Chapter 12
Inoculation Impact of Phosphate-Solubilizing Microorganisms on Growth and Development of Vegetable Crops

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Abstract Vegetables are one of the important food components of dietary systems in many countries including Asian regions. It provides some of the essential nutrients such as carbohydrates, proteins, and fats and therefore plays a critical role in the human health. Vegetables while growing in soil require significant amounts of phosphorus for better biological growth and optimum yields. The soluble and available forms of phosphorous in soil are, however, limited and not accessible for uptake by vegetable crops. To this end, apart from chemical phosphatic fertilizers, one strategy to provide phosphorus to vegetable crops is the use of phosphate-solubilizing microorganisms, which are ubiquitous and both inexpensive and safe to the environment. Phosphate-solubilizing microorganisms secrete organic acid which in turn solubilizes the complex forms of phosphorus and makes it available to vegetable plants, besides exhibiting other growth-promoting activities. Here, the impact of phosphate-solubilizing microorganisms onto the growth and yield of vegetables is discussed and considered. This approach of using PS microorganisms in vegetable cultivation is likely to help in reducing, if not completely eliminating, the use of synthetic fertilizers in vegetable production across different regions of the world.

Keywords PSM • Vegetables • Synthetic fertilizers • Brinjal • Potato • Tomato

12.1 Introduction

Vegetables are the source of several important nutrients and form an intricate part of our daily routine diets. For proper development and higher yields, vegetable crops grown in different production systems rely hugely on various plant nutrients

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(Solaiman and Rahbbani 2006) including the major element phosphorous (P). Even though the vegetables require high amounts of P for its luxuriant growth and development, the accessibility of P to such crops is restricted due to its rapid fixation ability (Khan et al. 2007; Bushman et al. 2009). Therefore, the deficiency of soluble P in soil has become one of the most limiting factors in crop production in different agroclimatic regions. The plants obtain their P requirements from the soil pool where it occurs as inorganic P, produced as a result of weathering of parent rock or as organic P derived from decayed plants, animal remains, or microorganisms. Mineral forms of P present in soil are apatite, hydroxyapatite, and oxyapatite, while organic P occurs chiefly in the form of inositol phosphate. Other organic P compounds in soil are in the form of phosphomonoesters, phosphodiesters (including phospholipids, nucleic acids), and phosphotriesters (Paul and Clark 1988). Although P is present in soil in abundance, yet it is the least soluble and majority of it is immobilized and rendered unavailable for plant uptake. Plants acquire P from soil solution as phosphate anions which are extremely reactive and are immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Al^{3+}. And hence, the soluble fraction of P within the soils is usually very low relative to other mineral nutrients.

Deficiency of P is a common and quite widespread problem among many soils including the Indian soils, because of which, the growth of vegetable suffers heavily. Phosphorous, therefore, needs to be applied frequently and externally in the form of phosphatic fertilizers in order to maintain a lavish crop growth. The chemical fertilizers when used, however, also become rapidly immobilized soon after application and thus remain unavailable to the plants (Sanyal and Datta 1991; Rodriguez and Fraga 1999). In this context, soil microorganisms play an important role in phosphate solubilization by mineralizing the organic P in the soil and thus making it available to the plants. Some microbes, isolated from various rhizospheric soils including those of vegetable rhizospheres, popularly known as the phosphate-solubilizing microorganisms, have this ability of solubilizing insoluble mineral P by various mechanisms (Sung-Man et al. 2010; Varsha et al. 2010; Sagervanshi et al. 2012; Sharma et al. 2012; Alia et al. 2013; Onyia and Anyanwu 2013; Karpagam and Nagalakshmi 2014). Some of the important genera of phosphate-solubilizing bacteria include *Achromobacter*, *Aerobacter*, *Alkaligenes*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Xanthomonas* (Li 1981; Sharma et al. 2005; Chen et al. 2006; Ivanova et al. 2006). Besides providing P, PSM also facilitate the growth of vegetables by other mechanisms (Jeon et al. 2003; Lucy et al. 2004; Egamberdiyeva 2005; Calvo et al. 2010; Kang et al. 2010; Sung-Man et al. 2010; Dastager et al. 2011; Sagervanshi et al. 2012).

