Role of Nitrogen-Fixing Plant Growth-Promoting Rhizobacteria in Sustainable Production of Vegetables: Current Perspective

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Almas Zaidi, Mohammad Saghir Khan, Saima Saif,
Asfa Rizvi, Bilal Ahmed, and Mohammad Shahid

Abstract

Vegetables due to high nutritional value comprising of carbohydrates, proteins, vitamins and several other essential elements are considered one of the important dietary constituents. In order to achieve optimum yields, agrochemicals are frequently used in vegetable cultivation. However, the excessive and inappropriate use of agrochemicals has been found deleterious for both soil fertility and vegetable production. The negative impact of agrochemicals in vegetable production practices can be avoided by the use of biofertilizers involving nitrogen-fixing plant growth-promoting rhizobacteria. The use of non-pathogenic nitrogen-fixing plant growth-promoting rhizobacteria to enhance vegetable production is, therefore, currently considered as a safe, viable and inexpensive alternative to chemical fertilization. Even though there are no direct connections between nitrogen-fixing organisms and vegetables, both symbiotic and asymbiotic/associative nitrogen-fixing bacteria have been used to facilitate the growth and yield of non-legume crops like vegetables through mechanisms other than nitrogen fixation. Indeed, there are numerous reports on the effect of plant growth-promoting rhizobacteria on vegetable production, but the information on nitrogen-fixing bacteria employed in vegetable production is scarce. Considering these gaps and success of nitrogen-fixing bacteria application in vegetable production achieved so far, efforts have been directed to highlight the impact of nitrogen fixers on the production of vegetables. Here, efforts will be made to identify most suitable nitrogen fixers which could be used to improve the health and quality of vegetables grown in different regions. The use of nitrogen fixers is also likely to reduce the use of chemicals in vegetable production.

A. Zaidi (✉) • M.S. Khan • S. Saif • A. Rizvi • B. Ahmed • M. Shahid
Faculty of Agricultural Sciences, Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India
e-mail: zaidi29@rediffmail.com

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3.1 Introduction

Nitrogen-fixing plant growth-promoting rhizobacteria including both symbiotic and asymbiotic/associative bacteria have been used in agricultural practices to promote growth and yield of many crops (Ahmad et al. 2013) including vegetables (Antoun et al. 1998; Lamo 2009; Vikhe 2014; Ziaf et al. 2016). Of these, bacteria that form root nodules on leguminous plants and transform atmospheric nitrogen (N) into usable form of N are collectively known as rhizobia: a general term used to denote all rhizobial genera together (Lindstrom and Martinez-Romero 2005). Beijerinck (1888) first of all isolated a bacterium from root nodules, which he identified as *Bacillus radicicola*. In the late nineteenth century, Frank (1889) named this bacterium *Rhizobium leguminosarum* and identified other species belonging to the same group. The term 'rhizobia' was originally used to name bacteria belonging to the genus *Rhizobium*, but nowadays, rhizobia also include other genera, for example, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, etc. (Sahgal and Johri 2003; Graham 2008). The designation rhizobia currently includes more than 70 species distributed over 13 genera including some *Betaproteobacteria* such as *Burkholderia* and *Cupriavidus* (Chen et al. 2007; Barrett and Parker 2006). Other nitrogen-fixing bacteria are free-living nitrogen-fixing bacteria such as *Azotobacter* and *Azospirillum*. They also have the ability to fix nitrogen and to release certain phytohormones, i.e. GA3, IAA and cytokinins (Vikhe 2014) which could stimulate plant growth and increase the availability of nutrients for plant roots. Traditionally, nitrogen fixers have largely been used to supply nitrogen to plants. However, more recently, some nitrogen fixers including both symbiotic (e.g. rhizobia) and asymbiotic (e.g. *Azotobacter*) have also attracted the attention of vegetable growers due to their positive effects on nonlegumes (Antoun et al. 1998; Bhadoria et al. 2005; Lamo 2009; Ramakrishnan and Selvakumar 2012). Vegetable growers on the contrary have long been using agrochemicals (Guertal 2009) in order to obtain maximum yields. The extensive use of fertilizers in vegetable production is, however, at present under debate due to environmental distress and problems to consumer health. Consequently, there has recently been a growing level of interest in environmentally friendly sustainable vegetable practices. In this regard, the integrated use of biofertilizers and chemical fertilizers is considered as the best choice not only to reduce the intensive consumption of chemical fertilizers but also to sustain soil with minimum undesirable impacts and to maximize fertilizer use efficiency in soil (Singh et al. 1999; Palm et al. 2001). Accordingly, soil microorganisms especially PGPR become important in horticultural practices because they are inexpensive and do not cause soil pollution. Among nitrogen-fixing PGPR, rhizobia are reported to possess many desirable plant growth-promoting traits (Ghosh et al. 2015) apart from their normal nitrogen fixation ability. When applied properly, they have been found to exert diverse positive effects on many important nonlegume crops (García-Fraile et al. 2012) including vegetables (Islam et al. 2013; Silva et al. 2014). Mechanistically, nitrogen-fixing PGPR can improve the growth and development of vegetables by producing compounds such as the phytohormone indole acetic acid (Sahasrabudhe 2011) or the enzyme ACC deaminase (Bhattacharjee et al. 2012) involved in the metabolism of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene. They can also mobilize certain major nutrients to the plants such as phosphorous via solubilization of soil insoluble phosphates (Singh et al. 2014a). Nitrogen-fixing PGPR expressing one or multiple plant growth-promoting activities can directly or indirectly promote vegetable growth. Also, some nitrogen-fixing PGPR secrete antimicrobial compounds like siderophores (Singh et al. 2014a), a low-molecular iron-chelating molecules, which restrict the growth of phytopathogens in soils with low content of this ion promoting indirectly the plant growth (Bhattacharjee et al. 2008; Lugtenberg and Kamilova 2009). Considering the importance of nitrogen-fixing PGPR in vegetable production, efforts are made here to collect information on the impact of nitrogen-fixing PGPR on different vegetables grown in different ecological niches.

3.2 Rationale for Using Nitrogen Fixers in Vegetable Production

Vegetables are one of the most important food commodities that significantly affect human health. Due to constantly increasing health awareness among masses, there is greater demand of quality vegetables on regular basis. In order to fulfil the growing demands of vegetarians, vegetable growers have increased the use of synthetic fertilizers to achieve optimum vegetable yields (Abayomi and Adebayo 2014; Guo et al. 2011). The intensive use of chemical fertilizers, however, is reported to cause soil/underground water pollution, destructs microbial composition and their functions, reduces soil fertility and human health (via food chain) problems, makes plant more susceptible to the attack of diseases (Abdelaziz et al. 2007) and leads to ecological risks and poor quality and lesser vegetable yields (Olowoake and Adeoye 2010). Furthermore, higher rates of fertilizer application in vegetable cultivation result in reduced ascorbic acid (vitamin C) content, accumulation of higher level of nitrates especially in leafy vegetables, altered flavour, delayed maturity and increased weight loss. Considering the deleterious effects of fertilizers, and challenge to produce fresh and healthy vegetables, there is urgent need to find suitable alternatives that could help to implement need-based nutrient management (NBNM) practices in order to achieve optimum quality vegetables without any dangerous impact of such chemicals on vegetables. In this context, the use of microbial preparations often called biofertilizers (Dixit et al. 2007) has been found safe for supplying the nutrients to crops besides limiting the problems associated with the use of conventional chemical fertilizers. Biofertilizer is essentially a natural product carrying living microorganisms recovered from various sources including rhizospheres or cultivated soils. Indeed, biofertilizers prepared from nitrogen-fixing PGPR don't have any ill effect on soil fertility and environment instead they improve the soil quality. A small dose of biofertilizer is sufficient to produce desirable results because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain (Anandaraj and Delapierre 2010). Taking into consideration the success of PGPR achieved so far with other crops (Ahmad et al. 2013; Zaidi et al. 2015), different workers have applied nitrogen-fixing PGPR including rhizobia (García-Fraile et al. 2012), *Azotobacter* (Bhadoria et al. 2005) and *Azospirillum* (Ramakrishnan and Selvakumar 2012) along with (Bhadoria et al. 2005) or without (Sharafzadeh 2012) fertilizers for enhancing the production of different vegetables. Apart from their main role in nitrogen fixation, they also stimulate plant growth by other mechanisms such as providing hormones, better nutrient uptake and increased tolerance towards drought and moisture stress. Other major problem in vegetable production is the occurrence of diseases caused by many phytopathogens such as *Pythium aphanidermatum* causing damping-off disease of cucumber (Elazzazy et al. 2012), *Ralstonia solanacearum* causing wilt of brinjal (Chakravarty and Kalita 2012), *Fusarium oxysporum* f.sp. *lycopersici* causing tomato wilt (Loganathan et al. 2014), etc. Traditionally, such diseases are controlled by agrochemicals (pesticides), using sanitary/cultural practices and developing resistant varieties (Sharma and Saikia 2013; Sahar et al. 2013). These disease control measures have, however, neither been promising nor successful. Therefore, the secretion of physiologically active biomolecules such as siderophores (Panhwar et al. 2014), antibiotics (Keel et al. 1992), cyanogenic compounds (Ruangsanka 2014) and lytic enzymes (Nabti et al. 2014) by some nitrogen-fixing PGPR such as rhizobia (Datta and Chakrabartty 2014), *Azotobacter* (Shimaa et al. 2015) or *Azospirillum* (Tortora et al. 2011) has been considered a viable, inexpensive and most effective option for controlling such lethal diseases. More importantly, the use of nitrogen fixers has been found safe for human health after several decades of crop inoculation ensuring that they are optimal bacteria for biofertilization.

