ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 13(6) 2014, 71-85*

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON GROWTH, NUTRIENT, ORGANIC ACID, AMINO ACID AND HORMONE CONTENT OF CAULIFLOWER (*Brassica oleracea* L. var. *botrytis*) TRANSPLANTS

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Abstract. This study was conducted to determine the effect of different plant growth promoting rhizobacteria (PGPR) strains on growth and quality of cauliflower transplants under greenhouse conditions. The strains of *Bacillus megaterium* TV-3D, *B. megaterium* TV-91C, *Pantoea agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10 were used in this study. The results of this study showed that different bacterial inoculations increased plant growth parameters such as fresh shoot weight, dry shoot weight, root diameter, root length, fresh root weight, dry root weight, plant height, stem diameter, leaf area and chlorophyll contents of cauliflower transplant respectively. Except for abscisic acid (ABA), the values of gibberellic acid (GA), salicylic acid (SA), indole acetic acid (IAA) was increased by ratio of 23.64, 89.54 and 25.63%, respectively in compared to the control by application of *B. megaterium* KBA-10 and *P. agglomerans* RK-92. The amount of organic acids with *B. subtilis* TV-17C PGPR applications have increased at a ranging ratio from 9.63 to 186.02%. Also, PGPR inoculations increased the macro and micro nutrient content of cauliflower transplants. As a result, the use of bacteria treatments may provide a means of improving transplant growth and quality in cauliflower.

Key words: brassica transplants PGRR, greenhouse conditions, phytohormone production, plant growth parameters

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INTRODUCTION

The vegetables are very important foods for human nutrition and also their production, marketing and processing are significant contributors to income for growers. Different management, techniques and applications are used to increase vegetable production. Production of vegetable crops with transplants has recently been preferred by growers because reduce the time needed to crop, and also to provide low seed cost of establishing a vegetable planting, more efficient use of fertilizer and irrigation water during early growth stages. The use of quality transplant is important to obtain uniform plant growth, increase of yield and quality of vegetable crops, and to protect efficiently against environmental stress, disease insect, pathogens, to establish near-perfect stands, uniform physiological plant age for field growth to produce early season markets. Greenhouse grown transplants is an increasingly popular way to establish vegetable crops which have several advantages compared to field-grown transplants. They are more uniform, stable for environmental stress, better stands and earlier harvests than field-grown plants. Commercial vegetable transplant production in Turkey getting increase significantly, and recently reached two and a half billion. A large number of brassica transplants, including cauliflower, are produced commercially [Turan et al. 2014].

For the main goal of, intensive farming practices are getting to high yield, and high yield require extensive use of chemical fertilizers, which are costly and create environmental problems. Therefore, there has recently been increased interest in the environmentally friendly, sustainable and organic agricultural practices in the world [Esitken et al. 2005, 2006]. There are limited sources used in sustainable and organic agricultural practices. So, growers and scientists have been trying to increase of limited fertilizer sources. Application of Plant Growth Promoting Rhizobacteria (PGPR) is very important source for environment friendly agriculture. Importance of PGPR has increased worldwide and are currently used as biofertilizer on plant production. PGPR can facilitate plant growth indirectly by reducing plant pathogens, or directly by facilitating the uptake of nutrients from the environment, by influencing phytohormone production (e.g. auxin, cytokinin, or giberallin), and/or by enzymatic lowering of plant ethylene levels. But, the mechanisms of plant growth promoting by rhizobacteria is still completely unknown [Lucy et al. 2004, Bashan and Bashan 2005, Cakmakci et al. 2006, Ibiene et al. 2012]. It was reported that inoculation of PGPR has significant increase in yield and growth of crops by different researchers [Garcia et al. 2003, Kokalis-Burelle et al. 2003, Esitken et al. 2006, Karlidağ et al. 2007, Dursun et al. 2008, Yildirim et al. 2008, Cakmakci 2009, Ekinci et al. 2009, Dursun et al. 2010, Misra et al. 2010, Yildirim et al. 2011, Ibiene et al. 2012].