12.2 Importance of Vegetables in Human Dietary System

Vegetables are considered a cheap source of energy as compared to other foods (Alertor et al. 2002; Hussain et al. 2009) but serve as a rich source of essential nutrients such as carbohydrates, carotene, protein, vitamins, calcium, iron, ascorbic acid, dietary fiber, and concentration of trace minerals (Salunkhe and Kadam 1995). Major nutritional components of some of the widely consumed vegetables are listed in Table 12.1. Indeed, vegetables are one of the important food components of human diets world over and have numerous health benefits. Cruciferous vegetables, for instance, contain protein, carbohydrate, and vitamins (ascorbic acid, folic acid, tocopherols, and provitamin A). The cruciferous vegetables contain both major essential mineral elements such as Ca, K, P, and Na, Mg, etc. (Singh et al. 2001), while Fe, Se, Cu, Mn, and Zn are micronutrients found in these vegetables. Among the root vegetables, carrot is rich in carotenoids and dietary fibers with high levels of several other functional components that aid in improving human health. Spinach, on the other hand, has a high nutritional value and is extremely rich in antioxidants and has vitamin A, vitamin C, vitamin E, vitamin K, Mg, Mn, folate, betaine, Fe, vitamin B_2, Ca, K, vitamin B_6, Cu, protein, P, Zn, niacin, Se, omega-3 fatty acids, and folic acid. Spinach also has a high Ca content. Potato contains several vitamins and minerals along with carbohydrate (starch) ($\approx$26 g/medium-sized potato). The starch of potato has the similar physiological effects and health benefits as fiber and offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, and reduces even fat storage. Tomatoes on the contrary are versatile vegetable in daily dietary practice and contain lycopene, one of the most powerful natural antioxidants. Lycopene has also been shown to protect against oxidative damage in many epidemiological and experimental studies. In addition to its antioxidant activity, other metabolic effects of lycopene have also been demonstrated. Tomato consumption has been associated with decreased risk of breast, head, and neck cancers and might be strongly protective against neurodegenerative diseases. In general, diets rich in these foods are associated with a lower risk of the chronic disease of cancer (Hennekens 1986) and heart diseases (Vanpoppel et al. 1994). Apart from human health benefits, vegetables in general improve the quality of the soil where they are growing (Hussain et al. 2010).

12.3 Importance of P to Some Vegetable Crops

Worldwide crop production remains limited due to low phytoavailability of P (Abd El-Salam et al. 2005; Khan et al. 2010). Therefore, it is required at regular basis to overcome the P deficiency to crop plants in P-deficient soils. Among different vegetables, potato, for example, has a relatively high P requirement, but it uses soil P inefficiently due to the limited accessibility of P. However, from the primary

Table 12.1 Nutritional value of some common vegetables

	Vegetables			
Nutrient components (g/100 g)	Brinjal	Cabbage	Tomato	Potato
Energy (kcal)	25	25	18	77
Carbohydrate	5.88	5.80	3.9	17.47
Protein	0.98	1.28	0.9	2.00
Fat	0.18	0.10	0.2	0.10
Dietary fibre	3.00	2.50	1.2	2.20
Sugars	3.53	3.20	2.6	15.44