3.3 Nitrogen Fixers-Vegetable Interactions: How Nitrogen Fixers Enter Vegetables

Nitrogen fixers in general have widely been used as biofertilizer to supply nitrogen to legumes or other associated crops. Among nitrogen fixers, the members of family *Rhizobiaceae* have also been found to form non-specific associative interactions with roots of other plants without forming nodules (Reyes and Schimidt 1979). Associative symbiosis refers to a wide variety of nitrogen-fixing species that colonize the root surface of nonleguminous plants without formation of differentiated structures (Elmerich and Newton 2007). In other words, these nitrogen-fixing soil bacteria possess the ability to promote the growth of nonlegumes by acting as PGPR (Noel et al. 1996). Indeed, rhizobia can attach to the surface of monocots in the same manner as they attach to dicot hosts (Shimshick and Hebert 1979; Terouchi and Syono 1990). Also, rhizobia grow readily in the presence of germinating seeds and developing root systems in a similar manner with legumes and nonlegumes (Pena-Cabriales and Alexander 1983). It is also interesting to note that the endophytic interaction of rhizobia and nonlegumes occurs without the involvement of genetic signals as observed between rhizobia and legumes during nodulation process (Reddy et al. 1997). Generally, the nitrogen fixers, for instance, rhizobia, enter inside nonlegume plant tissues mainly through cracks in epidermal cells of the roots and in fissure sites where lateral roots have emerged (Dazzo and Yanni 2006; Prayitno et al. 1999). Summarily, the rhizobial endophytic establishment being a dynamic process begins with root colonization which is followed by crack entry into the root interior through separated epidermal cells. Thereafter, endophytes consistently travel up to the stem base, leaf sheath and leaves where they grow rapidly to high population densities (Chi et al. 2005). After they enter inside the plant tissues and attain high population densities, they may influence plant growth by different PGPR mechanisms. Both rhizobia and *Azotobacter* species, apart from supplying N to their respective host plants, secrete some compounds like auxins, cytokinins and antibiotics which directly or indirectly promote the growth of nonlegume plants. For example, Sarhan (2008) indicated a positive effect of *Azotobacter* on growth and yield of potato plants.

3.4 Mechanism of Vegetable Growth Promotion by Nitrogen-Fixing Plant Growth-Promoting Rhizobacteria

Nitrogen fixers like many conventional free-living PGPR can affect plant growth via direct or indirect mechanisms. The direct mechanisms by which nitrogen fixers promote the growth of nonlegumes including vegetable include the solubilization of insoluble P by rhizobia (Singh et al. 2014a; Abd-Alla 1994; Halder and Chakrabarty 1991) and species of *Azotobacter* (Nosrati et al. 2014). Symbiotic rhizobia are advantageous than free-living PGPR in P solubilization as these bacteria are well protected inside the nodule tissues and face little/no competition from indigenous soil microbiota. Another important growth regulator that directly promotes the growth of vegetables is indole acetic acid secreted both by rhizobia (Kumar and Ram 2012; Sahasrabudhe 2011) and *Azotobacter* (Kumar et al. 2014). Indole acetic acid has been reported to play a central role in plant growth and development and acts as a signal molecule which is involved in plant signal processing, motility or attachment of bacteria in root which help in legume-*Rhizobium* symbiosis (Spaepen et al. 2009). On the contrary, the indirect mechanisms of plant growth promotion by rhizobia/*Azotobacter* involve the secretion of compounds that lessen or prevent the deleterious effects of one or more phytopathogenic organisms (Gandhi Pragash et al. 2009). Productions of siderophores (Greek for iron carrier), a low-molecular (500–1000 daltons) iron-chelating substance by *Azotobacter* (Muthuselvan and Balagurunathan 2013) or rhizobia (Ahmad et al. 2013; Datta and Chakrabartty 2014), may be considered a direct factor, since siderophores solubilize and sequester iron from soil and provide it to plant cells. But it can also be considered an indirect factor, since it is associated with suppression of plant pathogens by depriving them of iron uptake. Moreover, siderophore-producing ability helps in the sustenance of rhizobia in iron-deficient soils (Lesueur et al. 1995). The growth-promoting substances involved in vegetable production synthesized by various rhizobia/*Azotobacter* are summarized in Table 3.1.

Table 3.1 Examples of plant growth-promoting substances released by some commonly employed nitrogen-fixing plant growth-promoting rhizobacteria

Rhizobia	Source	Plant growth regulators	Reference
Rhizobium undicola, *Rhizobium* spp.	Nodules of aquatic legume	ACC deaminase, indole acetic acid	Ghosh et al. (2015), Bhagat et al. (2014)
Mesorhizobium, *R. leguminosarum*, *Bradyrhizobium*, *Sinorhizobium meliloti*	*Neptunia oleracea*, *Pisum sativum*, *Trifolium alexandrinum* L., *Cicer arietinum* L., *Trigonella foenum-graecum* L., *Medicago sativa* L., *Indigofera* spp. birdsfoot trefoil (*Lotus corniculatus*)	Exopolysaccharides, N_2 fixation, P solubilization, siderophores, ammonia, hydrogen cyanide, antifungals, volatile antifungal compounds, protease	Machado et al. (2013), Bhattacharjee et al. (2012), Sahasrabudhe (2011), Ma et al. (2004)
Azotobacter	Rhizosphere soil	P solubilization, siderophores, ammonia, hydrogen cyanide, IAA	Prasad et al. (2014)
Sinorhizobium sp. strain MRR101-KC428651, *Rhizobium* sp. strain 103-JX576499, *Sinorhizobium kostiense* strain MRR104-KC428653	Root nodules of *Vigna trilobata* plants	P solubilization, antifungal activity	Kumar et al. (2014)
Azotobacter	Rhizosphere soil	IAA	Kumar et al. (2014)
Azotobacter	Rhizosphere soil	Siderophores	Muthuselvan and Balagurunathan (2013)
Rhizobium psm6	Agricultural soil	P solubilization	Karpagam and Nagalakshmi (2014)
Mesorhizobium	Tunisian soils	P solubilization	Imen et al. (2015)
Rhizobium BICC 651	Root nodule of chickpea	Siderophores	Datta and Chakrabartty (2014)
Mesorhizobium spp.	Native isolates	HCN, siderphores, protease, cellulose, volatile antifungal compounds	Bhagat et al. 2014
Azospirillum brasilense	–	Siderophores, IAA antifungal activity	Tortora et al. (2011), Zakharova et al. (1999)

3.5 Nitrogen-Fixing Plant Growth-Promoting Rhizobacteria Improve Vegetable Production: A General Perspective

Conventional growers in order to achieve high yield and quality vegetables apply higher rates of chemical fertilizers, which are expensive and destructive to environment (Orhan et al. 2006). Considering the threat of the excessive use of fertilizers to soil fertility and vegetable production, vegetable growers have shown interest in applying environmentally friendly and sustainable nitrogen-fixing PGPR (Dixit et al. 2007; Shukla et al. 2012; Ziaf et al. 2016). Generally, the application of nitrogen-fixing PGPR in vegetable production has been found as an attractive alternative to replace chemical fertilizer, pesticides and other supplements. Nitrogen fixers including both symbiotic rhizobia and asymbiotic/associative nitrogen fixers, for example, *Azotobacter* or *Azospirillum*, have traditionally been used as biofertilizer to supply N to legumes and cereals/other crops. Among non-symbiotic N-fixing bacteria, *Azotobacter* and *Azospirillum* have widely been used for enhancing the production of vegetables (Doifode and Nandkar 2014; Solanki et al. 2010). The beneficial effects of *Azotobacter* and *Azospirillum* are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots, displacement of fungi and plant pathogenic bacteria and, to a lesser extent, biological nitrogen fixation (Okon and Itzisohn 1995). Besides N_2 fixation, *Azotobacter* synthesizes and secretes considerable amounts of biologically active substances like B vitamins, nicotinic acid, pantothenic acid, biotin, heteroxins, gibberellins, etc. which enhance root growth of plants (Rao 1986). Another important characteristic of *Azotobacter* association with crop improvement is secretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta 1986). The ability of *Azospirillum* to produce plant growth regulatory substances along (Tahir et al. 2013) with N_2 fixation stimulates plant growth and thereby productivity. Considering these, nitrogen-fixing PGPR for non-legumes especially vegetable production (Table 3.2) have attracted greater attention