These bacteria belong to different genera such as *Azotobacterium*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Clostridium*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Serratia* etc. [Bashan and Bashan 2005, Esitken et al. 2006, Adesemoye et al. 2008, Cakmakci 2009, Nezarat and Gholami 2009].

It was determined that the bacterial applications could be able to increase plant growth, germination rate of seed, improve transplant emergence, response to stress conditions and protect from disease. Bacteria strains of *Azospirillum*, *Pseudomonas* and

Azotobacter have significant impact on seed germination and transplant growth [Shaukat et al. 2006a, b, Nezarat and Gholami 2009]. In previous study with *Bacillus subtilis* and *Bacillus amyloliqufaciens* have been increased of plant growth and yield on different plants [Turner and Backman 1991, Vavrina 1999a, Kokalis-Burelle et al. 2003, Adesemoye et al. 2008].

Although there are many studies that made the effect of bacteria on plant growth and yield of some vegetables, there is no more research on vegetable transplant production. Whereas, it was reported that PGPR could be used to obtain standard sized transplant in less time and a more vigorous transplant for transplant production [Vavrina 1999a, b, Kokalis-Burelle et al. 2003]. Besides, it is stated that PGPR can applied at the sowing and transplanting stage wherefore used to control harmful microorganisms and can be increased growth in stress conditions as well as healthy plants also [Gül et al. 2008]. Turan et al [2014] reported that seed inoculation of the PGPR strains improved growth and quality of the cabbage transplants.

However, to our best knowledge there is no investigation on effect of PGPR on transplant growth and quality of cauliflower. The objective of this research was to determine effects of the bacterial strains of *B. megaterium* TV-3D, *B. megaterium* TV-91C, *Pantoea agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10 on transplant growth and quality of cauliflower in greenhouse conditions.

MATERIALS AND METHODS

Growth conditions. This study was conducted under greenhouse conditions at the Atatürk University in Turkey in 2013. Cauliflower (*Brassica oleracea* L. var. *botrytis*) seedlings were maintained under approximate day/night temperature of 26/13°C and 70% relative humidity during the experiment. Cauliflower seeds were sown into 45 celled-trays filled with peat. There was no nutrition application during the experiments.

Bacterial strains. All of the bacterial strains were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University. These bacterial strains had been isolated from the root of vegetables and foliage of some fruits growing in the eastern Anatolia region of Turkey [Kotan et al. 2005, Erman et al. 2010]. The identity of all bacterial strains used in this study was confirmed according to fatty acid methyl esters (FAME) analysis by using Sherlock Microbial Identification System (Microbial ID, Newark, DE, USA) [Miller 1982]. The bacterial cultures were grown on nutrient agar (NA) for routine use, and maintained in Luria Broth (LB) with 15% glycerol at -80ºC for long-term storage at the Department of Plant Protection, Faculty of Agriculture in Atatürk University. In previous studies, all strains used in this study were determined that they showed capacity to grow in N-free conditions and to solubilise phosphate (tab. 1) [Kotan et al. 2014, Turan et al. 2014].

Table 1. Nitrogen fixation and phosphate-solubilising activity of the tested bacterial strains

Bacterial strains	Isolated from	Nitrogen fixation	Phosphate solubilization
B. megaterium TV-3D	rice roots	s+	+
B. megaterium TV-91C	wheat roots	+	w+
P. agglomerans RK- 92	pear roots	+	s+
B. subtilis TV-17C	raspberry roots	s+	w+
B. megaterium TV-87A	sugar beet roots	+	-
B. megaterium KBA-10	apricot roots	s+	+

- – negative reaction, + – positive reaction, s+ – strong positive reaction, w+ – weak positive reaction

Identification of the bacterial strains by microbial identification system (MIS). Identification of the tested bacterial strains was confirmed by using MIS systems. Preparation and analysis of FAMEs from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMEs were separated by gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m × 0.2 mm, with cross-linked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package.

