isolates found to be improved growth, physiological, quality parameters and grain yield through symbiotic N₂-fixation capacity and plant growth promoting abilities. Co-inoculation of rhizobial and rhizobacterial isolates enhanced the growth and grain yield of French bean. These isolates may be used as consortium to improve the growth of French bean, which may reduce the dependency of farmer on chemical fertilizer as well as risk of pollution. In this chapter characterization of *Rhizobium* and plant growth promoting rhizobacteria and their effect on plant growth has been reviewed.

Keywords: *Rhizobium*, PGPR, biofertilizer, consortium

1. Introduction

In the present intensive agriculture practices leguminous plants play a critical role in natural ecosystem, agriculture and agroforestry because of their ability to fix nitrogen (N₂) in symbiotic relationship with *Rhizobium* and *Bradyrhizobium*. In addition to its role as a source of protein in the diet, biologically fixed N₂ is essentially free for use in economic terms by the host plant and by associated subsequent crops. This association improves the soil quality vis-à-vis sustainability. Among the legumes, French bean (*Phaseolus vulgaris* L.) is used profusely by the common people as an alternative diet of protein. It is very nutritious and contains 22.9 per cent protein, 1.2 per cent fat, 60.6 per cent carbohydrates and a large number of minerals like Ca (260 mg 100 gm⁻¹ of seed) P (101 mg 100 g⁻¹ of seed) and Fe

(5.8 mg 100 g⁻¹ of seed). French bean is sparsely nodulated throughout the India including North-West Himalayas putting it to disadvantage of biologically fixed-N₂ [1, 2] and thus responds to the enhanced levels of nitrogen [3]. In India, it is grown on an area of about 1 lakh hectare (ha) mainly in the states of Maharashtra (60,000 ha), Jammu and Kashmir (10,000 ha), Himachal Pradesh, Uttarakhand, Nilgiri (Tamil Nadu), Palni (Kerala) hills, Chickmagalur (Karnataka) and Darjeeling hills (West Bengal). The sparse nodulation in French bean mainly may be due to lack of threshold level of specific rhizobial cells in soil at the time of sowing. Recently different rhizobial strains have demonstrated various other plant growth promoting activities in addition to biological nitrogen fixation (BNF). This necessitates the isolation and development of the efficient multi-trait rhizobial isolates for French bean for economizing the nitrogen fertilizer, environmental safety and sustainable production.

Rhizobium plays a significant role in agricultural ecosystem services due to their ability to form symbiotic association with a wide range of leguminous plants that results in biological nitrogen fixation. Some of the rhizobial strains are reported to enhance the production of phytohormones, mineral uptake and reduce toxic effects of metals, thereby, indirectly promote growth and development of plant in polluted agricultural soils [4]. According to Tsai et al. [5] most commonly used French bean variety exhibit a high dependence on nitrogen fertilizers for growth and yield, and show considerable variation in their ability to nodulate and fix nitrogen, with the nitrogen percentage derived from atmosphere ranging from 68 to 72 per cent for the superior variety. This indicates that French bean needs starter nitrogen fertilization for sufficient nitrogen fixation. Inoculation of French bean with *Rhizobium* increases various plant growths, physiological, quality parameters and grain yield through symbiotic N₂-fixation capacity and plant growth promoting abilities. Plant growth promoting rhizobacteria is the beneficial rhizobacteria inoculation of which increases growth and yield of common bean through different direct and indirect mechanisms such as production of IAA, GA, HCN, Ammonia, siderophore and solubilization of phosphorus, potassium and zinc. Co-inoculation of *Rhizobium* with other PGPR; consortium enhanced the growth and grain yield of common bean [6]. By virtue of their rapid colonization of the rhizosphere and stimulation of plant growth, there is currently considerable interest in exploiting such microorganisms for enhanced crop yield. Therefore to harness the benefits of rhizobia and PGPRs in reducing the application of higher doses of inorganic fertilizers; the development of consortium comprising efficient multi-trait rhizobial isolates and efficient PGPRs are need of an hour for sustainable production of French bean. This chapter reviews the research reports relevant on the topic including (i) isolation and characterization of various isolates of French bean rhizobia (ii) authentication and evaluation of the efficacy of rhizobial isolates (iii) evaluation of the efficacy of potential multi-trait rhizobial isolates on growth and yield of French bean in relation to N dose (iv) isolation and characterization of PGPRs from the rhizosphere and the impact of various isolates of PGPR on growth of French bean (v) compatibility between selected rhizobial isolates and PGPRs; and development of consortium, and (vi) the interaction effect of efficient multi-trait *Rhizobium* and PGPR on French bean.

2. French bean and its use

Gramineae and Leguminosae are two major source of world's food supply, total 15 plant species of which account for more than 90 per cent of the total production of the major seed crops [7]. According to Harlan [8], three major cereal crops such as wheat (*Triticuma estivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) account