Table 3.2 Some examples of vegetable inoculation with nitrogen-fixing plant growth-promoting rhizobacteria

Host vegetables	Botanical name	Inoculant nitrogen fixers	Reference
Potato	*Solanum tuberosum*	*Rhizobium* sp. TN42, *Azotobacter chroococcum*	Naqqash et al. (2016), Meshram (1984), Hussain et al. (1993)
Radish	*Raphanus sativus*	*Azotobacter* + PSB	Ziaf et al. (2016)
Tomato	*Solanum lycopersicum*	*Bradyrhizobium japonicum*; *Azotobacter*	Parveen et al. (2008), El-Sirafy et al. (2010),
Okra	*Abelmoschus esculentus*	*Rhizobium meliloti*	Tariq et al. (2007)
Eggplant	*Solanum melongena*	*Azotobacter* and *Bacillus polymyxa*	Doifode and Nandkar (2014), Bhadoria et al. (2005),
Cabbage	*Brassica oleracea*	*Azotobacter*, *Azospirillum* and VAM	Sharma et al. (2013)

in recent times. Nitrogen-fixing PGPR have been found to colonize and survive in the rhizosphere of the nonlegumes plant to act as PGPR in the rhizosphere of non-host legumes and nonlegumes (Wiehe and Höflich 1995). Nitrogen-fixing plant growth-promoting rhizobacteria when used alone or in combination with other free-living PGPR have also caused a dramatic increase in vegetable production (Noel et al. 1996; Antoun et al. 1998). Mechanistically, as inoculant, nitrogen-fixing PGPR facilitate the vegetable growth by mechanisms other than nitrogen fixation (Trabelsi et al. 2012). When used as mixture, the composite nitrogen fixers provide multiple benefits to crops in addition to their normal physiological activity of N fixation (Iqbal et al. 2012). And hence, the synergistic effects of nitrogen fixer and other free-living PGPR/AM fungi have been found more effective than single inoculation and massively increase vegetable production largely due to enhanced synthesis of phytohormones and nutrient absorption and mobilization (Reimann et al. 2008; Yu et al. 2012). As an example, the composite application of rhizobia (*Bradyrhizobium japonicum*), *Pseudomonas aeruginosa* and mineral fertilizers (urea and potash) has been reported to suppress the deleterious impact of root-rotting fungi and root-knot nematode leading consequently to enhanced tomato production (Parveen et al. 2008). Conclusively, due to their variable growth-promoting activities, nitrogen fixers can be used either alone or in combination with other free-living PGPR/AM fungi for enhancing the production of vegetable in different vegetable production systems.

3.6 Effects of Nitrogen-Fixing Plant Growth-Promoting Rhizobacteria on Important Vegetable Crops

Vegetables are one of the most important food commodities which have occupied a central place in human dietary systems. Production of fresh and quality vegetables is, therefore, required in order to fulfil the demands of vegetarian around the world. Therefore, considering the importance of nitrogen-fixing plant growth-promoting rhizobacteria in vegetable growth, an attempt is made in the following section to highlight the impact of nitrogen-fixing PGPR on some vegetables grown in different production systems.

3.6.1 Potato (*Solanum tuberosum*)

Potato is a starchy and tuberous crop of the Solanaceae family. Potato, ranking fourth in production among vegetables, is a high-yielding, nutrient-exhaustive and short-duration crop. Potato requires higher quantities of nitrogen and phosphorus fertilizers for optimum production (Igual et al. 2001). Therefore, to reduce fertilizer application, nitrogen-fixing PGPR have been employed as a biofertilizer or as bacterial inoculum in potato production (Sidorenko et al. 1996; Kumar et al. 2001; Shafeek et al. 2004). For example, in order to investigate the effects of natural and chemical fertilizers on yield and quality of potato, Mohammadi et al. (2013) conducted a study at the Agricultural Research Farm of Razi University, Kermanshah, Iran. The experiment included three factors: (1) nitragin biofertilizer (a combination of *Azotobacter* species and *Azospirillum* species), (2) HB-101 (a completely organic natural extract) and (3) chemical urea fertilizer (500 kg/ha). Generally, all factors showed significant effects on tuber yield, tuber weight, number of tuber per plant, biological yield, harvest index and tuber nitrate content of potato. However, the highest tuber yield and the number of tuber per plant were obtained when tubers were inoculated jointly with nitragin, urea and HB-101. On the contrary, the lowest tuber nitrate content was obtained when HB-101 was sprayed two times and the tubers were inoculated with nitragin biofertilizer. From this study, it was concluded that the composite application of natural and biological fertilizers along with urea can be useful to enhance potato yield and quality. In a similar study, Verma et al. (2011) conducted an experiment on potato variety Kufri Jawahar to assess the effect of organic components on growth, yield and economic return in potato. The results revealed that combination of crop residues + *Azotobacter* + phosphobacteria + biodynamic approach was the best among all the treatments for most of the growth and yield parameters and gave highest net return and B:C (benefit/cost) ratio. Thus, it can be concluded that the biofertilizers (*Azotobacter*, phosphobacteria, microbial culture and biodynamic approach) are an advantageous source for sustainable organic agriculture, especially for heavy feeder crops like potato. Zahir et al. (1997) also conducted a pot experiment to evaluate the effects of an auxin precursor L-tryptophan (L-TRP) and *Azotobacter* inoculation on yield and chemical composition of potato grown with varying rates of fertilizers. Inoculated (with *Azotobacter*) and uninoculated potato tubers were sown in fertilized (with NPK 250:150:150 kg/ha, respectively) pots, and 1-week-old seedlings were treated with different concentrations of L-TRP (10^{-4}–10^{-7} g/kg soil). Results revealed that L-TRP application alone had no significant effect on tuber and straw yield and PK uptake; however, N uptake and NPK concentrations in the potato tubers were significantly increased at some of the L-TRP levels. *Azotobacter* inoculation significantly increased tuber yield by 28.5%, N uptake and NPK concentrations relative to control. Also, *Azotobacter* inoculation in the presence of L-TRP was found more effective and considerably increased the tuber and straw yield by 62.9 and 47.8%, respectively, and NPK uptake compared to sole application of *Azotobacter*. Hussain et al. (1993) conducted a field experiment to assess the ability of *Azotobacter* inoculation for enhancing yield and other growth parameters on a sandy loam soil treated with NPK (250:125:125 kg/ha, respectively). Shoot, root, single tuber weight, tuber yield plant and R/S ratio increased significantly following inoculation with all *Azotobacter* strains, and maximum tuber yield (18.13% higher than control) was observed with *Azotobacter* strain. The increase in potato growth was possibly due to the production of plant growth regulators since there was no possibility of N_2 fixation in the presence of such a high dose of N. Similar increase in growth and yield and other components of potato due to inoculation with biofertilizer (*Azotobacter chroococcum* with *Azospirillum brasilense*) is reported (Osman 2007). Mirshekari and Alipour (2013) evaluated the bio-priming effect of three different types of biofertilizers: *Azotobacter*, super nitro plus and super nitro on three potato cultivars—Agria, Satina and Kuzima—grown under field conditions. The number of tubers per plant in potato inoculated with *Azotobacter* and super nitro was 8.2, while non-inoculated seeds produced seven tubers per plant. However, seed inoculation with biofertilizers reduced the tubers size considerably over control. Among all treatments, seeds inoculated with *Azotobacter* had higher tuber yield (18,840 kg/ha), while the lowest was recorded for control (15,380 kg/ha). The stepwise regression analysis further verified that the tubers with diameter of greater than 40 mm and mean of tuber weight per plant had a marked increasing effect on the seed yield of potato. The present findings suggested that the tested biofertilizers could be used by farmers before sowing for enhancing potato production. In a follow-up study, Naqqash et al. (2016) inoculated potato with five bacteria belonging to genera *Rhizobium*, *Azospirillum*, *Agrobacterium*, *Pseudomonas* and *Enterobacter* under axenic conditions and observed differential growth responses of potato. Of these, associative nitrogen fixer *Azospirillum* sp. TN10 showed the highest increase in fresh and dry weight of potato over control plants. Also, the N contents of shoot and roots were found maximum following *Azospirillum* sp. TN10 application. Additionally, bacterial strains did colonize and maintained their population densities in the potato rhizosphere for up to 60 days, with *Azospirillum* sp. and *Rhizobium* sp. showing the highest survival. Since all strains showed variable impact, it was suggested that *Azospirillum* and *Rhizobium* could be used to develop biofertilizer for the production of potato.

