Turkish Journal of Agriculture - Food Science and Technology, 11(6): 1183-1194, 2023
DOI: https://doi.org/10.24925/turjaf.v11i6.1183-1194.6033

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Role of N_2-Fixing Plant Growth-Promoting Rhizobacteria in Some Selected Vegetables

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ARTICLE INFO

Review Article

Received : 07-03-2023
Accepted : 16-05-2023

Keywords:
Biological nitrogen fixation
Bio-fertilizers
Sustainable production
Vegetables
PGPR

ABSTRACT

Due to the increase in food-borne diseases, especially in recent years, consumers' orientation to healthy products and their emphasis on consumption force producers to environmentally friendly products. Nitrogen is an essential nutrient for plant growth, but excessive use of nitrogen fertilizers can pollute the environment and cause nitrate accumulation in plants Therefore, vegetable growers strive to replace chemical fertilizers such as nitrogen with environmentally friendly and cost-effective sources. PGPRs stand out in this regard and at the same time, their potential in environmentally and consumer-friendly vegetable production needs to be revealed. In this study, the importance and potential role of N_2-fixing PGPR are discussed for the improvement of yield and yield components in environment-friendly vegetable production for healthy human nutrition.

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Introduction

Nitrogen, next to phosphorous, is one of the major and key nutrients limiting plant growth and development because researchers have shown that crop yield especially vegetable yields, and some quality parameters of vegetables can be increased with appropriate fertilization with nitrogen and other mineral nutrients (Custic et al., 2000; Turan and Sevimli, 2005, Korkmaz et al., 2010). For this reason, nitrogen is the most used agricultural chemical in the world. Different forms of nitrogen can be used in plant nutrition worldwide, but the most important thing is to use it as much as the need by the grown vegetable species. Indeed, greenhouse vegetable growing, even all types of vegetable growing, requires the use of more fertilizers, especially nitrogen per unit production area than other crop production systems, and the use of high rates of nitrogen results in high costs and serious environmental (Korkmaz et al., 2008; Carillo et al., 2019; Colla et al., 2011). As a result, nitrogen fertilization is not only one of the high input costs for farmers but it is also responsible for very serious environmental effects and health risks (İbrikci et al., 2012; Karnez et al., 2013; Gupta et al., 2017). Similarly, some researchers have reported that excessive use of N, especially beyond the needs of the produced crop, has negative impacts on the environment such as especially on groundwater pollution, health hazards, and increased risk of chemical residues. In other words, they reported that nitrogen, which also has high input costs, causes environmental problems and degradation of natural resources (Sahin et al., 2004; Abramovic et al., 2018). On the other hand, as it is known, despite all these negative effects and high costs, nitrogen has an important role as the basic element of protein, nucleic acids, chlorophyll, and growth hormones for plants and is necessary during the growth periods of vegetables and other plants. An adequate supply of nitrogen can stimulate plant growth and increase crop production, but the problem arises with excessive nitrogen fertilization. Because in addition to the aforementioned problems, especially vegetables can accumulate high levels of nitrate under excessive nitrogen fertilizer application (Kaymak, 2013). As a result, growers had to turn to alternative fertilization methods, especially biological new sources, in order not to harm the environment and not leave any toxins behind, as well as to meet the needs of plant nutrients. Because consumers have focused on healthy products due to food-borne diseases that have increased recently, crop producers have entered these searches as a result of this pressure (Kaymak, 2019). Similarly, Zaidi et al. (2017) reported that recently there has been an increasing interest in environmentally friendly, sustainable, and healthy vegetable production. Therefore, efforts are being made by vegetable growers to replace chemical fertilizers with environmentally friendly and cost-effective sources such as plant growth-promoting rhizobacteria. This type of environmentally friendly fertilization method, bio-fertilizers, both reduces the consumption of chemical fertilizers and allows the soil to be used sustainably by protecting it from undesirable effects (Singh et al., 1999; Palm et al., 2001, Korkmaz et al., 2021). Consequently, the importance of alternative fertilization methods such as PGPR, which has great potential and was first introduced by Kloepper and Schroth (1978), has been increasing in recent years both for the healthy production of vegetables and for a healthy environment. Zaidi et al. (2017) have also reported that PGPRs, which enable environmentally friendly production as they are cheap and do not cause soil degradation, have gained importance in horticultural crops such as vegetables.

Soil bacteria, including PGPRs, which are one of the greatest richness of microbial diversity in the rhizosphere, can directly or indirectly affect the growth, development, and health of plants (Gowtham et al., 2017, Dinler et al., 2021). It has been known since the late 1970s that plant growth-promoting rhizobacteria (PGPR) are beneficial for plant growth, yield, crop quality, and plant health (Kloepper and Schroth, 1978). PGPRs include numerous species of the genera such as *Azospirillium, Azotobacter, Azoarcus, Bacillus, Burkholderia, Clostridium, Enterobacter, Erwinia, Flavobacterium, Gluconacetobacter, Klebsiella, Pseudomonas*, etc. (Kaymak, 2019; Kaymak, 2010).