Source: USDA Nutrient Database

growth until the maturity stage, an adequate supply of P is required by the plant (Grant et al. 2001). When sufficient concentration of P is taken up by potato, it promotes rapid canopy development, root cell division, tuber set, and starch synthesis in potato. An ample amount of P is therefore essential for optimizing the tuber yield, nutritional quality, and resistance of potato to some diseases also. Other studies have also demonstrated a significant increase in yield, number of tubers, and tuber size distribution due to fertilizer P application (Jenkins and Ali 1999; Maier et al. 2002; Sanderson et al. 2003). Even though an inverse relationship between tuber number and tuber size is reported (Knowles and Knowles 2006), an increase in tuber number with P fertilization has shown both an increase and decrease in tuber size (Freeman et al. 1998; Jenkins and Ali 1999). Also, P is an important constituent of nucleoproteins and nucleic acids of other vegetables such as brinjal (Parihar and Tripathi 2003). Onion (*Allium cepa* L.) is yet another most important commercial bulbous vegetable which requires sufficient amount of P among other macro- and micronutrients, from very early stages of growth for optimum production (Grant et al. 2001).

12.3.1 Relevance of Phosphate-Solubilizing Bacteria to Vegetable Crops

Vegetable crops require highest quantity of N, P, and K, while other nutrients, including Fe, Cu, Mn, and Zn, are needed in much smaller amounts. Apart from N and P, majority of these nutrients are most likely available in the soil in adequate or even excessive amounts. When nutrients are not needed by vegetables yet they are added to soil, may lead to deficiencies of other nutrients and can result into nutrient imbalance within soils. For example, when P is applied, but not needed, it can kill off the symbiotic mycorrhizal-forming fungi required by the plant and reduce the ability of vegetables to absorb iron and other micronutrients. Similarly, excess soil P also shuts down the plant's ability to produce phytochelates, organic molecules produced by roots to increase its iron uptake. Considering the cost and some side effects of excessive application in vegetable cultivation, there is an urgent need to protect the vegetable crops from deleterious impact of chemical fertilizers. In this regard, bacteria possessing the capability to solubilize/mineralize insoluble/organic forms of P, known as phosphate-solubilizing bacteria (PSB), have provided some solutions to the expensive synthetic P fertilizers (Khan et al. 2009, 2010; Calvo et al. 2010; Madgaonkar and Lakshman 2013). Phosphate-solubilizing bacteria isolated from different ecological habitats have been used for improving crop production including vegetables (Han and Lee 2005; Turan et al. 2007; El-Tantawy and Mohamed 2009) since 1903 (Khan and Joergensen 2009). The PSB are ubiquitous with variation in forms and population in different soils. These, PS bacteria are being used as biofertilizer since the 1950s (Kudashev 1956; Krasilinikov 1957) to supply soluble P to vegetable crops in an environment friendly and sustainable manner (Khan et al. 2007) by production of organic acids (solubilization) or by catalyzing organic P by enzymes (mineralization) (Khan et al. 2009, 2013).

12.4 Examples of Effects of PS Bacteria on a Few Notable Vegetable Crops

12.4.1 Brinjal

Brinjal (*Solanum melongena* L.) is one of the most popular and widely grown vegetables in the world. Generally, solanaceous vegetables require larger quantities of major nutrients like N, P, and K for optimum yields. In this context, PSB strain has been used to provide P to plants (Han and Lee 2005; Turan et al. 2007). The PS bacterium *Bacillus megaterium*, for example, when used as microbial inoculant against brinjal plants grown in nutrient-deficient soils, resulted in a higher P availability in the soil, and consequently there was more uptake of P by brinjal plants leading eventually to enhanced growth. Furthermore, the shoot and root dry weight of eggplants were increased substantially by 30 and 27 %, respectively, due to sole application of PSB or inoculation combined with RP after 30 days of planting. A significant increase in plant height, dry weight, and rate of photosynthesis was also observed following PSB application. Also, photosynthetic rates were enhanced by 12 % under the influence of PSB inoculation. From this study, it was suggested that *Bacillus megaterium* could be used as a biofertilizer to enhance various growth parameters and yield of eggplant in P-limited soils (Han and Lee 2005). Single and composite inoculation effects of some other PSB on two varieties of brinjal, viz., "Muktajhuri" and "VNR60," were found to be greatly variable. There was a significant positive effect of PSB on the vegetative growth of brinjal plants, and hence, an increase in fruit yield was observed when compared with uninoculated control. The PSB isolates when used either alone or in combination had a pronounced impact on growth and yields. However, the tripartite combinations of all the three P solubilizers resulted in highest crop yield compared to single inoculation of PSB. In summary, there was an overall improvement in average plant height, plant canopy, and other measured yield parameters when the brinjal cultivars were inoculated with the PSB strains, indicating a clear-cut role of PS bacteria in the development of eggplants (Roy and Sengupta 2008).