Apart from directly affecting the growth and yield of potato, nitrogen-fixing PGPR have also been used to facilitate the growth of potato indirectly by secreting siderophores (Muthuselvan and Balagurunathan 2013), HCN (Prasad et al. 2014) or antifungal metabolites (Bhosale et al. 2013). As an example, Meshram (1984) reported that isolates of *Azotobacter chroococcum* were found to be promising for the control of infestation of potato plants with *Rhizoctonia solani*. Inoculation with an isolate of *Verticillium biguttatum* in combination with isolates of *A. chroococcum* effectively protected sprouts, stems and stolons against infestation with *R. solani*. The effect of inoculation, however, varied with soil temperature. No sclerotia were formed on potatoes harvested from soil in pots inoculated with isolates of *A. chroococcum* plus *V. biguttatum* under glasshouse conditions, and the yield increased significantly over the control.

3.6.2 Tomato (*Lycopersicum esculentum* Mill.)

Tomato, the second-most important vegetable crops (Dorais et al. 2008), is cultivated throughout the world occupying an area of 3.5 × 106 ha with the production of 1×10^6 tons (FAO 2010). In India, it occupies an area of 0.54 million ha with a production of 7.60 million ton with an average yield of 14.074 tons per ha (Anonymous 2006). Tomato is a tasty and nutritious vegetable containing vitamins A and C and lycopene content. Due to these nutritive properties, the efforts to produce safe and quality tomatoes both in developing and developed countries have increased (Mahajan and Singh 2006; Flores et al. 2010). In order to reduce the cost and to avoid toxic impact of synthetic fertilizers on tomato production, Ramakrishnan and Selvakumar (2012) applied different biofertilizers to assess their effect on growth and yield of tomato plants. For this, 20-day-old seedlings were transplanted into field until the fruit ripening period. After transplanting, tomato seedlings were bacterized with *Azotobacter*, *Azospirillum* and mixture of both *Azotobacter* and *Azospirillum*. Microbial inoculations, in general, significantly enhanced the whole plant dry weight, plant height, number of leaves per plant, number of fruits per plant, yield per plant, average fruit weight per plant, chlorophyll and protein content. Among all treatments, the composite application of *Azotobacter* and *Azospirillum* showed maximum yield relative to single inoculations and control. The overall results suggest that biofertilizer inoculation improves plant mineral concentration through nitrogen fixation and thereby alters fruit production in tomato plants.

In a similar study, Islam et al. (2013) used 13 nitrogen-fixing bacterial strains belonging to 11 different genera which were positive for 1-aminocyclopropane-1-carboxylate deaminase (ACCD), IAA, salicylic acid and ammonia production. The strains RFNB3 of *Pseudomonas* sp. and RFNB14 of *Serratia* sp. most effectively solubilized both tricalcium phosphate and zinc oxide. In addition, all strains except *Pseudomonas* sp. RFNB3 oxidized sulphur, and six strains were positive for siderophore synthesis, and each strain expressed at least four PGP properties in addition to N_2 fixation. Of these, nine strains were selected based on their multiple PGP potential and evaluated for their effects on early growth of tomato and red pepper under gnotobiotic conditions. Bacterial inoculation considerably influenced root and shoot length, seedling vigour and dry biomass of the two crop plants. Three strains demonstrating substantial performance were further selected for greenhouse trials with red pepper. Of the selected strains, *Pseudomonas* sp. RFNB3 resulted in significantly higher plant height (26%) and dry biomass (28%) compared to control. The highest rate of N_2 fixation as determined by acetylene reduction assay (ARA) occurred in *Novosphingobium* sp. RFNB21-inoculated red pepper root (49.6 nM of ethylene/h/g of dry root) and rhizosphere soil (41.3 nM of ethylene/h/g of dry soil). Moreover, the inoculation with nitrogen-fixing bacteria significantly increased chlorophyll content and the uptake of different macro- and micronutrient contents leading to enhanced red pepper shoots compared to uninoculated controls. The findings of this study suggest that certain nitrogen-fixing strains possessing multiple PGP traits could be used as biofertilizers for enhancing the production of vegetables. Likewise, Bhadoria et al. (2005) conducted a field trial to assess the effect of three *Azotobacter* inoculation (without inoculation, soil inoculation and seedling inoculation) and five levels of N (0, 25, 50, 75, and 100 kg/ha) on tomato and red pepper. A basal dose of P (80 kg/ha) and K (80 kg/ha) along with 50% N was applied at the time of field preparation. Remaining dose of N was top-dressed after 30 days of transplanting. *Azotobacter* culture was used as soil inoculant (5 kg/ha) and seedling inoculant (2 kg/ha), and fresh and dry weight of fruit, ascorbic acid content, total soluble solids (TSS) and cracking percentage of fruits were recorded. Maximum fresh and dry weight, ascorbic acid, TSS (%) and minimum percentage of fruit cracking were observed under the seedling treatment with *Azotobacter* culture over soil inoculation and without inoculation. Favourable environments like proper aeration around roots and considerably greater food materials near roots might be the possible reasons for better bacterial activity resulting in more N_2 fixation and higher growth attributes with the seedling inoculation as compared to soil inoculation of *Azotobacter* culture (Martinez et al. 1993). Increase in quality characters might also be due to growth-promoting substances released by bacterial strains which could have accelerated the synthesis of carbohydrates, vitamins and other characters (Balakrishnan 1988). However, with increase in N concentration, there was a corresponding increase in fresh and dry weight, TSS and cracking percentage of fruit. The maximum fresh weight of fruit and ascorbic acid content were recorded with the application of 75 kg N/ha + seedling inoculated with *Azotobacter* culture, while maximum TSS and dry matter of fruit were observed for 100 kg N/ha + seedling inoculated with *Azotobacter*, which was at par with 75 kg N/ha + seedling inoculated with *Azotobacter*. Also, fruit cracking (%) was increased significantly with increasing dose of N. Similar results have also been reported by Singh and Singh (1992), Chattoo et al. (1997) and Fageria et al. (1992). Apart from asymbiotic/associative nitrogen-fixing PGPR, the symbiotic rhizobia have also been reported to influence tomato production (Ibiene et al. 2012). For instance, García-Fraile et al. (2012) in seed inoculation assays demonstrated that strains TPV08 and PETP01 of *R. leguminosarum* promoted the growth of both tomato and pepper. The dry biomass of shoots and roots of inoculated seedlings was two times higher than uninoculated seedlings. Also, there was a significant increase in the number of flowers and fruits of inoculated plants measured at harvest relative to control plants. The N, P, K and Mg concentrations significantly differed in inoculated and uninoculated plants. This finding consolidated the facts that rhizobia could also be developed as an efficient biofertilizer for augmenting the growth, yield and quality of tomato and pepper in different horticultural practices.

In addition to synthetic fertilizers, plant pathogens also affect very badly the production of vegetables (Singh et al. 2014b). Root diseases caused by root-rotting fungi and root-knot nematodes, for example, are a serious problem in tomato production throughout the world. To overcome disease problems, pesticides are applied on regular basis, but due to numerous problems like cost, emergence of resistance among insect pests and soil pollution associated with the use of pesticides, alternative strategies for disease management including the use of nitrogen-fixing PGPR are required. In this regard, Parveen et al. (2008) employed various treatments containing *Bradyrhizobium japonicum*, *Pseudomonas aeruginosa* (PGPR) and mineral fertilizers (urea and potash) in the management of root-rotting fungi and root-knot nematodes. *P. aeruginosa* and *B. japonicum* when used alone or with mineral fertilizers significantly reduced infection of tomato roots by the root-rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani*. Furthermore, the composite culture of *P. aeruginosa* and rhizobia in the presence of urea only or both urea and potash together resulted in greater suppression of *M. phaseolina* than sole application of each organism. Single application of *P. aeruginosa* or *B. japonicum* or with mineral fertilizers also suppressed the root-knot nematode *Meloidogyne javanica* by reducing numbers of galls on roots, nematode establishment in roots and nematode populations in soil. The maximum shoot fresh weight was recorded when treatment of *P. aeruginosa* or *B. japonicum* was applied with urea and potash or with urea alone. This study thus revealed that rhizobia could also be used in the management of certain diseases affecting vegetables in a big way.