Glick (1995) stated that PGPRs can support plant growth directly and indirectly, and summarized the mechanism of action of PGPRs as synthesizing certain compounds for plants, increasing the uptake of certain nutrients, and protecting plants against diseases. Production of plant hormones (auxin, ACC deaminase, cytokinin, gibberellin), modulating of these plant hormones, nitrogen fixation, phosphorous solubilization, and sequestration of iron (Glick,2012; Olanrewaju et al., 2017) can be explained as the direct effects of PGPRs on plants. The indirect effect of PGPRs is expressed as the suppression of bacterial, fungal, and viral pathogens or the inhibition of the functioning of these pathogenic organisms (Olanrewaju et al., 2017; Kirankumar et al., 2008). The ability of PGPRs to reduce the harmful effects of plant pathogens is closely related to their properties such as ACC deaminase activity, antibiotic production, and synthesis of hydrolytic enzymes (Parewa et al., 2018; Aloo et al., 2019).

Nitrogen fixation stands out as one of the most important and remarkable direct effects of PGPRs in promoting plant growth. The importance of biological nitrogen fixation by PGPRs emerges, as less than half of the applied inorganic nitrogen is effectively absorbed by plants, while the remainder is lost through volatilization or leaching and subsequently pollutes the environment (Aloo et al., 2019). In addition to *Rhizobia* spp., several free-living bacteria, such as *Azospirillum* spp., can also fix nitrogen and supply it to plants (Bashan and Levanony, 1990). *Azospirillum* is the first mechanism proposed to stimulate the growth of plants for nitrogen fixation (Kaymak, 2019; Bashan et al., 2004). In the following years, many studies have been carried out due to the importance of biological nitrogen fixation. However, it is generally believed that free-living bacteria provide only a small amount of the fixed nitrogen required by the bacterial-associated host plant (James and Olivares, 1997). Similarly, according to the reports of the researchers, it was reported that nitrogen transfer by *Azospirillum* spp. is not sufficient for the plant (Bashan et al., 2004; Bashan and Holguin, 1997). Yet, it has been determined that bacteria cannot fulfill all the nitrogen needs of plants, nevertheless, they contribute a significant amount of nitrogen (Kaymak, 2010). On the other hand, nitrogen fixation is the conversion of atmospheric nitrogen into usable nitrogen that converts to ammonia. This is essential for all life forms because nitrogen is the basic building block of plants, also all life forms (Kundan et al., 2015).

Biological nitrogen fixation, which takes place with enormous energy consumption in the form of ATP (Glick, 1995; Kundan et al., 2015), usually takes place at moderate temperatures by nitrogen-fixing microorganisms commonly found in nature (Raymond et al., 2004). In other words, Kundan et al. (2015) reported that since nitrogen fixation is a very energy-consuming process, at least 16 moles of ATP are required for each mole of reduced nitrogen. In addition to these, to explain briefly and simply, N2 fixation is carried out with the nitrogenase enzyme, which has a complex structure (Kim and Rees, 1994). The structure of nitrogenase has been determined as a two-component metalloenzyme consisting of dinitrogenase reductase and dinitrogenase (Stacey et al., 1992). During biological nitrogen fixation, dinitrogenase reductase provides electrons with high reducing power. Dinitrogenase uses these electrons to reduce N_2 to NH_3 (Kundan et al., 2015).

According to Vessey (2003), report use of PGPR for various crops is important and beneficial not only for increasing crop production but also for the restoration and health of the environment. The direct effect of PGPRs on plant growth, such as biological nitrogen fixation, production of plant hormones, and solubilization of phosphorus, is known to increase the growth and yield of vegetables and has been proven by many researchers. Additionally, when the studies with PGPRs are examined, it will be seen that many studies have been carried out, from increasing the tolerance of vegetables to different stress conditions such as salinity and drought to controlling many plant diseases. In these studies, some properties of PGPRs such as ACC deaminase activities, production of plant hormones, and phosphorus solubilizing were utilized. However, when making this review, it was evaluated only if the N_2-fixing ability of the PGPRs used in the research was specified. Therefore, in this review, the importance and potential role of N_2-fixing PGPR is discussed for the improvement of yield and yield components in environment-friendly vegetable production for healthy human nutrition.

N_2-Fixing Plant Growth Promoting Rhizobacteria (PGPR) and Vegetable Production

The potential of N_2-Fixing PGPR for cost effective and environment-friendly sustainable vegetable production in some selected vegetable species is discussed below.

Tomato

The tomato, a member of the Solanaceae family, is native to tropical coastal regions of Ecuador, Peru or Bolivia and parts of northern Chile, and tropical America in general (Decateau, 2000; Welbaum, 2015). Tomato, which is the second most important vegetable after potato in world vegetable production, is produced over 180 million tons with a production area of 4.8 million hectares worldwide (Aksoy and Kaymak, 2021). Nitrogen is very important for vegetative growth in tomato production. On the other hand, excessive vegetative growth induced by excessive nitrogen fertilization may reduce early and subsequent fruit sets (Rubatzky and Yamaguchi, 1997). However, considering the harmful effects of excess nitrogen on the environment, the importance of different nitrogen sources such as N_2-fixing PGPRs becomes apparent for environmentally friendly tomato production.

The direct and indirect effects of PGPRs are known to have great potential to improve tomato growth and yield under field and greenhouse conditions. In previous studies, it was reported that *Pseudomonas fluorescens* PF15, *P. putida* PP27 and PCI2, *P. aeruginosa* PM12 showed significant protection against Fusarium wilt in tomato (Pastor et al., 2016; Fatima and Anjum, 2017). Similarly, *Bacillus subtilis* B-001, *B. amyloliquefaciens* SQY162 and *B. amyloliquefaciens* strain SQRT3 can effectively controls tomato bacterial wilt (Peng et al., 2017; Li et al., 2017). The examples of indirect effect of different PGPR's can be increased.