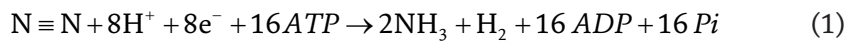
for three quarters of the total food supply. The grains of these cereals provide carbohydrates for human and are complemented by the legumes [9] which vary in their carbohydrate and oil content but have high protein content [7]. In addition to important source of food, feed and fuel legumes are also a renewable source of nitrogen through atmospheric N_2 -fixation for agriculture [10]. Incorporation of legume crops in field improves soil fertility and yield sustainability. Among the legumes, French bean (*Phaseolus vulgaris* L.), of American origin, is the most edible pulse in the world and is second only to the soybean (*Glycine max* L.) [11]. According to Broughton et al. [12], french bean (*Phaseolus vulgaris* L.) has been reported as an important legume for human nutrition and a major protein and calorie source in the world. It is cultivated in the sub-Himalayan and higher Himalayan altitudes between 1200 and 1800 m. In India, French bean covers an area of 2.3 mha with production of 1.1 million tonnes and productivity of 478 kg ha⁻¹ [13]. French bean is popular among Indian farmers due to its high lucrative features such as short life cycle, good adaptability, high market value and the most important for poor farmers, particularly women, hence it is also known as woman's crop. French bean is a self-pollinating leguminous crop which belongs to the family Fabaceae and considered as an important crop in high population density areas of the world [14]. French bean is used both as a pulse and as a green vegetable [15]. In both the developed and the developing countries, French bean is consumed in different forms [16]. Seeds can be consumed as immature green grain. Dehulled seeds may be boiled, parched, roasted, germinated, fermented or cooked in different ways to suit specific tastes. In some parts of the tropics, the young leaves are used like spinach. Common bean seeds are also cooked with tomato sauce and canned. The residual straw can be used as fodder and forage [16] as well as to incorporate in the soils to improve the soil health.

3. Adaptation

French beans are well adapted to tropics, subtropics, and warm temperate regions, grown from 40°S to 40°N latitude. French bean completes their life cycle within 80 to 110 days which depends on variety and night temperatures during the growing season. Suitable temperature for growth of French bean varies between 20 and 22°C. The maximum temperature during flowering of French bean must be under 28°C. It requires a minimum of 500 to 600 mm of rain during the growing season if the crop is cultivated under rainfed conditions whereas an annual total of 600 to 700 mm is considered ideal. They are planted in warm soils with minimum temperatures preferably above 15°C after all danger of frost has passed. Soil texture such as sandy loam, sandy clay loam or clay loam with good drainage and clay content between 15 and 35 per cent is supposed to be best for cultivation of this crop. Soil pH of 6.0 to 6.5 is considered to be the best for the cultivation of French bean.

4. Biological nitrogen fixation

Biological N_2 -fixation is a biological phenomenon, which involves some legumes, whether grown as pulses for seed or as pasture in agro-forestry or in natural ecosystems [17]. Biological nitrogen fixation is very efficient in satisfying the high nitrogen requirements of legumes because of the conversion of gaseous nitrogen (N_2) to ammonia (NH_3) making it available to plant use. Enzyme nitrogenase facilitated the process of BNF. Many N_2 -fixing prokaryotes are diazotrophic, *i.e.* they can grow using dinitrogen gas as their sole source of N while other organisms can fix N_2 only in symbiosis with another eukaryotic organism. The equation for the reaction is.



Two protons are reduced by hydrogen for fixation of one molecule of dinitrogen and because of high stability of dinitrogen the reaction needs high energy [18]. Dupont et al. [19] reported that soon after germination of legume seeds, rhizobia present in the soil or added as seed inoculum invade the root hairs and move through an infection thread to the root. The bacteria multiply rapidly in the root, causing the swelling of root cells to form nodules. Nitrogen in the air of soil pores around the nodules is fixed by binding it to other elements and thus changing it into a plant available form. Some of the carbohydrates manufactured by the plant photosynthesis process are transported to the nodules where they are used as a source of energy by the rhizobia. The rhizobia also use some of the carbohydrates as a source of hydrogen in the conversion of atmospheric nitrogen to ammonia. Despite BNF being a naturally occurring process, many soils do not harbor sufficient numbers of appropriate rhizobia for effective symbioses. Inoculation of leguminous crop with appropriate and compatible rhizobia ensures maximum BNF. Inoculation is generally needed when certain new leguminous crops are introduced to new areas.

5. *Rhizobium*

Rhizobiaceae family is a physiologically heterogeneous and genetically diverse group of soil organisms, which are called rhizobia [20]. Rhizobia include a group of soil bacterial genera viz.; *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium* and *Azorhizobium* which have ability to nodulate symbiotically the members of the plant under Leguminosae family [21, 22]. *Rhizobium*-legume associations are very specific therefore the nodules will be formed in the legume only when infected with a specific *Rhizobium* [23]. According to Broughton et al. [12] specificity involves the recognition of the bacterium by the host and of the host by the bacterium through the exchange of signal compounds which induce differential gene expression in both partners. The bacteria which are able to form root nodules in French bean have been classified into five species of the genus *Rhizobium*, *R. leguminosarum* biovar (bv.) *phaseoli* [24], *R. tropici* [25], *R. etli* bv. *Phaseoli*, *R. gallicum* bvs. *Gallicum* and *phaseoli* and *R. giardinii* bvs. *Giardinii* and *phaseoli*. From various studies, it has been observed that *Phaseolus* rhizobia are very diverse at the species, intra-species and population levels. According to Aguilar et al. [26], current evidence refers difficulty to recognize factors which involved in the distribution of the different rhizobial species among sites, although there is increasing evidence in the literature of parallel evolution between bacteria and the French bean.