3.6.3 Eggplant (*Solanum melongena*)

Eggplant also known as garden egg, aubergine, brinjal or Guinea squash is one of the non-tuberous species of the nightshade family Solanaceae (Kantharajah and Golegaonkar 2004). Eggplant is one of the top ten vegetables grown in the world. Even though it is of considerable economic importance in Asia, Africa and the subtropics (India, Central America), it is also grown in some warm temperate regions of the Mediterranean and South America (Sihachkr et al. 1993). Globally, Asia accounts for 92.4% of the total world production. Nutritive value of eggplant is comparable to any other common vegetables but is less than tomato. Eggplant fruits are low in calories, but the mineral composition of eggplants is important for human health. Eggplant is composed of 92.7% moisture, 1.4% protein, 1.3% fibre, 0.3% fat and 0.3% minerals, and the remaining 4% consists of various carbohydrates and vitamins (A and C). They are also a rich source of potassium, magnesium, calcium and iron (Zenia and Halina 2008). Apart from these, it also contains beta-carotene (34 mg), riboflavin (0.05 mg), thiamine (0.05 mg), niacin (0.5 mg) and ascorbic acid (0.9 mg) per 100 g of fruit (Choudhary 1976). Among plant nutrients, nitrogen is required by eggplants in comparatively larger amounts than other elements (Marschner 1995), and deficiency of it generally results in stunted growth and chlorotic leaves that lead to premature flowering and shortening of the growth cycle. As an example, Bobadi and Van Damme (2003) investigated the effect of varying level of N (50, 75, 100, 125, 150, 175 and 200 kg N/ha) on number of flowers per plant, number of different types of flowers per plant, length of style, number of fruits per plant and fruit yield/ha of eggplant under controlled greenhouse conditions. Of the varying N concentrations, 200 kg N/ha showed the best performance and significantly produced the highest number of flowers per plant, fruits per plant and yield (32.24 ton/ha) over control plants. However, there was no visible effect of N on style length and type of flowers (long, medium, pseudo-short and short-styled flowers). Also, the results on the measured parameters were comparable when N was applied at 150 and 175 kg/ha. In order to reduce dependency on chemical N fertilizers, Nanthakumar and Veeragavathatham (2000) assessed the effect of integrated nutrient management on the growth and yield of aubergine (cv. Palur 1) during kharif, rabi and summer seasons in Tamil Nadu, India. The results clearly indicated that combining organic fertilizers such as farmyard manure (12.5 t/ha) and 2 kg each of *Azospirillum* and phosphobacteria, with inorganic fertilizers at 75% of the recommended dose of N and P and 100% of K (75 kgN, 37.5 kg P and 22.5 kg K/ha), favourably affected the growth parameters leading to a maximum increase in yield (36.48 t/ha) of eggplant. Bhakare et al. (2008) conducted an experiment in Rahuri, Maharashtra, India, during kharif with aubergine cv. Mahyco-10, involving different N levels, nitrogen-fixing PGPR, *Azotobacter chroococcum* biofertilizer and phosphate-solubilizing bacteria. The application of *Azotobacter* biofertilizer caused a significant increase in plant height, branch number, fruit number per plant and yield/ha compared to the uninoculated control. The inoculation effect was maximum in the treatment containing 100% recommended dose of NPK (NPK 100:50:50 kg/ha) + *A. chroococcum* biofertilizer. Subsequently, the *A. chroococcum* inoculation resulted in consistent increase in yield attributes with gradual increase in the level of N. The yield obtained with 75% RDN+ *A. chroococcum* was almost equal to control. From this study, it was obvious that 25 kg N/ha could be saved if supplemented with *A. chroococcum* inoculation. Similarly, the application of *Azospirillum* and *Azotobacter* along with recommended dose of fertilizer resulted in maximum plant height, number of branches per plant, number of fruits per plant, fruit yield per plant and per ha and TSS in brinjal plants. Whereas days to initiation of flowering, fruit weight and crude protein did not change significantly (Solanki et al. 2010).

In a recent study, Latha et al. (2014) observed that the sole/composite application of microbial and chemical fertilizers had a great effect on the measured stages of eggplant growth. However, the total biomass differed significantly among treatments. Among all treatments, the maximum biomass was observed for treatment containing urea, super phosphate, muriate of potash, *Azospirillum*, phosphobacteria and potassium mobilizer (each 5 g/pot), and the fresh weight was 89.67 g/plant and dry weight 6.15 g/plant at harvest. Maximum chlorophyll content (1.7490 mg/g), protein content (18.2 mg/g), phenol content (19.6 mg/g) and carbohydrates (92 mg/g) in inoculated eggplant were recorded at flowering stage. In a follow-up study, Doifode and Nandkar (2014) evaluated the effect of biofertilizer like *Azotobacter* and *Bacillus polymyxa* (PSB) used alone and in different combinations with recommended dose of chemical fertilizer (NPK) on brinjal crop during kharif season to explore the possibility of reducing doses of chemical fertilizers and for better soil health. The growth characters such as height of plant (11.03–37.54%), stem diameter (6.38–23.79%), length of root (5.56–36.93%), number of functional leaves (5.67–51.51%), weight of fresh shoot (7.90–35.91%) and weight of dry shoot (7.14–46.94%) were significantly improved following microbial inoculations over control. Similarly, number of fruits per plant (11.3–52.81%) and yield of fruits (11.89–54.61%) were more in inoculated crop, and the attack of shoot-root borer, fruit borer and little leaf infestation was less (26.71–50.14%) as compared to uninoculated condition.

3.6.4 Cabbage (*Brassica oleracea*)

Cabbage is yet another important vegetable that requires proper nutrients for optimum production. And hence, nutrient management involving the use of chemical fertilizers coupled with inexpensive biofertilizers and environmentally safe organic manures in balanced proportion may be effective in augmenting the cabbage production (Hussein and Joo 2011). Considering this strategy, Sharma (2002) in a field trial assessed the impact of nitrogen-fixing PGPR (*Azospirillum* and *Azotobacter*) and different levels of N (0.30, 45 and 60 kg N/ha) on growth and yield of cabbage. *Azospirillum* application significantly increased the number and weight of non-wrapper leaves/plant, head length and width, gross and net weight of head/plant and yield/ha. Similarly, N at 60 kg/ha produced maximum number and weight of non-wrapper leaves/plant, head length and width, gross and net weight of head/plant and yield/ha. In addition, *Azospirillum* in the presence of 60 kg N/ha resulted in maximum yield/ha with benefit:cost ratio of 2.9. Similarly, Sarkar et al. (2010) assessed the influence of varying dose of N (0, 60, 80 and 100 kg/ha) and biofertilizer (*Azotobacter*) on growth and yield of cabbage grown at Horticulture Research Station, Mondouri of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, using a plot size of 4.2 × 3.6 m. Application of both N and biofertilizer in general displayed a significant impact on growth and yield attributes of cabbage. In terms of plant improvement, 100 kg N/ha was found to be superior which was followed by 80 kg N/ha. *Azotobacter*-inoculated cabbage plants performed better than non-inoculated plants, and statistical differences were noted in this respect except the number of outer leaves. Plants inoculated with *Azotobacter* had head yield of 31.77 t/ha which was 19.66% higher than non-inoculated plants. The increase in plant growth has been attributed to the fact that N increases the chlorophyll content of the leaves which in turn ensure production of more carbohydrates and, hence, accelerated the growth and head yield of cabbage (Sharma 2002 and Lopandic and Zaric 1997). Other factors by which *Azotobacter* might have promoted the growth and development of cabbage could be the synthesis of auxin, vitamins, growth substances, antifungal and antibiotics by *Azotobacter*. The better results obtained due to *Azotobacter* inoculation are also supported by the findings of Jeevajohti et al. (1993) in cabbage where they reported that growth-promoting substances secreted by microbial inoculants might have led to better root development, transport of water, uptake and deposition of nutrients. The composite application of N and biofertilizer however resulted in significant increase in head weight and head yield of cabbage. The combined application of 100 kg N/ha and biofertilizer recorded highest head yield of 37.80 t/ha which was significantly higher than the other combination treatments. Verma et al. (1997) also recorded highest vegetable and seed yield of cabbage due to application of 60 kg N/ha along with *Azotobacter* inoculation. These studies together suggest that *Azotobacter* in the presence of 100 kg N/ha could be the best option to achieve highest head yield of cabbage. In other report, Sharma et al. (2013) observed the effects of single and composite culture of *Azotobacter*, *Azospirillum* and VAM on cabbage crop. The results showed that 4 kg/ha dose of each biofertilizer resulted in maximum plant height, number of leaves per plant, diameter of stem, length and width of longest leaf and plant spread compared to other doses. Among biofertilizers, *Azospirillum* was found superior and significantly enhanced the growth and fresh weight of green leaves per plant to the extent of 25.85 and 15.24% over *Azotobacter* and VAM, respectively. Also, *Azospirillum* significantly enhanced the total production of trimmed head of cabbage to the extent of 7.06% compared to those observed with *Azotobacter* application. Among various doses of biofertilizers, 4 kg/ha dose of each biofertilizer demonstrated greatest favourable effect on field-grown cabbage production than 2 kg/ha or even 6 kg/ha dose of *Azotobacter* and *Azospirillum*. In yet other microbial approach, Ishfaq et al. (2009) applied vermicompost (0, 5 and 10 t/ha) and *Azotobacter* (0, 5 and 10 kg/ha) against cabbage cv. 'Pride of India'. Application of vermicompost at 10 t/ha resulted in the tallest plant, maximum plant spread, largest size of head and highest yield of heads per plant and per hectare. The number of leaves/plant and number of wrapper leaves/head were, however, maximum with 5 t Vc/ha. Among various levels of biofertilizer inoculation, 10 kg/ha of *Azotobacter* application gave maximum plant height and diameter of head, maximum number of leaves/plant and number of wrapper leaves/head, while the length of head and head yield/plant were maximum with 5 kg *Azotobacter*/ha. Results by Hussein and Joo (2011) showed that seedling inoculation with bacterial (*A. chroococcum*) and fungal effective microorganisms (EM) significantly enhanced Chinese cabbage growth. Shoot dry and fresh weight and leaf length and width were significantly increased by both bacterial and fungal inoculation. However, the NPK chemical fertilizer decreased microflora inhabiting the soil, while the effective microorganisms either fungi or bacteria increased the microbial density significantly. This study implies that both fungal and bacterial EM are effective for the improvement of the Chinese cabbage growth and enhance the microorganisms in soil.