For example, according to Sharma and Sharma (2017) report, PGPR's can also be used against the nematodes and *P. jessenii* strain R62 and *P. synxantha* strain R81 have a great potential to reduce nematode infection in tomato in greenhouses.

Besides, one of the biggest problems for today's world is the continuous use of chemicals such as inorganic fertilizers for plant growth and development in agricultural production (Kılıç ve Korkmaz, 2012). One of the necessary keys to overcoming this problem can be PGPRs. For example, Kumar et al. (2017) reported that bioformulation prepared from biochar and *Burkholderia* sp. strain L2 has effectively increased seed germination, promoted plant growth and yield, and improved soil physical, chemical and dehydrogenase activity, and has the potential to tremendously enhance productivity of tomato and can act as a sustainable substitute for chemical fertilizers. It was also stated that the combined use of PGPR (*Paenibacillus polymyxa* and *Bacillus amyloliquefaciens*) and biochar can improve the soil microbial community structure and increase the nitrogen use efficiency of tomato (Wang et al., 2021). Application of biochar with PGPR may cause these effects as it increases the relative abundance of Nitrospira, which is a group of Gram-negative bacteria that acts as nitrification and can oxidize nitrite to nitrate in the soil, promoting nitrification and increasing NO-3-N content in the soil (Wang et al., 2021; Posmanik et al., 2014). In addition, Adesemoye et al. (2010) declared in their study tomato was used as a test plant that PGPR increased N uptake and maintained productivity despite the use of less fertilizer and the use of PGPR can help reduce N leaching during the growing season and reduce residual N in the soil. In another research, tomato plants inoculated with *Pantoea agglomerans* MVC 21 and *Pseudomonas putida* MVC 17 showed better performance in the shoot length and biomass compared to the control plants, and the most significant increase in shoot length was determined in treated tomato plants with *Pantoea agglomerans* MVC 21 (Vasseur-Coronado et al., 2021). According to the results of another study, although it was determined that inoculation with PGPR could increase plant growth, N and P uptake in tomatoes grown in calcareous soils, it was reported that the effect of PGPR could vary according to the nitrogen source and rate (Fan et all., 2017). In the study conducted to determine the effects of PGPRs on plant growth, yield and tomato quality under simulated seawater irrigation, it was determined that *Erwinia persicinus* RA2 and *Bacillus pumilus* WP8 can promote tomato growth and improve fruit quality more than *Pseudomonas putida* RBP1 (Shen et al., 2012). Reyes-Castillo et al. (2019) reported that although *Pseudomonas gessardi* Tmt-16 increased the root growth of tomato by 23.57% compared to the negative control, the lowest plant height was also detected in *Pseudomonas gessardi* Tmt-16. In addition, *Pseudomonas gessardi* Tmt-16 increased the N content in plant tissue by 2.60% compared to the control. In an experiment about the effect of PGPR's as a biofertilizer on processing tomato cultivars, tomato seedlings were inoculated with 1% liquid commercial solution of PGPR (*Pseudomonas putida*, *Azotobacter chroococcum*, *Bacillus circulans*, *B. megaterium*). At the end of this experiment, it was determined that PGPR treatment was increased the marketable yield, total biomass, and water use efficiency of tomato (Le et al., 2018). Similarly, as in previous records on *Azotobacter chroococcum* and *Bacillus circulans*, the applied PGPR solution can increase tomato yield and mineral nutrient availability by improving root growth (Mehta et al., 2015; Baba et al., 2018). Moreover, the plant growth promoting and high yielding effects of *Bacillus megaterium* and *Pseudomonas putida* on tomatoes may be due to phytohormonal effects such as decreasing ethylene-related processes (Aslam et al., 2018; Hernández-Montiel et al., 2017). Also, the nitrogen fixing activity of bacteria can promote growth in tomato by enriching the soil composition with the nutrients provided by organic compost (Jyolsna et al., 2021). In addition, when 75% of the recommended fertilizer dose is used together with nitrogen-fixing PGPRs (*Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4), values close to the recommended fertilizer dose can be obtained in tomato growth parameters such as plant height, shoot dry weight, root dry weight, yield, and nutrient intake (Adesemoye et al., 2009). Furthermore, *Pseudomonas brevicompactum*, *Pseudomonas marginalis*, *Pseudomonas putida* and *Trichoderma atroviride* under hydroponic conditions, and *Pseudomonas fluorescence* can increase yield in tomato under insufficient light conditions (Gagné et al., 1993; Gravel et al., 2007). PGPRs can also be used in organic tomato cultivation. As a matter of fact, it was determined that different PGPR applications in organic agriculture improved the yield and quality parameters of tomato and the highest yield was obtained from foliar application of *Bacillus megaterium* M-3 (Yagmur and Gunes, 2021).

Pepper

The pepper, *Capsicum annuum* L., is an important species of the Solanaceae family, which is grown commercially around the world due to its better adaptability to different agro-climatic zones. Furthermore, pepper is an excellent source of antioxidant compounds and an economically important agricultural crop (Islam et al., 2013). It is recommended that pepper which requires less fertilizer than tomatoes to fertilized according to soil and leaf test results in commercial production (Welbaum, 2015). The need for nitrogen is more evident in the first fruit set of peppers. On the other hand, since excessive nitrogen use causes strong vegetative growth, it has a negative effect on early fruit set, total yield, and fruit quality in peppers (Decateau, 2000). Indeed, Islam et al. (2013) stated that PGPRs with nitrogen fixation capability can play an important role in providing essential nutrients for plant growth and yield, and it is necessary to screen and select new and effective nitrogen-fixing PGPRs for sustainable crop cultivation practices in pepper cultivation. Nitrogen-fixing PGPRs can play an important role in reducing chemical nitrogen use and promoting plant growth in pepper.