6. Nitrogen fixation by *Rhizobium*

French beans have low ability to fix nitrogen symbiotically and surprisingly larger rates of N₂-fixation can be obtained under appropriate conditions [27]. In the light of the poor nodulation in French bean, in general in India, it is feasible that under these situations BNF technologies can become extremely important in order to reduce the use of chemical nitrogenous fertilizers, improve the soil health and enhance the yield levels. Hence, inoculation with the effective rhizobial inoculum presents a great potential for increasing food production in N-W Himalayas and other parts of the French bean growing area. The number of nodules in the plant decreases with the

higher rates of soil N application at planting. In leguminous plants, Nitrogen fixation is a symbiotic process between nitrogen fixing bacteria and legume roots, and occurs within specialized root nodules. Hungria et al. [28] observed an adverse effect on leguminous root nodule development at low temperature stress.

7. Morphological and biochemical characteristics of rhizobia

Morphological characteristics of rhizobia refers to external appearance of rhizobia *viz*; shape, size and color; and biochemical characteristics refers to different characteristics which are produced by rhizobia through their chemical and microbial activities such as acid or alkali production, CRYEMA test, GPA test, carbohydrate utilization, enzymatic activity and plant growth promoting traits. Yadav et al. [29] studied 50 rhizobial isolates separated from the nodules of French bean (*Phaseolus vulgaris* L.) were tested and exhibited typical characteristics of *Rhizobium* sp. on yeast extract mannitol agar media supplemented with Congo red. In Kenya, genetic characterization and diversity of *Rhizobium* isolated from root nodules of climbing bean (*Phaseolus vulgaris* L.) varieties were studied by Koskey et al. [30] and they found that none isolates absorb Congo red dye when incubated in the dark on CRYEMA medium found Gram +ve rods. All isolates were found to be acid producers and fast growers by turning BTB indicator from deep green to yellow when grown on YEMABTB. Most of the isolates showed a mucoid texture because of the exopolysaccharides production. In the study of biochemical characterization of French bean associated rhizobia, Rai and Sen [31] observed and reported that colonies of *Rhizobium* were circular, convex, semi-translucent, raised, single and mucilaginous in nature. According to Vincent [27] and Holt et al. [32] the colonies were large (2–4 mm in diameter) mucilaginous, circular, convex with smooth edges, glistening translucent or white and precipitated calcium glycerophosphate present in YEM agar. *Rhizobium* test in Congo red showed that the colonies did not absorb the congo red color which differentiates *Rhizobium* from *Agrobacterium* [33]. According to Deka and Azad [34] *Rhizobium* cannot grow in Hoffer's medium, however; in contrast Rai and Sen [31] studied and observed that few of the isolates like S-3, CBR and K-1 showed mild growth. The growth of *Rhizobium* in the Hoffer's medium was also observed by Dubey et al. [1]. Deshwal and Chaubey [33] observed no yellow zone around the colonies of *Rhizobium* and such negative ketolactase activity confirmed the isolates to be free from any contamination of *Agrobacterium*.

The isolates changed to yellow color showed the production of acid which is the characteristic of *Rhizobium* [35]. Similarly, isolates of *Rhizobium leguminosarum* by *trifolii* associated with clover showed growth and turned the yeast extract mannitol agar media containing BTB to yellow color indicated all were fast growers and acid producers. It was reported that the utilization of glucose as a carbon source is a confirmatory test for *Rhizobium* [35]. Utilization of different carbon sources is an efficient tool to characterize the isolates [36]. Only four isolates obtained in the study were able to use dextrin as a carbon source, which is in accordance with other works indicating that dextrin is rarely utilized by *Rhizobium* [24, 31]. The utilization of majority of carbon and sodium organic salt sources by *Rhizobium* has also been reported [37].

In the glucose-peptone agar medium, growth of the *Rhizobium* has been observed by [1]. Hunter et al. [38] observed the negative gelatinase activity which is a feature of *Rhizobium*. Yellow slants and red butt were obtained showing the utilization of glucose and sucrose in the triple sugar iron agar medium [35]. De Oliveira et al. [39] also observed that *Rhizobium* strains obtained from different sources can utilize starch. Rhizobial isolates may not grow on lactose [35]. As the pH becomes

high, color of the media changes from yellow to pink which indicates the production of ammonia because of urease enzyme secretion by the incubated isolates which is a positive reaction for the test [40]. Gauri et al. [37] observed that all isolates of rhizobia showed a positive test for urease.

Biochemical characterization and protein profile by sds-page of French bean (*Phaseolus vulgaris* L.) associated rhizobia conducted by Kumari et al. [41] in Andhra Pradesh. They isolated total of six isolates. All the rhizobial isolates were positive to the indole acetic acid, nitrate reduction, urease, catalase and oxidase. Some of the isolates did not produced H₂S and consumed citrate as a sole source of carbon. Positive results were found from the starch hydrolysis assay. On subjecting inoculated plates to iodine test, clear zones from place to place were observed and the colonies changed to yellow color, however blue color appeared on no growth areas. It designates the isolates have the potential to hydrolyze starch present in the medium.

8. Morphological and biochemical characteristics of PGPR

The plant growth promoting rhizobacteria must be defined by some important attributes such as (a) an efficient tool to colonize the root surface (b) to survive, multiply and compete with other microorganisms and (c) to promote plant growth [42]. Anitha and Kumudini [43] reported that the isolates of fluorescent *Pseudomonas* on King's B agar produced creamy, convex colonies having 1–2 mm diameter and at 265 nm appeared yellowish-green fluorescence. Microscopic studies showed that the isolates were gram negative and rod shaped. These isolates were found to utilize sucrose, mannitol and lactose to varying extent and showed that the bacterial isolates were positive only for catalase, oxidase, organic acids, citrate, amylase, indole and caseinase. According to Battu and Reddy [44] gram negative and rod shaped colonies, produced yellowish green pigment on King's B medium were positive for gelatinase and oxidase which were identified as *Pseudomonas fluorescens*. Rodríguez-Cáceres [45] determined morphology and motility for each isolate and also performed biochemical tests such as nitrate reductase and urea hydrolysis [46]. Qualitative analysis showed that all bacterial isolates produced IAA, ammonia, siderophore and hydrogen cyanide.