3.6.5 Broccoli (*Brassica oleracea*)

Broccoli is an important winter season vegetable crop which is cultivated widely in many European and American countries. It is an edible green vegetable belonging to cabbage family Brassicaceae whose large flowering head is eaten as a vegetable. Broccoli has many nutritional and medicinal values due to its high content of vitamins (A, B1, B2, B5, B6, C and E), minerals (Ca, Mg, Zn and Fe) and a number of antioxidants (Talalay and Fahey 2001; Rangkadilok et al. 2002; Rozek and Wojciechowska 2005; Wojciechowska et al. 2005). Broccoli is a rich source of sulphoraphane, a potent anticarcinogenic compound. It is a low-sodium, fat-free and low-calorie food (Decoteau 2000). Due to its variable use and great nutritional value, broccoli has attracted greater attention in recent times. For enhancing the growth, yield and head quality of broccoli, higher rates of plant nutrients are applied (Brahma and Phookan 2006). In order to reduce the usage of fertilizers in broccoli production, Abou El-Magd et al. (2014) conducted two field experiments in newly reclaimed land during two winter seasons in Egypt to study the effect of bio-nitrogen (*Azospirillum brasilense* and *A. chroococcum*) and different levels of mineral N [60, 90 and 120 kg N per feddan (one feddan = 0.42 ha)] on vegetative growth, yield and head quality of broccoli (cv. Hybrid Decathlon). Plants treated with nitrogen-fixing PGPR *A. brasilense* and *A. chroococcum* (bio-nitrogen) had higher vegetative growth, i.e. plant length, number of leaves, fresh weight of leaves, stems and total plant. The dry matter accumulation in leaves and heads, main head yield and physical head quality (weight and diameter) as well as N, P and K contents of leaves and heads were greater in nitrogen-fixing PGPR-inoculated broccoli plants compared to those found in untreated control plants. Of the two inoculants, *A. chroococcum* was found superior and resulted in dramatic increase in vegetative growth, main head yield and physical head quality (weight and diameter), as well as N, P and K content of leaves and heads of broccoli compared to those recorded for *A. brasilense* or non-inoculated control plants. Varying levels of N, however, differed statistically in their effects on the measured parameters of broccoli plants. Among N levels, 120 kg N/feddan showed the highest vegetative growth which was followed by 90 kg N/feddan. The lowest vegetative growth, main head yield, physical head quality and N, P and K of broccoli leaves and heads were, however, obtained by 60 kg N/feddan application. The present findings showed that the composite application of nitrogen-fixing PGPR and mineral N caused statistically a significant positive impact on vegetative growth, yield and nutrient uptake of broccoli. However, among all single or multiple inoculation treatments, the combined application of 120 kg N/feddan with bio-nitrogen *A. brasilense* resulted in the highest vegetative growth, yield and chemical contents of broccoli. Yildirim et al. (2011), on the contrary, investigated the effects of root inoculations with *B. cereus* (N_2 fixing), *Brevibacillus reuszeri* (P solubilizing) and *Rhizobium rubi* (both N_2 fixing and P solubilizing) on growth, nutrient uptake and yield of broccoli, grown in field soils, treated with manure and some fertilizers. Bacterial inoculations with manure significantly increased the yield, plant weight, head diameter, chlorophyll content and N, K, Ca, S, P, Mg, Fe, Mn, Zn and Cu contents of broccoli over control. Among different treatments, manure with sole culture of *B. cereus*, *R. rubi* and *B. reuszeri* increased the yield by 17, 20.2 and 24.3%, respectively, and chlorophyll content by 14.7, 14 and 13.7%, respectively, over control. It was suggested from this study that seedling inoculation with P solubilizing (*B. reuszeri*) and both N_2 fixing and P solubilizing (*R. rubi*) could be employed as an alternative to partially reduce the use of costly fertilizers in broccoli production.

Biofertilizers prepared from *Azospirillum*, PSB, *Azotobacter* and VAM applied alone and in combinations with/without inorganic fertilizer had variable impact on yield and quality of broccoli (Singh et al. 2014a). The composite application of *Azospirillum* + *Azotobacter* (50% each) significantly increased the curd size (15.17 cm diameter) and curd yield (1.17 kg and 0.93 kg curd with and without guard leaves, respectively) of broccoli, and this combination was found superior compared to other microbial or fertilizer applications. The results further revealed and showed that 100% application each of *Azospirillum*, PSB and *Azotobacter* also had better performance than the recommended dose of fertilizers. However, all other treatment combinations except *Azospirillum* + *Azotobacter* (50% each) performed poor than the recommended dose of fertilizer. Among the biofertilizer, the coculture of *Azospirillum* and *Azotobacter* (50% each) increased the protein and lipid profile along with phosphate and sulphate content of broccoli curd. Conclusively, the composite application of *Azospirillum* + *Azotobacter* applied each at 50% level was found better for enhancing the curd yield of broccoli and its active biomolecules.

3.6.6 Okra (*Abelmoschus esculentus* L.)

Okra is an annual flowering vegetable grown for its edible pods which can be used as fresh, canned, frozen or dried food worldwide. The approximate nutrient content of the edible okra pods is as follows: water, 88%; protein, 2.1% m; fat, 0.2%; carbohydrate, 8.0%; fibre, 1.7%; and ash, 0.2% (Tindall 1983). Besides these, okra also contains minerals and vitamins. For production and maintenance, okra requires nutrients such as N, P, Ka, Ca, Na and S (Ahmed et al. 2015; Hooda et al. 1980). Deficiency of any of these nutrients resulted in poor growth and leads to a lower yield (Shukla and Nalk 1993). Therefore, an integrated approach involving bio-inoculants/bioagents and fertilizers has been practised over the years for okra production (Singh et al. 2010). The biological potential of different microbial antagonists like, *Bacillus thuringiensis*, nitrogen-fixing PGPR *Rhizobium meliloti*, *Aspergillus niger* and *Trichoderma harzianum* in the suppression of root-rotting fungi like *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. inflicting losses to okra and sunflower plants, was evaluated by Dawar et al. (2008). All biocontrol agents enhanced the germination, growth, length of plant organs (shoot and root) and dry matter accumulation in shoot and root of both okra and sunflower compared to control. The length and weight of shoot and root were significantly increased in sunflower and okra when seeds were coated with *R. meliloti* and *B. thuringiensis*. Also, *Rhizobium* used alone as seed dressing also significantly improved plant growth and reduced disease intensity of plants. *Rhizobium meliloti* significantly inhibited the infection of *R. solani* on okra plant when *R. meliloti* was multiplied on leaves powder of *Rhizophora mucronata* plant (Tariq et al. 2007). Rhizobia which are good rhizosphere organism for leguminous or nonleguminous plants presumably prevent the contact of pathogenic fungi on roots by covering the hyphal tips of the fungus and parasitizing it. Maximum plant height was observed where seeds of okra and sunflower were coated with *T. harzianum* using 2% of glucose followed by gum arabic, mollases and sugar solution. Gum arabic was found more effective in reducing infection by root-rotting fungi, viz. *M. phaseolina*, *R. solani* and *Fusarium* spp. Of the different microbial antagonists used, *T. harzianum* was found more effective followed by *B. thuringiensis*, *R. meliloti* and *A. niger* in the control of root-rotting fungi. Similarly, Ehteshamul-Haque and Ghaffar (2008) reported that *Rhizobium meliloti* inhibited growth of *M. phaseolina*, *R. solani* and *Fusarium solani* while *B. japonicum* inhibited *M. phaseolina* and *R. solani* producing zones of inhibition. In field, *R. meliloti*, *R. leguminosarum* and *B. japonicum* used either as seed dressing or as soil drench reduced infection of *M. phaseolina*, *R. solani* and *Fusarium* spp., in both leguminous (soybean, mung bean) and nonleguminous (sunflower and okra) plants. Likewise, the antagonistic effects of *Bacillus subtilis*, *B. thuringiensis*, *B. cereus* and *R. meliloti* against the control of root-infecting fungi on mash bean and okra were reported by Tariq et al. (2007). Germination of seeds, shoot and root length and shoot and root weight of okra and mung bean were significantly improved following *B. subtilis*, *B. thuringiensis*, *B. cereus* and *R. meliloti* application. Infection of *R. solani* was significantly inhibited on okra when *R. meliloti* was used at 1% w/w, whereas all biocontrol bacteria, viz. *B. subtilis*, *B. thuringiensis*, *B. cereus* and *R. meliloti*, completely suppressed the infection of *R. solani* and *M. phaseolina* on mung bean.