In a greenhouse experiment, thirteen nitrogen-fixing bacterial strains belonging to 11 different genera were used to test their potential as biofertilizers in pepper production. *Ochrobactrum* sp. RFNB9 and *Novosphingobium* sp RFNB21 increased the root length of red pepper by more than 50%. All tested strains (*Pseudomonas* sp. RFNB3, *Ochrobactrum* sp. RFNB9, and *Novosphingobium* sp. RFNB2) increased plant height and dry biomass, and also it was determined that *Pseudomonas* sp. RFNB3 was more effective than others. In addition, at the end of the study, it was clearly stated that three of the nitrogen-fixing bacterial strains (*Pseudomonas, Ochrobactrum, and Novosphingobium*) can be used for the development of efficient bio-inoculants for capsicum and other plants (Islam et al., 2013).

Russo (2006) reported that an organic certified seedling growing medium was used in the production of organic bell pepper seedlings by inoculating it with a mixture of arbuscular mycorrhizae and *Sinorhizobium* sp. The use of bacteria improves plant height and dry weight, and interactions of bacteria and fertilizer ratio or irrigation regime have been reported to affect plant height or dry weight. It has been determined that the use of *Sinorhizobium* sp. provides significant benefits in seedling production in bell pepper. Similarly, Marquina et al. (2018) also reported that inoculation of bell pepper seeds with rhizobacteria (*Rhizobium tropici* ME01, *Sinorhizobium* sp. RmB, *Bradyrhizobium* sp. Nod 2R, *Sinorhizobium* sp. Leu2A(1)2 and *Azospirillum* sp. YAC1), especially with Leu2A(1)2 can be used to increase the germination and growth of the plant. In addition, due to its high colonization capacity and ability to function in phosphate solubility, sulfur oxidation, nitrogen fixation, and IAA production in plant tissues, *Pantoea anantis* B1-9 increased yield and growth in pepper and it has been reported that it can be used in different plants (Kim et al., 2012).

The soil environment and crop yield are affected negatively when the intensive cropping of red pepper. Therefore, *Bacillus licheniformis* K11, Bacillus subtilis AH18, and *Pseudomonas fluorescens* 2112, were used to overcome these adverse effects. As a result, it has been reported that the application of the microbial agent has a positive effect not only on the rhizospheric soil bacterial community structure but also on the yield of red pepper (Jung et al., 2015).

The effects of organic and inorganic nutrient sources on nutrient uptake, residual soil fertility and bell pepper crop production were investigated by combining chemical and organic N with PGPR. The physical and biological health of the soil has been improved with the use of 75% chemical N and 25% organic N such as FYM, VC and PM together with PGPR. With this application, both the yield and the uptake of plant nutrients in bell peppers increased and the use of chemical fertilizers was reduced by 25% (Sharma et al., 2020).

In previous studies, it was determined that inoculation with Pseudomonas sp. increased plant height, stem diameter and dry weight of *Capsicum chinense* seedlings, and increased the yield and size of fruits at the end of cultivation (Reyes-Ramírez et al., 2014). In a similar study, it was determined that the growth, nutrient uptake and photosynthesis activity of *Capsicum chinense* plants increased after the seeds were inoculated with PGPR (*Pseudomonas tolaasii* P61, *Pseudomonas tolaasii* A46, *Bacillus pumilus* R44, and *Paenibacillus polymyxa* BSP1.1) during planting. It was stated that *Paenibacillus polymyxa* BSP1.1 was better than the others and that fertilizer and water use in *Capsicum chinense* plants could be reduced by inoculation with BSP1.1 (Castillo-Aguilar et al., 2017).

The effect of inoculation with *Bacillus licheniformis* on the growth and development of pepper and tomato was investigated under greenhouse conditions; it was determined that the plant height and leaf area increased significantly and the positive effect was more in pepper than tomato. Furthermore, the total weight of pepper harvested from plants inoculated with *Bacillus licheniformis*, which has high colonization and competitiveness and can be used as a bio-fertilizer or biocontrol agent in greenhouses, increased significantly compared to the control (Garcia et al., 2004).

Commercial products prepared with various PGPR can also be used in pepper production. For example, it was reported that the yield per plant and the yield per ha were higher in the biofertilizer (containing 109 cfu of *Bacillus siamensis* per mL) treatments (fertilized with 80% of the recommended N dose) than in the N 80% and N 100% control. Additionally, PGPRs can be used with commercial products containing arbuscular mycorrhizal fungi (Pastor-Bueis et al., 2017). In pepper production, different combinations of *Azotobacter chroococcum*, *Azospirillum brasilense,* a commercial product containing mycorrhizal fungi and chemical fertilizer applications at reduced doses were tested. It was clearly reported that the highest yields per plant in *Capsicum chinense* Jacquin were obtained with a combination of reduced chemical fertilization and inoculation with *Azotobacter chroococcum* (Constantino et al., 2008).