9. Authentication and evaluation of *Rhizobium*

Authentication is the process by which we can ascertain that the isolates are *Rhizobium* or not through its infection capability under gonotobiotic conditions. Authentication of rhizobia to determine their symbiotic efficiency is required to screen out effective native rhizobial isolates [47]. In order to achieve maximum legume productivity, screening of native isolates for their N₂-fixation efficiencies [48] is important for the development of effective legume inoculum. Nodulation ability and effectiveness of native rhizobia from the seven districts of Uttarakhand in French bean was determined by Yadav et al. [29] and reported that out of fifty isolates 36 were authenticated as *Rhizobium* based on their ability to nodulate French bean. Morphological assessment and effectiveness of indigenous rhizobial isolates nodulating *Phaseolus vulgaris* in water hyacinth compost testing field in Lake Victoria basin was studied by Muthini et al. [49] and reported that the isolates obtained in his study had the ability to renodulate (Infectiveness) *Phaseolus vulgaris* under bacteriologically controlled conditions. Bala et al. [50], who reported that appropriate rhizobial isolates nodulate and fix N₂ on the target host and that each

isolate was able to form nodules with the host plant was identified as *Rhizobium*. High degree of symbiotic efficiency of the specific indigenous strain S_{21/6} was recorded indicating different symbiotic potential of indigenous strains and confirmed the importance of rhizobial strain selection [51].

Evaluation or screening of the authenticated isolates can be done on the basis of plant dry matter response (effectiveness), nodule number and nodule dry weight of the inoculated isolates. Isolation, authentication and evaluation of rhizobial isolates from the soils of North-West Himalayas in French Bean (*Phaseolus vulgaris* L.) studied by Yadav et al. [29] and reported that French bean inoculation with the rhizobial isolates significantly increased the dry shoot, root and total biomass; and shoot: root ratio. The inoculation of French bean with rhizobial isolate RA6 produced total dry biomass significantly higher by 154.4 per cent over reference strain i.e. MTCC 10096. This results demonstrated the presence of native rhizobia in soils of N-W Himalayas capable of nodulating French bean that are either superior or at par with reference strain in improving the overall growth and synthesis of higher biomass in French bean. Muthini et al. [49] reported that nodulated plants had higher shoot dry weight, than the non nodulated plants, however, the mean shoot dry weights was not directly related to the nodule number or nodule dry weight.

10. *Rhizobium* inoculation, N-levels and interaction impact

10.1 *Rhizobium*

Grain legumes have been recognized worldwide as an alternative means of improving soil fertility through their ability to fix atmospheric nitrogen, increasing soil organic matter and improving soil structure [52]. Several studies have sought to identify efficient and competitive strains of rhizobia to cope the nitrogen requirements of common bean [53]. Deshwal et al. [54] observed that in addition to BNF rhizobia can promote plant growth by different direct or indirect mechanisms such as production of IAA, GA, solubilization of inorganic phosphates and biocontrol of plant diseases. Beneficial effects of rhizobium on French bean have been reported by various workers under different climatic and soil conditions [55, 56]. Ndlovu [57] reported that nodulation was significantly affected by inoculation with *Rhizobium phaseoli*. Nodule dry biomass plant⁻¹ was significantly increased by approximately 51.11 per cent with inoculation compared to uninoculated treatment in 2012–2013. Several workers reported that inoculation of legumes with *Rhizobium* isolates improved nodulation and had a positive effect on a number of plant growth parameters [58]. Das [59] also reported that higher number of nodules plant⁻¹ was observed in inoculated plants than the un-inoculated. This might be due to application or introduction of inoculants that increased number of the *Rhizobium* bacteria which infect the roots to form nodules. The higher number of bacteria resulted in higher number of vigorous nodules plant⁻¹. The number and size of nodules indicated the amount of plant tissue available for nitrogen fixation. Thus, the results of this study also suggested a good symbiotic association between *Rhizobium phaseoli* and the host French bean. The presence of nodules in uninoculated treatments during both seasons might be due to the result of existing indigenous *Rhizobium* present in the soil. The increase in dry biomass of nodules which was formed by the inoculation with *Rhizobium phaseoli* might be results of efficiency of the strain.

There were significant differences in shoot, root and total dry biomass of *P. vulgaris* inoculated with variable rhizobial isolates. The significant differences in the shoot dry biomass showed clear differences in the ability of the isolates to

fix nitrogen and are among the preferred methods for determining symbiotic effectiveness of rhizobial isolates [3]. According to Meena et al. [60], application of *Rhizobium* significantly increased the plant height, germination, number of branches plant⁻¹, number of leaves, leaf length and leaf width. The improvement in plant height and dry biomass production at flowering as well as at harvest might be due to the plant growth promoting capabilities, carbohydrates utilization abilities and improved nodulation. The differences in plant growth due to rhizobial inoculation have been attributed to changes in assimilate partitioning [58]. The higher dry shoot biomass of common bean by *Rhizobium* inoculation seems to be due to the supply of N to the crop through symbiotic N₂-fixation [61].