Seed surface disinfection with sodium hypochlorite. The seeds were surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the seed surface. Seed disinfection was performed by dipping the seeds for 3 min in 3% sodium hypochlorite and washing four times in sterilized and distilled water (sd. H_2O). Seeds were left to dry on sterile filter paper sheets overnight in the laminar flow hood for using further studies.

Application of bacterial strains. All bacterial isolates were incubated in Tryptic Soy Agar (TSA, Oxoid) at 27°C for 24 h. After incubation period, a single colony was transferred to 500 ml flasks containing Tryptic Soy Broth (TSB, Oxoid), and grown aerobically in the flasks on a rotating shaker (150 rpm) for 48 h at 27°C (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water (sdH_2O) to a final concentration of 1×10^8 cfu ml^{-1} with a turbidimeter. The suspension of bacteria were used to treat cauliflower plants. The suspension of bacteria (10^8 cfu ml^{-1}) was applied twice as drench into root zone after germination of seed at one week intervals and the applications of control plants were made with water.

Growth parameters. Approximately forty days after sowing, twenty plants from each repeats were harvested, and parameters of plant growth (shoot fresh weight and root fresh weight, shoot dry weight and root dry weight, root diameter, root length, stem diameter, plant length) were examined. The plant samples for dry weight were dried at 65°C for 48 hours. The area of the transplant leaf was measured with leaf area meter (LI-3100, LICOR). The leaf greenness was determined with a portable chlorophyll

meter (SPAD-502; Konica Minolta Sensing, Inc., Japan). For this, measurements were taken at four locations on each leaf, two on each side of the midrib on all fully expanded leaves [Khan et al. 2003].

Mineral analysis. Leaf tissue samples were taken during harvest, then oven dried at 68°C for 48 h and ground, and passed a 1 mm sieve size. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter,Germany) were used to determine total N [Bremner 1996]. Macro- (P, K, Ca, Mg, and sodium (Na)) and microelements (Fe, Mn, Cu and Zn) were determined after wet digestion of dried and ground subsamples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three steps (first step: 145 C, 75% radio-frequecy power (RF), 5 min; second step: 180 C, 90% RF, 10 min and third step: 100 C, 40% RF, 10 min)) in microwave digestion (Bergof Speedwave Microwave Digestion Equipment MWS-2; Berghof Products and Instruments, Eningen, Germany) [Mertens 2005a]. Tissue P, K, Ca, Mg, Na, Fe, Mn, Cu and Zn were determined using an inductively coupled plasma spectrophotometer (Optima 2100 DV, ICP/OES; Perkin-Elmer, Shelton, CT) [Mertens 2005b].

Hormone analysis. Extraction and purification processes were executed as described by Kuraishi et al. [1991] and Battal and Tileklioglu [2001]. 80% methanole adjusted to -40ºC was added in one gram fresh samples [Davies 1995]. After solution was homogenised for 10 min with ultraturraks, it was incubated for 24 h in dark condition. The samples was filtered through filter paper and then supernatants were filtered second at 0.45 µm pore filter [Cutting 1991]. Supernatants were dried in 35ºC by evaporator pumps. Dried supernatants were desolved using 0.1 M KH_2PO_4 (pH 8.0). Extracts were centrifuged at 5000 rpm for 1 hour at 4ºC for separating fatty acids [Palni et al. 1983]. Polvinilpolipirilidon (PVPP), 1 g, was added to supernatant for separating phenolic and colour matters [Chen 1991, Hernandez-Miana 1991, Qamaruddin 1996]. Supernatant with PVPP was filtered (Whatman No. 1) to separate PVPP [Cheikh and Jones 1994]. For further specific separation, Sep-Pak C-18 (Waters) cartridge was used. Hormones adsorbed by cartridge transferred to vials using 80% methanole. The hormone was analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC) and absorbance of 265 nm in UV detector. Flow speed was set to 1.2 ml/min and at column temperature of 25ºC. Giberallic acid, salisilic acid, indol acetic acid (IAA), absisic acid (ABA) were determined by using 13% acetonitrile (pH 4.98) as mobile phase.