Broccoli

Broccoli (*Brassica oleracea* L. var. *italica* Plenck), belonging to the Brassicaceae family and mostly extended in the Mediterranean region, is an important vegetable due to being rich in vitamins and minerals, high fiber, and low-calorie properties (Decateau, 2000; El-Nemr et al., 2011). Broccoli grows well in both organic and mineral soils with proper fertilization and adequate irrigation. Soil test results should be taken into account when fertilizing broccoli, as the soil structure varies according to the region. Soil pH in the range of 6.0-6,5 is preferred, but broccoli can tolerate a pH of 5.5. Fertilizer should be placed near the plants because of its high N requirement and relatively small root zone. If enough N fertilization is not done, the yield will decrease, maturity will be delayed, and the taste will deteriorate. N is usually applied in parts, during the seedling period and when the heads begin to form. However, the N requirement is lower in organic soils. Excessive N fertilization results in rapid growth with poor quality heads with low shelf life. When excessive N fertilization is combined with high temperatures, rapid growth is promoted and some physiological disorders such as tip burn may occur (Welbaum, 2015). Broccoli is an important and valuable vegetable crop in different regions of the world whose cultivation demands high quantities of N, usually obtained from chemical sources. Therefore, different sustainable and environmentally friendly N sources are needed in broccoli cultivation. In addition, it can be clearly said that nitrogen-fixing PGPRs can be extremely beneficial for the growth and high yield of broccoli, and can be used for sustainable N fertilization.

In a field experiment, it was investigated that the combined use of nitrogen-fixing *Bacillus cereus*, P-solubilizing *Brevibacillus reuszeri* and N_2-fixing and P-solubilizing *Rhizobium rubi* with manure on plant growth, nutrient uptake, and yield of broccoli. In addition, it was reported that bacterial inoculations with manure increased yield, plant weight, head diameter, chlorophyll, and mineral content of broccoli when compared with control. At the end of the research, it was stated that inoculation with *Brevibacillus reuszeri* and *Rhizobium rubi* can partially replace costly chemical fertilizers for successful broccoli production (Yildirim et al., 2011). Similarly, inoculations with *Bacillus cereus*, *Brevibacillus reuszeri* and *Rhizobium rubi* promoted plant growth and resulted in a yield increase in organic broccoli production in field conditions. It was indicated that the great potential of these PGPR's increase the yield, growth, and nutrition of broccoli under organic growing conditions (Yildirim et al., 2010).

In many studies to date, the promoting effects of Bacillus genus bacteria on plant growth have been determined and demonstrated. New strains of these species also continue to be identified. For example, in a study, the capacity of phosphate solubilization, biological nitrogen fixation, and the production of plant growth-regulating hormones of new species belonging to the *Bacillus* spp. was identified and was determined for possibilities for use in broccoli cultivation. In this research, *Bacillus licheniformis* IB10, which has nitrogen fixation, auxin production, and phosphate dissolving properties, increased plant height by 13.7% in broccoli in field experiments. *Bacillus megaterium* CT11, which had the same properties, significantly increased the dry matter content, length, and root weight of broccoli compared to the control. The researchers stated that the newly identified these PGPRs and used in this study could be a biological alternative to chemical fertilizers in broccoli production (Acurio Vásconez et al., 2020). Similarly, it was reported that *Bacillus subtilis* strain QST 713 when used as biofertilizers with manure and chemical fertilizers has great potential to increase growth, yield, ascorbic acid content, and nutrient uptake of broccoli (Altuntas, 2018).

Cauliflower

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important vegetable species belonging to the Brassicaceae family. Although Asian countries make up 70% of the world's cauliflower production, it is produced and consumed in various parts of the world. High-yielding cauliflower cultivars bred in recent years have led to an increase in cauliflower production in general, but it can cause health hazards and environmental pollution as high rates of inorganic fertilizers are used in cauliflower production (Kaushal and Kaushal, 2013). The N requirement of cauliflower is high and as mentioned before, a similar method should be followed in fertilization since its root system is similar to broccoli. Nitrogen deficiency causes yield losses, delayed maturity, and reduce storage in cauliflower. Nitrogen fertilization in cauliflower is often applied at planting. It may be necessary to fertilize again when the plants are growing rapidly and when the requirement for nitrogen is high. In addition, excessive use of nitrogen promotes rapid growth of cauliflower and affect adversely curd quality (Welbaum, 2015; Peirce, 1987; Swaider et al., 1992). To put it briefly, although cauliflower constantly needs large amounts of water and nutrients to get high yields (Bashyal, 2011), high doses should be avoided while fertilizing with nitrogen. It is extremely important to provide balanced nutrition through different inorganic and organic food sources for high yield and quality in cauliflower (Thakur et al., 2018). In order to make cauliflower cultivation sustainable, environmentally friendly, and less dependent on inorganic fertilizers, PGPRs, which can biologically fix nitrogen, solubilize phosphorus, and produce some phytohormones, can contribute to environmentally friendly production by reducing the use of chemical fertilizers (Kaushal and Kaushal, 2013).

As the positive effects of PGPRs on environmentally friendly and sustainable agriculture continue to gain importance day by day, the production and use of commercial formulations containing different PGPRs have also started to become widespread. For instance, in a recent research, the effects of commercial formulation biofertilizer containing *Bacillus megaterium*, *Azospirillum brasilense* and *Pseudomonas fluorescens* and the combined use of decreasing nitrogen and phosphorus doses on the growth and development of cauliflower were investigated. The efficiency of biofertilizers increased with the increase in the dose of chemical fertilizers. And the highest yield and other growth parameters such as leaf length and width, stem height, plant weight were determined in 75 and 100% of inorganic fertilizers along with biofertilizer (Salim et al., 2020).