Yadegari and Rahmani [62] recorded the positive effect of inoculation with rhizobial strains Rb-133 and Rb-136 on plant growth. Rhizobial strain increased the seed yield, number of pods plant⁻¹, number of seeds pod⁻¹, weight of 100 seeds, seed protein yield, total dry matter over uninoculated control plants. During two years of study they registered the seed yield in inoculated plants ranging from 1221 to 4693 kg ha⁻¹ depending on the strain and cultivars. Inoculation with suitable strains of *Rhizobium* has been recognized as prerequisite for increasing the yield and quality of legumes [63].

Inoculation of seeds by *Rhizobium* sp. prior to planting has also been reported to be a key factor in enhancing nodulation, early emergence, crop vigor and high grain yield [64, 65]. Bambara and Ndakidemi [66] also reported high common bean seed yield of 1679 kg ha⁻¹ with inoculation compared to 758 kg ha⁻¹ from the uninoculated control. The inoculation of seeds with *Rhizobium* increased nodulation, protein and chlorophyll content, nitrogen uptake, growth and yield parameters of legume crops [67]. In Iran, Namvar et al. [67] reported that *Rhizobium* inoculated plants showed more chlorophyll content and LAI than uninoculated plants. *Rhizobium* inoculation increased chlorophyll content and LAI by 5.43 and 6.99 per cent, respectively as compared to uninoculated plants. In West Bengal, varietal performance of bush type French bean (*Phaseolus vulgaris* L.) for growth, fresh pod yield and quality was studied by Das [59]. Their results revealed that *Rhizobium* inoculation increased the yield and quality parameters *viz*; protein content, vitamin-A content and ascorbic acid content in the fresh pods of the French bean varieties. Under *Rhizobium* inoculation special jhhati beans recorded higher pod yield (23.05 t ha⁻¹) over uninoculated control (20.05 t ha⁻¹). Meena et al. [68] observed and reported, among the six different biofertilizers the best biofertilizer B3 (*Rhizobium*) is recorded significantly improvement in various yield and quality traits. Higher yields obtained with inoculation confirm that the *Rhizobium* technology is efficient in supplying nitrogen to legumes and is a better option for resource-poor farmers who cannot afford to purchase expensive inputs as well as potential strategy to nullify the adverse impact of chemical fertilizer on environment.

Number of pods, number of grains, grain yield, protein content and protein yield in French bean was influenced significantly due to inoculation with rhizobial isolates over uninoculated control [69]. This positive effect due to inoculation with rhizobial isolates attributed to nodulation and nitrogen fixing capability which enhances the nitrogen supplement to plants, resulted higher vegetative growth and carbohydrate partitioning and more protein formation. Phosphorus solubilizing capability of rhizobial isolates helps to solubilize inorganic fixed phosphate and make it available for plant uptake. Higher availability of phosphorus improves N₂-fixation, root proliferation which results higher uptake of nutrient from soil and phosphorus also acts synergistically with nitrogen and helps in carbohydrate translocation and protein synthesis. An increase in number of pods plant⁻¹, number of grains pod⁻¹ and pod yield due to *Rhizobium* inoculation might be due to more availability of nitrogen inside the plant bodies [59]. Koskey et al. [30] reported that inoculation of climbing beans in the field

and green house significantly enhanced nodule and shoot dry biomass, number of pods plant⁻¹, seed yields and nitrogen content in shoot of MAC 13 and MAC 64 climbing beans. Ali (1998) also reported the same results and he concluded that inoculation increased pod yield plant⁻¹. With respect to *Rhizobium* inoculation treatment, higher pod yield was recorded in *Rhizobium* inoculation (20.73 t ha⁻¹) compared to without *Rhizobium* inoculated plants (17.68 t ha⁻¹). Increase in total pod yield is due to more nitrogen availability in the inoculated plants. The higher values of protein content are estimated in *Rhizobium* inoculated plants (2.09%) in comparison to without *Rhizobium* inoculation (1.44%). Increase in protein content due to *Rhizobium* inoculation might be the result of increased nitrogen content inside the plant body which is main element for protein synthesis.

10.2 Nitrogen levels

Nitrogen is an essential nutrient for plant growth and development. Nitrogen deficiency is frequently a major limiting factor for crop production all over the world [70, 71]. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in plants which usually depend upon combined or fixed form of N such as NH₄⁺ and NO₃⁻ because it is unavailable in its most prevalent form as atmospheric N. The sparse nodulation in French bean needs more amount of nitrogen for growth and development in comparison to other legumes.

In French bean all the plant growth parameters except nodule number and nodule biomass were significantly improved with higher level of nitrogen application (from 0 to 120 kg N ha⁻¹), reported in several studies (**Table 1**). This improvement is attributed to the high vegetative growth and higher formation of photosynthates. Nitrogen plays an important role in the formation of protein and nucleic acids structure, the most important building material for every cell. In addition to it nitrogen is also a component of chlorophyll that enables the plant to capture energy from sunlight thorough photosynthesis. Thus, nitrogen supply to a plant increased the concentration of protein, amino acids, protoplasm and chlorophyll which influenced cell size, leaf area and photosynthetic activity [91, 92]. Increased level of nitrogen application in French bean resulted in increased plant height [81]. The negative effect of N fertilizer on French bean nodulation is well documented [23]. However, farmers have gradually adopted the use of N fertilizers with French bean crops, to maximize yields, particularly when irrigation is used. According to Yadav [69] number of trifoliolate leaves, leaf area and chlorophyll content of French bean was significantly increased with higher levels of nitrogen (from 0 to 120 kg N ha⁻¹), the reason behind that N is chief constituent of amino acids which is the building unit of protein, protoplasm leading to improved vegetative growth. Nitrogen being constituent of chlorophyll higher dose of nitrogen increases chlorophyll concentration resulted in more photosynthesis and enhanced number of trifoliolate leaves and leaf area which was reported by various workers (**Table 1**). N fertilization upto 120 kg N ha⁻¹ in French bean increased number of pods plant⁻¹ [77–79, 85]. Nitrogen supply affects a wide range of physiological processes in higher plants [87].