12.4.2 Potato and Tomato

Globally, approximately 40 % of world's land has low crop production efficiency especially for potato because its roots have limited access to P in the soil (Igual et al. 2001). Moreover, potato needs high amounts of P because of its high biomass producing ability. In order to circumvent this P deficiency, chemical fertilizers are used, but due to rapid fixation ability, P is not available for consumption by potato plants. PSM here play an important role and supply P to potato by secreting certain organic acids (Rashid et al. 2004; Uma and Sathiyavani 2012). Three PS bacterial strains, namely, *Pantoea agglomerans*, *Microbacterium laevaniformans*, and *Pseudomonas putida*, when used singly or in combination against potato (*Solanum tuberosum*), demonstrated a positive response under three sets of experiments, i.e., laboratory, greenhouse, and fields. The combinations of either *P. agglomerans* or *M. laevaniformans* strains with *P. putida* led to higher biomass and potato tuber growth in greenhouse and in field trials. This increase was attributed to the fact that mixture of an acid- and a phosphatase-producing bacterium might have allowed the simultaneous utilization of both inorganic and organic P compounds by potato plants. On the contrary, the Pi levels of soil or application of chemical Pi fertilizer, however, did not cause much difference in potato yields. Of all the three PSB, *P. agglomerans* significantly increased the growth and yield of potato plants by about 20–25 % (Malboobi et al. 2009). Likewise, the dry weight of creole potato roots, and the soil available N, showed better results with the inoculation of 50 % of the inoculum consisting of PSB (*Pseudomonas cepacia*, *Xanthomonas maltophilia*, *Enterobacter cloacae*, and *Acidovorans delafieldii*, formerly called *P. delafieldii*) and four strains of *Azotobacter chroococcum* plus 50 % of chemical fertilizer. A dual inocula of PSB and *A. chroococcum* resulted in significant production of "criolla" potato, Yema de Huevo variety (*Solanum phureja*), at a level matching that of crops grown solely with 100 % NPK fertilizer. Furthermore, approximately 7.4 % reduction in costs of production was observed following microbial inoculation (Faccini et al. 2007). According to Naderi et al. (2012) in a follow-up study, the tuber number per plant, stem number per plant, and plant height of potato were not affected, but the PSB application had significant effects on tuber formation (yield) and tuber mean weight. Leaf area index (LAI), crop growth rate (CGR), and relative growth rate (RGR) were all higher in the first stage of growth due to PSB application which further increased at later stages of plant growth. Among all treatments, spraying PSB on the soil treated with 100 kg/ha P chemical fertilizer displayed the best production of potato, suggesting that this combination of fertilizer and PSB could serve as a sound strategy for sustainable production of potato in any conducive environment.