In an interesting study, it was determined that novel PGPRs isolated from cauliflower rhizosphere soil have the ability to dissolve phosphorus, fix nitrogen, and produce auxin and siderophore. The combined use of these newly isolated PGPR and N and P fertilizers significantly increased the curd diameter, depth, curd weight and yield of cauliflower (Kaushal et al., 2011). In another study conducted in the continuation of this study, it was reported that the combined application of PGPR isolates with 75% of the recommended dose of N and P increased the yield of cauliflower by about 27% compared to the control (Kaushal et al., 2013). Similarly, the bio formulations prepared with *Bacillus cereus*, *Pseudomonas rhodesiae* and *Pseudomonas rhodesiae* were found to be effective in increasing the growth parameters of cauliflower (Kalita et al., 2015). It is also known that inoculation of *Azospirillum and Azotobacter* together with nitrogen increases the curd diameter of cauliflower and promotes plant growth (Bashyal, 2011). It was also determined that the combined use of organic fertilizers, chemical fertilizers, and *Azotobacter* increased the curd size of cauliflower (Choudhury et al., 2004). In addition, the use of PGPR as a biofertilizer can be an effective approach to replace chemical or inorganic fertilizers for sustainable and environmentally friendly cauliflower cultivation (Kaushal and Kaushal, 2013).

Nutrient management in cauliflower cultivation is partially dependent on the microbial population in the rhizosphere. Therefore, the plant growth-promoting rhizobacteria SB5, SB8, SB10, and SB11 (*Bacillus* spp.) were compared to the reference strain *Bacillus pumilus* (JN559852) in cauliflower cultivation. As a matter of fact, one of the rhizospheric isolates of cauliflower, SB11 (*Bacillus* spp.) application both increased yield and showed the best plant growth promoting performance in field experiments of 75% N and P application. At the end of this research, it was stated that the combined application of SB11 isolate at 75% N + P could reduce the use of chemical fertilizers in cauliflower production without compromising yield and soil fertility (Bhardwaj et al., 2018).

PGPRs can be used not only to improve yield or curd characteristics but also to produce quality seedlings in cauliflower. PGPR strains such as *Bacillus megaterium* TV-3D, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, *Bacillus subtilis* TV-17C, *Bacillus megaterium* TV-87A, *Bacillus megaterium* KBA-10 can be used to increase seedling growth and quality of cauliflower in organic agriculture. Considering the high costs in seedling production, these PGPR's can reduce the input cost of crop production without harming the plant and the environment (Ekinci et al., 2014).

Cabbage

Cabbage (*Brassicae oleraceae* var. *capitata* L.) is also an important species of the Brassicaceae family, like broccoli and cauliflower, and is perhaps the most produced species of this family. Just like broccoli and cauliflower, cabbage has a high nitrogen requirement and needs sufficient nitrogen to maximize yields. Yield losses, delayed maturity, and quality losses of leaves are the main indicators of nitrogen deficiency in cabbage. On the other hand, excessive nitrogen applications can reduce head density and storage life of cabbage, and also can promote secondary growth and split heads (Welbaum, 2015). It is a well-known fact that excessive amounts of chemical fertilizers, especially nitrogen, are harmful to the environment in today's agricultural systems. Not only cabbage producers but also most of vegetable producers are trying to produce that does not harm both the environment and human health due to increasing consumer pressure. In recent years, it can be easily shown nitrogen-fixing PGPRs among environmentally friendly nitrogen sources. And these nitrogen sources can be used by vegetable producers not only in cabbage but also in many vegetable crops both for environmentally and human-friendly production and to reduce the use of chemical fertilizers.

As with many types of vegetables, seedlings are widely used in cabbage production, and seedling quality is at least as important for successful production as good fertilization. N_2-fixing and P-solubilising PGPR, *Paenibacillus polymyxa* RC14, improved the growth characteristics such as fresh and dry shoot and root weight, stem diameter, seedling height, and leaf area of cabbage seedlings. At the end of this research, it was stated that *Paenibacillus polymyxa* RC14 could be an alternative to chemical N and P fertilizer sources for seedling production and seedling quality in cabbage (Yildirim et al., 2015). Similarly, the possibilities of using *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, and *Bacillus subtilis* TV-17C in cabbage seedling production were investigated. These PGPRs, which P-solubilizing and phytohormone-producing as well as N_2-fixing properties, promoted root and shoot growth. *Bacillus megaterium* TV-91C showed the best performance in increasing nutrient content and growth parameters such as fresh and dry shoot and root weight. Also, the highest leaf area, gibberellic acid, salicylic acid, and indole acetic acid contents of seedlings were obtained from *Pantoea agglomerans* RK-92. Inoculation of seeds of cabbage with these PGPRs may help to reduce the total amount of chemical fertilizers necessary to obtain the highest seedling performance in sustainable and environmentally friendly agriculture (Turan et al., 2014).

PGPRs that can fix nitrogen may also have the capacity to solubilize phosphorus and produce phytohormones. This can add value to them in promoting plant growth by overcoming some stress factors such as drought and salt stress. For example, it was investigated that the effect of P-solubilizing and phytohormone-producing and N_2-fixing *Bacillus subtilis* TV-13B, Bac*illus pumilus* TV-67C, and a PGPR mixture (*Bacillus megaterium* TV-6D + *Pantoea agglomerans* RK-92 + *Brevibacillus choshiensis* TV-3D) on the physiological and biochemical development of cabbage seedlings under drought stress. It has been reported that *Bacillus pumilus* TV-67C is beneficial in reducing the effects of drought stress by accelerating superoxide dismutase, catalase and peroxidase levels, hormone and amino acid accumulation in cabbage seedlings (Samancıoglu et al., 2016). Also, seed coating and seedling dipping applications with nitrogen-fixing plant growth-promoting rhizobacteria provide means of improving amino acid contents in cabbage and cause an increase in values of histidine, glycine, thionin, arginine, and alanine (Dursun et al., 2017).