10.3 *Rhizobium* x N-level interaction

An inoculation of rhizobial isolates in combinations with different levels of nitrogen significantly improved the various plant growth parameters in French bean as compared to uninoculated control [61, 69, 90, 93, 94]. Growth, symbiotic and yield response of N-fertilized and *Rhizobium* inoculated common bean (*Phaseolus vulgaris* L.) was conducted by Yoseph and Shanko [94] at Hawassa University, Ethiopia and reported that N fertilization and *Rhizobium* inoculation had significant effect on

Growth parameters	References
Plant height	Negi and Shekhar [72]; Dwivedi et al. [73]; Kushwaha [74]; Saxena and Verma [75]; Singh and Rajput [76]; Singh and Singh [77]; Dhanjal et al. [78]; Prajapati et al. [79]; Veeresh [80]; Jagdale et al. [81]
Number of branches plant ⁻¹	Dhanjal et al. [78]; Prajapati et al. [79]; Veeresh [80]
Dry biomass production	Veeresh [80]; Prajapati et al. [79]; Singh et al. [82]
Number of pods plant ⁻¹	Sharma et al. [83]; Rajput et al. [84]; Singh and Singh [77]; Behura et al. [85]
Number of seeds pod ⁻¹	Dhanjal et al. [78]; Prajapati et al. [79]; Veeresh [80]; Behura et al. [85]; Singh et al. [82]
Grain yield	Singh and Singh [77]; Singh et al. (2000); Dhanjal et al. [78]; Veeresh [80]; Singh et al. [82]
Physiological parameters	
Number of trifoliolate leaves	Escalante et al. [86]; Cechin and Fumis [87]; Yadav [69]
Leaf area plant ⁻¹	Hegde and Srinivas [88]; Rana and Singh [89]; Escalante et al. [86]; Veeresh [80]; Namvar et al. [90]
Chlorophyll content	Escalante et al. [86]; Rana and Singh [89]; Namvar et al. [90]
Quality parameters	
Protein content	Verma and Saxena [75]; Singh and Singh [77]; Veeresh [80]
Water soluble carbohydrate	Namvar et al. [90]; Yadav [69]

Table 1.

List of plant growth, physiological and quality parameters of French bean reported by various workers.

plant height, dry shoot weight, nodule number plant⁻¹, nodule dry weight plant⁻¹, number of pods plant⁻¹, number of seeds pod⁻¹ and grain yield. Omeregie and Okpefa [95] observed that when initial levels of available soil nitrogen were low, a period of nitrogen hunger can reduce nodulation. Kucuk [96] studied the effect of *Rhizobium* inoculation either alone or in combination with nitrogen applications on French bean (*Phaseolus vulgaris* L.) and reported that plant heights were significantly affected by the control, nitrogen, inoculation and different treatment x variety interactions. Interaction effect between nitrogen and rhizobial isolates significantly improved plant height, number of trifoliolate leaves, dry biomass production, and leaf area, chlorophyll content, nitrogen status, grain yield, protein content and protein yield. This could be attributed to additional external supply of nitrogen for vegetative growths of plants and to be used by microbes to fulfill its requirement as starter in initiation of nitrogen fixation. Combined application of rhizobia and nitrogen may be beneficial for improvement of root proliferation and plant growth due to plant growth promoting ability of rhizobial isolates which need more nitrogen for their metabolic activity, might be fulfilled through external supply. Generally, inoculation with *Rhizobium* at all levels of nitrogen application increased biomass production over uninoculated plants [61, 67, 97]. Sajid et al. [98] concluded that the *Rhizobium* inoculation produced higher grain yield than without inoculation. It might also be due to more number of pods and seeds due to *Rhizobium* inoculation and applied N.

11. Compatibility between *Rhizobium* and PGPR

Under natural soil conditions microorganisms are effective to colonize the plant roots for function. Compatibility between the PGPR and other microorganism to

colonize the root system without inhibiting each other is a prerequisite for getting beneficial result using multiple microbes in a crop field. The use of mixed cultures of beneficial microorganisms as soil inoculants is based on the principles of natural ecosystems which are sustained by their constituents such as the quality and quantity of their inhabitants and specific ecological parameters [99]. In the rhizosphere, PGPR and nodule promoting rhizobacteria induce phytoalexins production by the plant, creating antibiosis in the rhizosphere for pathogenic forms, siderophores production to chelate insoluble cations and associative action with the plant [100, 101]. *Rhizobium* as a gram -ve bacteria, is able to establish symbiosis with leguminous plants such as *Cicer* as well as many other rhizobacterial strains, and develops positive interactions with legumes by inhabiting root nodules. Within these nodules, bacteria reduce atmospheric nitrogen to ammonia which acts as a sufficient useable nitrogen source [102]. Studies on legume rhizosphere bacteria have shown that besides indigenous rhizobia interacting and competing for nodulation with an inoculant strain by antagonistic or synergistic interactions, other diazotrophs such as *Azotobacter* and *Azospirillum* as well as rhizosphere fungi and bacteria especially species of *Pseudomonas* and *Bacillus* do interact with *Rhizobium* affecting nodulation and nitrogen fixation [58].