Amino acid analysis. 0.1 N HCl added in one gram fresh sample, homogenized with ultraturraks, and incubated in 4ºC at 12 hours. Samples were vortexed. After samples were centrifuged at 1200 rpm for 50 min, supernatants were filtered through 0.22 µm (Millex Millipore). Then supernatants were transferred to vial and vials for aminoacid acid analysis in HPLC as described by Aristoy and Toldra [1991], Antoine et al. [1999] and Henderson et al. [1999]. Briefly, Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 µm columns (Agilent 1200 HPLC) were used and reading was recorded at 254 nm, and the amino acids were identified by comparison with standards. O-phthaldialdehyde (OPA), fluorenylmethyl-chloroformate (FMOC) and 0.4 N Borate. The following were used as the mobile phase in the chromatography system: mobile phase. A: 40 mM NaH_2PO_4 (pH 7.8) and mobile phase B: Acetonitrile/Methanol/Water (45/45/10, v/v/v) solutions. The flow rate of the mobile phase moved through the system at 2 ml min^{-1} and the column temperature was 40ºC. Aspartate, glutamate, asparagine, serine, gluta-

mine, histidine, glycine, theonine, arginine, alanine, tyrocine, cystine, valin, methionine, tryptophan, phenylalanine, isoluecine, leucine, lysine, hydroxyproline, sarcosine and proline quantity of samples determined as nmol μl^{-1} after 26 minutes derivation process in HPLC.

Organic acid analysis. 10 ml deionized water was added to fresh sample (1 g), solution was then homogenized with ultraturraks. After centrifuging at 1 200 rpm for 50 minutes, supernatants were filtered through 0.22 µm pore (Millex Millipore). In vials, supernatants were subjected to HPLC using Zorbax Eclipse-AAA 4.6 × 250 mm, 5 µm column (Agilent 1200 HPLC) and absorbance of 220 nm in UV detector. Flow speed was 1 ml min^{-1}, column temperature was 25ºC. Oxalic, propionic, tartaric, bütyric, malonic, malic, lactic, citric, maleic, fumaric and succinic acid were determined by using 25 mM potassium phosphate (pH 2.5) as mobile phase.

Statistical analysis. Research consisted of a completely randomized design with 4 replicates per treatment and 45 plants per replicates. All data were subjected to analysis of variance using SPSS 18 statistical program. Means were separated by Duncan's multiple range tests (DMRT) [SPSS 2010].

RESULTS

Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* var. botrytis) transplants are shown in Table 2, 3, 4, 5 and 6. The effects of PGPR applications on hormones of cauliflower transplant were statistically significant. The highest content of gibberellic acid (168.68 ng μl^{-1}) was obtained from *B. megaterium* KBA-10 bacterial application; salicylic acid and indole-3-acetic acid (67.06 ng μl^{-1} and 7.50 ng μl^{-1} respectively) was obtained from *P. agglomerans* RK-92 bacterial application, and abscisic acid (0.24 ng μl^{-1}) was obtained from *B. subtilis* TV-17C bacterial application. Generally, with the exception of ABA, content was significantly lower than in the control treatments only after the application of the TV-91C isolate. The remaining differences were not statistically significant. The levels of gibberellic acid, salicylic acid, indole-3--acetic acid was increased by ratio of 23.64, 89.54 and 25.63%, respectively in compared to the control (tab. 2).