Moreover, it has been determined that *Paenibacillus polymyxa* RC14, which has nitrogen fixing and phosphate solubilization capacity, has positive effects on the growth, yield, and nutrient content of cabbage with seed and seedling inoculation. In this study, the application of mineral fertilizers together with *Paenibacillus polymyxa* RC14 provided 25% savings from the use of chemical fertilizers in cabbage production, while 33% more yield was obtained compared to the full dose chemical fertilizer application. In consideration of the environmental pollution, excessive use of chemical fertilizers, and the high costs of chemical fertilizers production, *Paenibacillus polymyxa* RC14 can be used combined with chemical fertilizers to achieve sustainable cabbage production (Yildirim et al., 2016).

Lettuce

Lettuce (*Lactuca sativa* L.), one of the most important leafy vegetables in the world, is a member of the Asteraceae family. Since lettuce completes 80% of its development 3-4 weeks before harvest, it is very critical to the fulfillment of the nutritional requirements such as nitrogen during this period for a successful production (Decateau, 2000). The most important factor affecting the yield and quality of lettuce is fertilization (Sarhan, 2012). Since lettuce responds to nitrogen fertilization with high efficiency and quality, this causes high rates of nitrogen fertilizer applications to be common (de Barros Sylvestre et al., 2019). However excessive use of chemical fertilizers is known to cause many negative results on both environmental and human health. Therefore, biological nitrogen sources such as N_2-fixing plant growth-promoting rhizobacteria can be used together with chemical fertilizers to at least reduce the use of chemical fertilizers.

Indeed, in a study conducted for this purpose, it was determined that mixture-3 (Pseu*domonas putida* RK-142 + *Pseudomonas fluorescence* TV-11D + *Bacillus megaterium* TV-91C) prepared with some N_2-fixing plant growth-promoting rhizobacteria have a great potential to reduce nitrogen use (25%) for environmentally friendly lettuce production. It was also stated that all of the plant growth-promoting rhizobacteria (*Agrobacterium rubi* RK-34, *Pantoea agglomerans* RK-79 and RK-92, *Pseudomonas putida* RK-142 and TV-42A, *Bacillus megaterium* TV-6D, TV-60D and TV-91C, *Pseudomonas flourescens* TV-11D and *Paenibacillus polymyxa* TV-12E) used in this study has the ability to fix nitrogen and the highest growth and yield values were obtained from mixture-3. These PGPR mixtures also reduced the accumulation of heavy metals such as Cd, Ni, and Pb in lettuce and increased the intake of other plant nutrients of lettuce (Kaymak et al., 2020). In a similar study using a PGPR mixture, a commercial biostimulant containing plant growth-promoting bacteria (*Bacillus amyloliquefaciens, B. brevis, B. circulans, B. coagulans, B. firmus, B. halodenitrificans, B. laterosporus, B. licheniformis, B. megaterium, B. mycoides, B. pasteurii, B. subtilis, and Paenibacillus polymyxa*) was used on lettuce production. This commercial biostimulant provided the highest biomass accumulation of the lettuce seedlings and positively affected plant growth as well as the yield and nitrate content of lettuce plants (Vetrano et al., 2020). Also, the use of the same bacterial biostimulant in the hydroponic cultivation of lettuce was determined to be a sustainable means to increase the crop yield of lettuce and alleviate salt stress (Moncada et al., 2020). The combined use of *Bacillus subtilis*, *Azotobacter* sp., *Penicillium*, and *Fusarium spoxalicum* as biofertilizers positively affected the yield of lettuce and the yield increase was recorded 17.7% more than the control (Tošić et al., 2016).

N_2-fixing plant growth-promoting rhizobacteria can also promote plant growth and yield under some abiotic stress conditions such as different water-deficit levels in lettuce. For example, inoculations with N_2-fixing PGPR *Bacillus megaterium TV-6D and Bacillus subtilis* TV-12H significantly increased growth, nutrient content, leaf relative water content, stomatal conductance, and yield of lettuce plants grown under lower irrigation levels. These PGPRs can increase the growth and yield of lettuce under lower irrigation conditions, and also can overcome the adverse effect of water deficit for lettuce production (Sahin et al., 2015).

Some researchers have reported that a more efficient and profitable nutrient management strategy for lettuce can be developed by verifying the beneficial effect of the combined application of humic fertilizers and PGPR on lettuce. Indeed, the synergistic effect of *Bacillus subtilis* No.2 and humic fertilizer has been proven with the increase in N and chlorophyll content and the decrease in nitrate content in lettuce leaves. Also, it can be said that according to the results of this research, B subtilis No 2 could decrease the application rate of humic fertilizer for cost-effective lettuce production (Pishchik et al., 2016).