3.6.7 Onion (*Allium cepa*)

Onion, a widely cultivated commercial bulbous vegetable and spice of the genus *Allium*, is grown worldwide. Among onion-producing countries, India ranks second and occupies 756,200 ha area with a production of 12.15 MT and productivity of 16.1 tons/ha (Anonymous 2010). Onion has stimulant, diuretic and expectorant properties and is considered useful in flatulence and dysentery. The shallow-rooted onion plants require large amounts of N for better growth, development and quality of bulb and consequently optimum production (Gamiely et al. 1991; Drost et al. 2002 and Woldetsadik et al. 2003). On the contrary, the inadequate or low N supply increases the incidence of onion bolting and limits bulb yield (Diaz-Perez et al. 2003). The application of super-optimal N has been reported to overstimulate growth and results in (1) extensive foliage growth, (2) delayed crop maturity and (3) poor bulb quality with increased storage losses (Brown et al. 1988; Brewster 1994 and Woldetsadik et al. 2003). Therefore, since both the lower and higher rates of N adversely affect the quality and quantity of onion, the careful application of N fertilizer becomes extremely important in order to improve the yielding ability and bulb quality of onion plants. Under these circumstances, synthetic nitrogenous fertilizer must be supplemented with biofertilizers especially those prepared from PGPR so that the cost of production could be reduced and quality of onion be maintained.

Like other vegetables, the production of onion is also greatly influenced by biofertilizers, organic manures and inorganic fertilizers (Banjare et al. 2015; Yeptho et al. 2012; Yadav et al. 2004). For example, the impact of single and composite culture of *B. circulans*, *Azospirillum lipoferum*, *A. chrococcoum* (nitrogen-fixing PGPR), *B. polymyxa*, *Rhizobium* sp. and AM fungi on growth and quality of onion bulbs was found favourable (El-Batanomy 2009). Vegetative growth and total bacterial populations in onion rhizosphere were increased due to PGPR inoculations. Additionally, the mixture of all cultures showed highest increase in dry matter and bulb diameter. The composite microbial cultures resulted in maximum nitrogenase activity (41.98 μmole C_2H_4/h/g RDW) and mycorrhizal infection (95%) in onion roots. The mixture of *B. circulans*, *A. lipoferum*, *A. chrococcoum*, *B. polymyxa*, *Rhizobium* sp. and AM fungi showed maximum NPK (4:1.97:2.91%) in dry onion shoots relative to fertilized control. Also, the total carbohydrate was highest (29.23 mg/g) in onion plants inoculated with six cultures together which was followed by co-inoculation of *Rhizobium* sp. and AM fungi (28.77 mg/g) and *B. circulans* used alone (24.9 mg/g). Similarly, Ghanti and Sharangi (2009) studied the effect of combinations of six biofertilizers [(1) *Azotobacter* + PSB, (2) *Azotobacter* + AM fungi, (3) *Azotobacter* + *Azospirillum*, (4) *Azospirillum* + PSB, (5) *Azospirillum* + AM fungi and (6) PSB + AM fungi)] and two levels of chemical fertilizers (NPK 100% and 50%) on onion cv. Sukhsagar under field experiment, carried out during the winter season. The co-inoculation of *Azotobacter* + VAM showed the maximum height (43.46 cm) of plants, while number of leaves, number of inflorescence/plot and bulb diameter were maximum due to inoculation with *Azotobacter* + *Azospirillum*. The composite application of *Azotobacter* and *Azospirillum* in the presence of 100% NPK produced maximum length of bulbs (6.03 cm), and the maximum number of scale per bulb (9.81) was recorded with 50% NPK. The plants grown with 100% NPK had maximum bulb weight of 67.45 g, maximum and TSS (12.29%), but the plants fertilized with 50% NPK had the highest reducing sugar (1.420%) and starch (6.27%). It was concluded from this study that the combination of *Azotobacter* and *Azospirillum* could be developed as an effective microbial pairing for enhancing the growth, yield and quality of onion. Furthermore, even though the 100% NPK fertilizer (recommended dose) produced the best result relative to combinations of biofertilizers, the application of biofertilizer should be preferred in order to achieve sustainable and safe production of onion. Balemi (2006) conducted a field experiment using four levels of N (0, 25, 50 and 75% recommended doses) and three strains (CBD-15, AS-4 and M-4) of *Azotobacter* with two uninoculated controls, one with the full dose of N and the other without NPK during summer season against onion cultivar Pusa Madhvi to identify a suitable *Azotobacter* strain and N level for better yield and quality of onion. Application of 75% recommended N along with *Azotobacter* CBD-15 or M-4 significantly increased the marketable yield and the N content in both leaves and bulbs, over control (full dose of N), whereas only 75% recommended N + *Azotobacter* CBD-15 significantly increased the total yield. However, total soluble solids and neck thickness were significantly reduced by 50% recommended N applied with CBD-15 or M-4 compared with the uninoculated control (full N dose). *Azotobacter* strains in the presence of 50 or 75% recommended N significantly reduced the sprouting loss during storage, while nitrogen-fixing PGPR in the presence of 50 or 25% recommended N doses significantly reduced rotting and total losses. Inoculation with a mixture of N-fixing bacteria (*Azospirillum*, *Azotobacter* and *Klebsiella*), biofertilizer (Halex 2) alone or combined with four levels of N (00, 30, 60 and 90 kg N/fed.) had a variable impact on growth, yield components and bulb quality of onion (Yaso et al. 2007). A significant increase in plant height and number of leaves, average bulb weight and marketable and total bulb yield were observed following consistent increase in N levels. Inoculation of onion transplants with Halex 2 significantly improved onion bulb yield and its components (average bulb weight and marketable yield), in both seasons, and accelerated the maturity of onion bulbs in the first season but did not significantly influence vegetative growth and bulb quality characters (plant height, number of leaves and percentages of single and double bulbs, bolters, TSS and sprouted bulbs). Among all treatments, combination of 60 kg N/fed and biofertilizer (Halex 2) was found as the best combination which gave the maximum marketable yield and total bulb yield. The use of Halex 2 could replace one-third of the used chemical N fertilizer and, consequently, improve the economics of onion production. In other study, significant increase in growth and yield of onion plants due to the synthesis of IAA, siderophores and P-solubilizing activity of *B. subtilis* and *A. chroococcum* is reported (Colo et al. 2014). The longest seedling was observed due to inoculation with *A. chroococcum*, while all inoculated plants had maximum height recorded 60 days after sowing. The onion yield was highest when plants were bacterized with *B. subtilis* and *A. chroococcum*.