Tomato (*Lycopersicon esculentum* Mill) is the other important vegetable crop, which contains some important minerals and vitamins. Tomatoes, eaten freely throughout the world, are believed to benefit the heart among other things. Lycopene is one of the most powerful antioxidants found in tomato, and, when cooked, tomatoes have been found beneficial in preventing prostate cancer. The NPK are the most important nutrients supporting its growth, while deficiency of any one of these nutrients limits growth and yield. To increase the availability of P for plants, large amounts of phosphatic fertilizer are used on a regular basis. However, due to reasons explained in the other section (Sect. 12.3.1), PS microorganisms are considered to supply P to tomato plants in a more economical and hazard-free manner. For example, the PS bacteria isolated from tomato rhizosphere efficiently promoted the growth of tomato plants under laboratory conditions. Moreover, shoot length, root length, fresh weight, dry weight, and P content of the plants were increased following PSB application over control. The concentration of available P in rhizospheric soil collected after 30 days growth of tomato plants was higher in rhizospheric soil samples of plants bacterized with PSB over control. The inoculated tomato plants accumulated more P than control plants. Subsequently, the PSB-inoculated plants were healthier and were protected well from diseases like *Fusarium* wilt and early blight, and hence, the overall disease incidence was significantly decreased in the inoculated plants (Hariprasad and Niranjana 2009). Also, two PS bacterial isolates (*Pantoea agglomerans* and *Burkholderia anthina*) in a pot experiment under greenhouse conditions remarkably enhanced plant height, root length, shoot and root dry weight, P uptake of tomato plants, and available P content of soil compared to the control. The enhancement was more pronounced in co-inoculation of PSB strains with TCP. It was, therefore, concluded that the PSB strains possessed greater potential to be developed as biofertilizers for enhancing soil fertility and concurrently the health of tomato plants (Walpola and Min-Ho 2013) Also, the impact of *Bacillus* application along with fertilizer treatment on growth and phosphorous content of tomato was studied. Similar increase in tomato growth and yields following *Pseudomonas* (El-Tantawy and Mohamed 2009) or other PSB inoculation is reported (Awasthi et al. 2011).

12.4.3 Cucumber and Pepper

The impact of a P solubilizer *Bacillus megaterium* var. *phosphaticum* on cucumber and pepper in nutrient-deficient soils was variable, but this strain enhanced nutrient P uptake from the soil and promoted the growth of plants. The availability of P increased further for plants inoculated with PSB when applied with RP (Han and Supanjani 2006). Plant growth-promoting rhizobacteria (*Pseudomonas* sp.), PS biofertilizer prepared from *Pseudomonas putida* strain P13 and *P. agglomerans* strain P5, and chemical fertilizers were used in a separate experiment to evaluate their effect on yield and yield components of cucumbers under field environment. The results clearly showed that the mixture of PGPR (*Pseudomonas* sp.), strains of *P. putida* and *P. agglomerans*, and chemical fertilizers demonstrated a profound increase in length, fresh and dry weight of roots and shoots, and yield of cucumber plants (Isfahani and Besharati 2012). Bacterial cultures, for instance, *Pseudomonas* sp., exhibiting high PS ability isolated from the rhizosphere soil and root cuttings of bush black pepper (*Piper nigrum* L.) when used in combination with N_2 fixing *Azospirillum sp.* and VAM showed additive effect on black pepper under greenhouse trials and reflected their potentiality as efficient P solubilizer for black pepper growing in soils (Ramachandran et al. 2007). In a similar way, a Gram-positive, rod-shaped potential PSB *Bacillus* strain which shared highest sequence similarity to *Bacillus tequilensis* NRRL B-41771T (99.5 %) produced good amount of IAA and was positive for siderophore production. The seed inoculation with this strain (NII-0943) resulted in significantly higher root initiation in black pepper cuttings grown under pot experiments. The soil N and P and P and N uptake by inoculated plants were also enhanced significantly following bacterial inoculation (Dastager et al. 2011).

12.5 Conclusion and Future Prospects

Even though phosphorus is an essential nutrient required for proper growth and development of vegetable plants, it is generally unavailable due to its rapid fixation ability with soil constituents. No doubt, PSM in this context can act as a better and viable substitute and may supply an ample quantity of P to vegetable crops in an inexpensive way. Therefore, researchers need to identify more and more potentially sound PSM so that they could be developed as microbial P inoculants for raising the production of vegetables in eco-friendly way under different agroecological regions. Broadly, the use of PSB in vegetable cultivation has genuinely provided an exciting and meaningful option for enhancing its production and simultaneously preserving the inherent characteristics of diverse agroecosystems from the unpleasant shock of synthetic fertilizers. The success of PSB application, however, depends on selection and delivery of quality PSB inoculants, which requires considerable attention of the scientists to overcome such challenges.

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