Spinach, Mint and Rocket

Spinach (*Spinacia oleracea* L.), belongs to the Chenopodiaceae family and a valuable member of leafy vegetables with an important nutrient value for humans such as minerals and vitamins. Spinach is quite resistant to low temperatures; it can be easily grown in winter as long as the temperatures do not drop to -12ºC. However, at high temperatures, leaf quality, and foliage colour decrease. For this reason, it is recommended to be produced in early spring and early summer, but it can be produced even in winter in production areas where winters are not severe (Welbaum, 2015; Rubatzky and Yamaguchi, 1997). Nitrogen fertilization is often preferred in winter cultivation, as it often increases yield and low soil temperatures cause little nitrification. In addition, spinach, which is very modest in fertilization, should be well fertilized to rapid growth and an increase in leafiness. However, sometimes high amounts of N-P-K may be needed in sandy soils (Welbaum, 2015). This modest fertilization requirement of spinach supports the idea that nitrogen-fixing PGPRs can be used with ease.

The effect of different N_2-fixing, phytohormone-producing, and P-solubilizing bacterial species on spinach growth and yield is extremely interesting. These PGPRs (*Bacillus cereus* RC18, *Bacillus licheniformis* RC08, *Bacillus megaterium* RC07, *Bacillus subtilis* RC11, *Bacillus* OSU-142, *Bacillus* M-13, *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14) increased spinach shoot fresh weight by 2.2%–53.4% over control. According to these research results, especially N_2-fixing bacterial strains RC05, RC06, RC14 and OSU-142 have greater potential than the others to be formulated and used as biofertilizers (Cakmakci et al., 2007). Similarly, *Pseudomonas putida* NWU12, *Pseudomonas fluorescence* NWU65, *Vibrio fluvialis* NWU37 and *Ewingella americana* NWU59, which have the potential to be used as biofertilizer by improving yield and yield components, increased plant height between 17.14% and 21.43% in spinach compared to control (Hou and Oluranti, 2013).

The number of studies on the effects of N_2-fixing PGPRs in other leafy vegetables such as rocket, and mint is more limited than in spinach and lettuce. Examples of studies conducted in these species, albeit limited ones, are as follows.

Mint, whose fresh leaves are used as a leafy vegetable in different countries such as Turkey, belongs to the Labiatae family and the genus *Mentha* (Kaymak et al., 2013). Mint is also the most important vegetable produced worldwide and used as an essential oil plant. Essential oils of the mint are used in many areas such as candy, chewing gum, toothpaste, pharmaceutical industry (del Rosario Cappellari et al., 2015). Although N_2-fixing ability was not specified, *Bacillus subtilis* GB03, *Pseudomonas fluorescens* WCS417r, *Pseudomonas putida* SJ04 increased shoot and root biomass, leaf area, and the number of nodes in mint (del Rosario Cappellari et al., 2015). In fact, this increase in vegetative parameters in mint can be considered as proof of the N_2-fixing ability of the PGPRs used in the study. In an interesting study comparing the competitiveness of *Pseudomonas putida* biotype B C3/101 and *Paenibacillus polymyxa* RC105, with urea in mint production, which have the nitrogen-fixing ability, the PGPRs provided a significant increase in total fresh and dry mint yield compared to the control. On the other hand, this increase in both fresh and dry yield according to the control was less than the urea application. The results obtained from this study are promising for the use of PGPRs as a bio-fertilizer in environmentally friendly mint production (Kaymak et al., 2013).

In the literature review, only one research could be reached on rocket (*Eruca vesicaria* subsp. *sativa*). In this study, *Burkholderia gladii* BA-7, *Pseudomonas putidae* BA-8, *Bacillus subtilis* OSU-142 and MFD-5, *Bacillus megatorium* M3, *Agrobacterium rubi* A-1, A-16, and A-18 which have nitrogen-fixing ability were used to increase the growth and yield of the rocket. Compared to other PGPR treatments, the highest yield, average leaf weight, length, dry matter content and area, and root weight were obtained from *Burkholderia gladii* BA-7 applications.

The results of this research showed that *Burkholderia gladii* BA-7, as well as *Pseudomonas putidae* BA-8, *Bacillus subtilis* OSU-142, have great potential to increase the growth and yield of rocket (Dursun et al., 2008).

Conclusions

In vegetable production, chemical fertilizers, especially nitrogen, is a necessity for high yield, but the damage they cause to the environment, together with their high prices, has made chemical fertilizers questionable. Excessive use of chemical fertilizers pollutes the soil and groundwater, and chemicals such as nitrogen can accumulate in vegetables in the form of harmful compounds such as nitrates. In order to overcome these problems, it is necessary to reduce chemical inputs and to spread environmentally friendly practices. In crop production, the cost and use of chemical fertilizers can be reduced without harming the plant and the environment by using N_2-fixing PGPRs. N_2-fixing PGPRs in the nutrition of plants, especially supplying the nitrogen requirement, have been tested with many researches and their contribution to the increase in yield in vegetable production has been revealed. The use of N_2-fixing PGPRs as an environmentally friendly nitrogen source has provided significant improvements in plant growth and yield. Thus, the expectations in the production of vegetables for the future, which are environmentally friendly and do not pose a health problem in human nutrition, may be to replace and reduce chemical fertilizers and to ensure the safety of the ecosystem with beneficial practices. However, the increasing world population and increasing food demand are the main problems facing environmentally friendly practices. However, there are many reasons why N_2-fixing PGPRs should be used as a nitrogen source in environmentally friendly vegetable production and these applications should be placed in the focus of production. Therefore, the future of using N_2-fixing PGPRs as an environmentally friendly nitrogen source and presenting these N_2-fixing PGPRs to farmers as biofertilizers in commercial preparations looks very bright.

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