3.6.8 Radish and Daikon (*Raphanus sativus*)

Radish, a native of Europe and Asia (Gill 1993), is a popularly grown root vegetable which belongs to the family Cruciferae. In many countries like India, it is grown almost everywhere throughout the year. The fusiform roots of radish are eaten raw as salad or as cooked vegetable. Its leaves are rich in minerals and vitamins A and C and are also cooked as leafy vegetable. Like many other nonlegumes, growth and development of radish are also influenced by some nitrogen-fixing PGPR. For instance, *B. japonicum* strain Soy 213 among 266 PGPR strains tested by Antoun et al. (1998) showed the highest stimulatory effect on radish plant. A maximum of 60% increase in stimulatory effect was obtained with *B. japonicum*, while about 25% of all strains of rhizobia and bradyrhizobia, in general, increased radish growth by 20% or more. Similarly, strain Tal 629 of *B. japonicum* significantly increased the dry matter yield of radish by 15% over control in a second plant inoculation assay. It was concluded from these experiments that rhizobia like many other PGPR could also be used as traditional PGPR for enhancing the production of vegetables. In a follow-up study, Basavaraju et al. (2002) reported the effect of asymbiotic nitrogen-fixing PGPR *Azotobacter* strains C_1 and C_2 on germination and seedling development of radish grown under controlled conditions. Of the two strains, strain C_2 of *A. chroococcum* maximally enhanced the germination percentage by 9.33%, radical length by 90.47% and plumule length by 54.37% over uninoculated control. Furthermore, inoculation of radish seeds with *Azotobacter* showed increase in plant height, number of leaves, leaf area, root girth, root length, fresh and dry weights of root and leaf and root N contents over uninoculated control. However, *Azotobacter* in the presence of 75% recommended dose of N per ha was found to be more advantageous and helped to reduce dependence on nitrogenous fertilizers while maintaining good yields. Shukla et al. (2012) carried out an experiment with radish cv. Chinese pink using synthetic fertilizers (N, P and K) and biofertilizers (*Azospirillum*, phosphorus-solubilizing bacteria and AM fungi). Seed yield (10.2 q per ha), 1000 seed weight and seedling vigour index-II were recorded maximum with the combined application of *Azospirillum* + recommended rates of NPK. Ziaf et al. (2016) in a recent study evaluated the effect of nitrogen-fixing PGPR like *Azotobacter* spp., PSB, germinator (Ger, a synthetic germination and early growth enhancer) and PSB + Ger in combination with full (recommended dose of fertilizer), half dose of N and half dose of P on yield of radish cv. 'Mino Early'. The results revealed that *Azotobacter* spp. improved plant- and yield-related attributes, while germinator negatively affected them. The combined application of PSB and recommended dose of fertilizer resulted in maximum number of leaves per plant, root fresh weight and marketable yield. On the contrary, the application of *Azotobacter* spp. in combination with half dose of N and half dose of P showed the highest leaf fresh weight, above ground plant biomass, biological yield, agronomic efficiency and yield response. Moreover, root diameter increased when PSB or *Azotobacter* spp. was applied with recommended dose of fertilizer, while plants treated with *Azotobacter* spp. along with a half dose of P had the longest roots. Correlation analysis revealed that marketable yield of radish was dependent on root fresh weight.

3.6.9 Lettuce (*Lactuca sativa* L.)

Lettuce is considered as one of the most important vegetable crops grown in many countries. It is reported that 100 g of lettuce contain 95% water, 1 g of protein, 3 g carbohydrate, Ca (22 mg), P (25 mg) and vitamin A (Work and Carew 1955). Among many factors, fertilizer application is the most important factor that affects greatly the quantity and quality of lettuce. However, the excessive use of fertilizers negatively affects its production, and hence, the combination of chemical and biological fertilizers is recommended for this crop so that the quality of lettuce is maintained while preserving the soil fertility (Forlin et al. 2008; Sarhan 2008). For example, Sarhan (2012) carried out an experiment during winter season to investigate the effects of nitrogen-fixing bacterium (*Azotobacter*) with different levels of N (100, 200, 300 kg/ha) and without *Azotobacter* (N alone) on growth, yield quantity and quality of lettuce. The results revealed a significant increase in measured characteristics such as plant height, leaves number, length of stem, fresh and dry weight of head, head diameter and head yield following application of *Azotobacter* with low levels of N. Chabot et al. (1996a) on the other examined the single and composite effect of symbiotic nodule forming *R. leguminosarum* bv. phaseoli strains P31 and R1, *Serratia* sp. strain 22b, *Pseudomonas* sp. strain 24 and *Rhizopus* sp. strain 68 on lettuce and forage maize, grown in field conditions having high to low amounts of available P. The composite inoculation of strains R1 of *R. leguminosarum* and 22b of *Serratia* sp. significantly increased the dry matter yield of lettuce shoots where lettuce inoculated with *R. leguminosarum* R1 had a 6% higher P concentration than the uninoculated control. Similarly, at other experimental site (poorly fertile soil), the dry matter of lettuce shoots was significantly increased by inoculation of *R. leguminosarum* strain P31 and *Pseudomonas* sp. 24 along with 35 kg/ha P superphosphate or with *Rhizopus* sp. strain 68 plus 70 kg/ha P superphosphate. The present findings clearly demonstrated that rhizobia expressing P solubilization activity can also function as PGPR with nonlegumes especially lettuce and maize. In a follow-up experiment, Chabot et al. (1996b) assessed the effects of two strains of *R. leguminosarum* bv. phaseoli and three other PGPR on maize and lettuce root colonization. Maize and lettuce seeds were treated with derivatives of all strains marked with lux genes for bioluminescence and resistance to kanamycin and rifampin prior to planting in non-sterile Promix and natural soil. The introduced bacterial strains were quantified on roots by dilution plating on antibiotic media together with observation of bioluminescence. Rhizobia were found as superior colonizers compared with other tested bacteria; rhizobial populations were 4.1 CFU/g (fresh weight) on maize roots 4 weeks after seeding, while 3.7 CFU/g (fresh weight) was found on lettuce roots 5 weeks after seeding. The average populations of the recovered PGPR strains were 3.5 and 3 CFU/g (fresh weight) on maize and lettuce roots, respectively. Bioluminescence also revealed in situ root colonization in rhizoboxes and showed the ability of rhizobial strains to colonize and survive on maize and lettuce roots. In a study, Galleguillos et al. (2000) observed that the rhizobial strains increased very efficiently the lettuce biomass and also induced modifications on root morphology, particularly in mycorrhizal plants suggesting that these strains behaved as PGPR. However, rhizobial strains differed in mycorrhizal plants with regard to (1) the biomass production, (2) the length of axis and lateral roots and (3) the number of lateral roots formed; effects which were, in turn, affected by the AM fungus are involved. Microbial treatments were more effective in terms of growth and morphology of roots at 20 days of plant growth, but after 40 days, the microbial inoculation profoundly increased plant biomass. The interaction between the AM fungi (*Glomus mosseae*) and rhizobial strain had the maximum growth-promoting effect (476% over control) despite the fact that *G. intraradices* showed a quicker and higher colonization ability than *G. mosseae*. Flores-Félix et al. (2013) assessed the impact of *R. leguminosarum* strain PEPV16 on crops like lettuce and carrot and observed a significant increase in macro- and micronutrients of both lettuce and carrots. Also, the rhizobial inoculation enhanced the N and P uptake by lettuce and carrot plants. The P uptake in lettuce shoots was increased by 15, while 40% increase in P concentration was recorded in carrot roots. Increase in Fe content of both crops was attributed to the production of siderophores by *R. leguminosarum* strain PEPV16.

3.6.10 Spinach (*Spinacia oleracea*)

Spinach, an annual member of Chenopodiaceae family, is a valuable leafy vegetable. It is a rich source of chlorophyll, which gives spinach a dark-green colour, good quality and consumer acceptance. Also, spinach is a low-calorie vegetable but contains unusually high minerals like iron, vitamin A and vitamin C contents, which add nutritive value to it. For enhancing growth, yield, seed production and quality of spinach, nitrogenous and phosphorus fertilizers are frequently applied. However, like other vegetables, the quantity and quality of spinach also suffer from uncontrolled application of such fertilizers. And hence, like many crops, the use of biofertilizers has also been suggested as a cheap and viable option for optimizing the production of spinach. For example, the application of nitrogen-fixing PGPR such as *Azotobacter chroccocum* and phosphorein when used singly or in combination with different rates of N and P fertilizers showed a variable effect on growth, yield, sex ratio and seeds (yield and quality) of spinach plants cv. Dokki (El-Assiouty and Abo-Sedera 2005). Seed inoculation with 300 g phosphorein inoculum/fed. in the presence of 40 kg N/fed. (100% of the recommended N dose) + 15 or 7.5 kg P/fed. (66.7 or 33% of the recommended dose of P_2O_5) and seeds inoculated with 300 g *Azotobacter* inoculum in the presence of the full dose of P_2O_5 (22.5 kg P_2O_5/fed.) + 50% of the full dose of N (20 kg/fed) demonstrated the optimum favourable impact on growth, yield, sex ratio and higher seed yield with the best quality relative to control (40 kg N + 22.5 kg P_2O_5 fed.). The populations of inoculated microbes were higher in spinach rhizosphere when seeds were inoculated with *Azotobacter* and phosphorein compared with uninoculated control. Among all treatments, application of 40 kg N + 15 kg P_2O_5 + 300 g phosphorein increased plant fresh yield by 27.2 and 42.3% and 16.3 and 10.4% seed yield over control in the first and second seasons, respectively.

Conclusion

Nitrogen fixers are well known for their beneficial effect resulting from the symbiotic and asymbiotic nitrogen fixation with legumes and other crops including vegetables. In this work, we have tried to showcase the beneficial activity of two contrasting nitrogen fixers on the overall performance of different vegetables grown distinctively in different agroclimatic regions of the world. The advantages of using nitrogen fixers as PGPR are the easy availability of the technology for inocula production and seed inoculation and the better understanding of the functional diversity and genetics of these bacteria. In addition, they have been used in agronomic practices since very long without any adverse impact, they can, therefore, be considered as environmentally benign PGPR for nonlegumes. The work presented here is likely to help vegetable growers to optimize the vegetable production through the use of inexpensive and environmentally safe nitrogen-fixing PGPR while reducing the dependence on chemical input in vegetable production system across the globe.

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