12. Impact of PGPR on French bean

The effect of Plant growth promoting rhizobacteria on plant growth is a well-documented fact. PGPR plays an important role in agricultural systems, especially as biofertilizer. A positive influence of inoculation with various PGPR isolates on shoots and roots length; dry biomass production; and shoot: root ratio was studied by Yadav [69]. The higher shoot length, root length, root volume and dry biomass production due to inoculation with various PGPR isolates could be attributed to their plant growth promoting traits such as IAA, GA, P-Solubilization, Zn-Solubilization, ammonia production, HCN production and nitrogen fixation. IAA and GA are the plant growth hormones in which IAA controls processes *viz*; differentiation, division and enlargement of cells which controls plant growth whereas GA plays pivotal role in growth and development of plants. In most of the observed studies, the growth controller especially IAA, influences most of the root system like primary root growth, side root and piliferous layer formation [103] helps in plant growth promotion. The significant increase in growth of shoot and root due to inoculation of isolates indicates that the bacterial isolates have ability to provide better nutrient flux to the plant host which resulted in the increase of the plant biomass and N accumulation. Beneficial responses of PGPR due to beneficial interaction with rhizobia on legumes have been reported by various workers [75]. An increase in shoot: root ratio of French bean due to inoculation with the PGPRs isolates indicated that carbohydrate might be translocated to shoot but increase in root: shoot ratio in plant due to inoculation with isolates indicated more accumulation of carbohydrates in root rather than its portioning. High root: shoot ratio of plant indicated that plant may be survived in water or salinity stress conditions. The high root: shoot ratio due to inoculation of PGPR was also reported by [69]. Kloepper [42] reported that various PGPR isolates can alter the root architecture and promote plant development through the synthesis of different phytohormones such as IAA, GA and cytokinin. Stefan et al. [104] studied the effects of inoculation with PGPR on photosynthesis, antioxidant status and yield of runner bean and reported that PGPR strains used for seed inoculation induced significant increase in photosynthetic rate at 20 DAI. Increased photosynthetic activity is a consequence of a higher N incorporation which contributed to the formation of chlorophyll [105].

Inoculation of PGPR strains increased the nutritive value of grains by increasing the soluble protein content up to 16.24 per cent and total reducing carbohydrates concentration up to 49.28 per cent.

13. Interaction effect of *Rhizobium* and PGPR on growth of French bean

An application of PGPR together with *Rhizobium* improved the growth and seed production of beans [62]. Plant growth parameters, physiological parameters and quality parameters of French bean were influenced due to consortium comprising of efficient rhizobial and PGPR isolates [69]. The plant growth parameters were significantly improved due to inoculation of rhizobial isolates and PGPR isolates could be attributed to plant growth promoting abilities of PGPR isolates and; nitrogen fixing and PGP traits of rhizobial isolates. Selected rhizobial isolates RD20–3 (R1) and RK3–1 (R2) being GA producer and ammonia producer, stimulate plant growth through plant growth hormone production and protecting plants against phytopathogens. Both isolates were also capable of utilizing highest number of carbohydrates which depicts their ability to proliferate in diverse soils having varying carbon sources. Selected PGPR isolates NAG-K3 (P1) and CRC-J2 (P2) being IAA, GA producer and P solubilizer; and IAA, GA producer, Zn solubilizer and HCN producer, respectively improved plant growth through beneficial effects on seed germination, differentiation, proliferations and division of root cells and through biocontrol activity against harmful fungus. Phosphorus solubilizing capability of isolates increased the availability of phosphorus and through Zn solubilizing ability improves Zn nutrition of plants. Higher root growth and phosphorus availability renders the plant to uptake more amount of other nutrients also from soil and also increase the nitrogen fixing potential of rhizobial isolates which makes more amount of atmospheric nitrogen available to the plants to increase their vegetative growth, chlorophyll content, no. of trifoliolate leaves, leaf area, root acid phosphatase activity and nitrogen and phosphorus status in plants. Higher root proliferation due to more differentiation and division of root cells increases the surface area which resulted higher water and nutrient absorption from soil. Higher uptake of nitrogen and phosphorus improved the starch synthesis and carbohydrate portioning in plant, resulted highest root and shoot length; dry biomass of root and shoot; and shoot: root ratio; when rhizobial (R1) and PGPR (P2) isolates applied conjointly at 100 kg N ha⁻¹ application. N being the chief constituent of amino acids and protein; and P as constituent of phospholipid, both are the building blocks of protoplasm which forms the body structure of plant. Thus N and P improves the body structure and increase number of trifoliolate leaves and leaf area of plants and various physiological parameters vis-à-vis dry biomass accumulation in plants. There are two types of phosphatases on the basis of pH as acid and alkaline phosphatase, because maximum activity occurs at low pH (6.5) and high range of pH (11.0), respectively. Acid level phosphatases secretion can vary with crop sp. [106]. Thus there is differential interspecific genetic variation in root enzyme secretion and acid phosphatase activity [107]. Co-inoculation of rhizobial isolates and PGPR isolates significantly improved root acid phosphatase activity in plants converting organic phosphorus compounds to inorganic-P. Concurrent exudation of organic acids and phosphatase by phosphate solubilizing microorganisms could enhance P solubility, by releasing bound organic phosphates and its mineralization by escalating the rate of hydrolytic cleavage [108].

As the main constituent of chlorophyll and an element of porphyrin ring, N content supplied by symbiotic nitrogen fixation and external application significantly improved the synthesis of chlorophyll 'a', chlorophyll 'b', chlorophyll 'a':'b' ratio in plant, when rhizobial and PGPR isolates applied in combination. Chlorophyll 'a' is always greater than chlorophyll 'b' and chlorophyll 'a' plays an important role in

photosynthesis through absorbing light energy and converting it into chemical energy while chlorophyll 'b' as an accessory pigment absorb more light energy and transfers it to chlorophyll 'a' for photosynthesis. The chlorophyll 'a': chlorophyll 'b' ratio could be a useful indicator of N partitioning within a leaf because this ratio is positively correlated with the ratio of PSII cores to light harvesting chlorophyll-protein complex.

Nitrogen and phosphorus uptake was significantly improved in plants could be attributed to higher N availability through symbiotic nitrogen fixation and high P availability through root acid phosphatase activity and phosphorus solubilizing ability of PGPR isolates. Significantly higher amount of fixed nitrogen in shoots, roots and grains of plant might be attributed to more nodulation and higher root growth due to phosphorus and nitrogen supplementation. The higher amount of nitrogen fixed in French bean due to combined inoculation of rhizobial isolate RD20-3 and PGPR isolate NAG-K3 over solitary inoculation of individual isolate is attributed to the higher phosphorus availability made due to P-solubilization and root acid phosphatase activity. The enhanced P-availability facilitates the more ATP synthesis which is required as a source of energy for carrying out the N₂-fixation by an enzyme nitrogenase. Number of pods plant⁻¹, pod yield, grains pod⁻¹ and grain yield was significantly increased due to inoculation of French bean with rhizobial isolates and PGPR conjointly at 100 kg N ha⁻¹ might be attributed to higher content of nitrogen and phosphorus in plant body which help in pod formation and grain formation in plants. Nitrogen as a chief constituent of protein and phosphorus also helps in protein synthesis, resulted highest protein content and protein yield plants receiving rhizobial and PGPR isolates both, at 100 kg N ha⁻¹ application. Co-inoculation of rhizobial isolate RK3-1 (R2) and PGPR isolate CRC-J2 (P2) significantly reduced all plant growth parameters, physiological parameters, quality parameters, grain yield and nutrient uptake in plants compared to application of RK3-1 (R2) and CRC-J2 (P2), alone [69] which depicts the non-synergistic interaction between these rhizobial and PGPR isolates. Thus these two isolates RK3-1 (R2) and CRC-J2 (P2) may not be used as consortium to improve plant growth.

Co-inoculation of *Rhizobium* and PGPR showed a better nodulation which resulted higher shoot dry biomass and seed yield production. Beneficial effects of PGPR on symbiotic efficiency of rhizobia nodulating legume crops have also been reported by various scientists [64, 109]. According to Samavat et al. [110] the significant correlation has been observed between nitrogen absorption and improvement in growth of plant roots and shoots, as a result of *Rhizobium* and *Pseudomonas* interaction. Lucas Guarcia et al. [111] reported that the co-application of rhizobacteria and *Rhizobium* might have effects on their symbiotic relation with the host legume, depending on the applied isolates. Co-application of *Rhizobium* and *Pseudomonas* improved nodulation, leaf chlorophyll content and other growth factors under greenhouse conditions [110]. The increased chlorophyll content in plant leaves as the result of bacterial isolates co-inoculation could be due to the increased plant nutrition and photosynthesis [112]. An application of PGPR together with *Rhizobium* improved the growth and seed production of beans [62]. Mishra et al. [113] reported that combined application of *Rhizobium* + PSB + PGPR improved plant height and number of pods significantly. An over view of the combined dual inoculation of *A. chlorophenolicus* and *Enterobacter*; and triple inoculation of strain *B. megaterium*, *A. chlorophenolicus* and *Enterobacter* gave significantly higher plant height.

14. Conclusion

This study revealed the presence of efficient multi-trait rhizobial and PGPR isolates in French bean rhizosphere. Those rhizobial isolates which nodulate the plant

under controlled conditions may be authenticated as *Rhizobium* and, having plant growth promoting traits may increase plant height, nodule number, dry biomass, chlorophyll content, grain yield, nutrient uptake and protein yield after inoculation. PGPR isolates from French bean rhizosphere possessed the efficient plant growth promoting traits viz; IAA production, GA production, P solubilization, Zn Solubilization, HCN production, ammonia production and siderophore production may be considered effective for improving plant growth, physiological and quality parameters after inoculation in plants. Inoculation of *Rhizobium* with higher levels of N application may have a positive influence on plant height, dry biomass production, leaf area content, chlorophyll content, grain yield, protein content and water soluble carbohydrate content whereas negative effect on nodule number and dry biomass of nodule. Co-inoculation of *Rhizobium* and PGPR improved the plant growth, physiological and quality attributes of French bean. Microbial consortium comprised of *Rhizobium* and PGPR may not only enhance growth and yield of French bean but may also reduce inorganic fertilizer application.

Conflict of interest

None.

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