Table 2. Effects of PGPR applications on hormones content in cauliflower transplant after 40 days (ng μl^{-1})

	Control	*B. megaterium* TV-3D	*B.megaterium* TV-91C	*P. agglomerans* RK-92	*B. subtilis* TV-17C	*B. megaterium* TV-87A	*B.megaterium* KBA-10
GA	136.43 b	163.65 a	155.92 ab	165.25 a	176.26 a	168.68 a	177.99 a
SA	35.38 d	41.15 c	43.85 c	67.06 a	41.55 c	44.93 c	54.80 b
ABA	0.21 a	0.23 a	0.16 b	0.23 a	0.24 a	0.22 a	0.23 a
IAA	5.97 bc	5.90 c	6.92 ab	7.50 a	5.80 c	4.74 d	6.70 abc

Means within rows not followed by the same letter differ significantly by DMRT at 5%

The concentrations of organic acids in cauliflower transplant in response to PGPR applications are shown in Table 3. Organic acids in cauliflower transplant were significantly affected with PGPR applications. The highest oxalic, propionic, malonic, lactic and maleic acid was obtained in *P. agglomerans* RK-92 bacterial application. On the other hand, the highest tartaric, butyric and citric acid was obtained in *B. subtilis* TV-17C bacterial application. While the highest malic and succinic acid was obtained in *B. megaterium* KBA-10 and bacterial application, the highest fumaric acid was obtained from *B. megaterium* TV-87A bacterial application. The amount of organic acids with PGPR applications has increased at a ranging ratio from 9.63 to 186.02% compared to control (tab. 3).

Table 3. Effects of PGPR applications on organic acids content in cauliflower transplant after 40 days (ng μl^{-1})

	Control	*B. megaterium* TV-3D	*B. megaterium* TV-91C	*P. agglomerans* RK-92	*B. subtilis* TV-17C	*B. megaterium* TV-87A	*B. megaterium* KBA-10
Oxalic acid	6.31c	6.37c	7.14ab	7.73a	6.79bc	6.39c	6.90bc
Propionic acid	3.82bc	3.54c	4.22ab	4.63a	4.23ab	3.81bc	3.84bc
Tartaric acid	1.86e	2.24d	3.22b	2.29d	3.46a	2.45d	2.77c
Butyric acid	2.81c	2.86c	3.15b	2.82c	3.43a	3.13b	2.83c
Malonic acid	5.87d	5.64d	6.25c	7.26a	6.70b	6.36bc	5.66d
Malic acid	5.64d	6.32b	5.28d	6.33b	6.03bc	5.78c	7.13a
Lactic acid	16.72ab	14.82b	18.16a	18.33a	16.88ab	16.84ab	17.38a
Citric acid	2.25de	2.74bc	2.23e	2.68bcd	3.27a	2.60cde	3.05ab
Maleic acid	0.15d	0.21bc	0.16d	0.39a	0.14d	0.18cd	0.23b
Fumaric acid	1.75c	1.99bc	1.82c	2.35a	2.14ab	2.42a	2.15ab
Succinic acid	15.19bc	13.62c	14.67bc	19.38a	16.78b	20.66a	20.83a

Means within rows not followed by the same letter differ significantly by DMRT at 5%

Amino acids content of cauliflower transplants was affected with PGPR treatments. Aspartate, serine, glutamaine, isoleucine, and leucine contents were lower than in the control treatment in all cases after the bacteria had been applied. The increase in arginine content after the application of *B. megaterium* was not significant relative to the control.

Particularly, application of *B. megaterium* TV-3D in terms of serine, glycine, arginine, tyrocine, valin, lysine and sarcosine, application of *B. megaterium* TV-91C in terms of histidine, alanine, cystine and phenylalanine, application of *B. subtilis* TV-17C in terms of theonine and hydoxyproline, application of *B. megaterium* TV-87A in terms of methionine and tryptophan, application *B. megaterium* KBA-10 in term of proline was higher than other applications (tab. 4).

Table 4. Effects of PGPR applications on amino acids content in cauliflower transplant after 40 days (nmol μl^{-1})

	Control	*B. megaterium* TV-3D	*B. megaterium* TV-91C	*P. agglomerans* RK-92	*B. subtilis* TV-17C	*B. megaterium* TV-87A	*B. megaterium* KBA-10
Aspartate	4.41a	4.26b	4.16bc	3.86d	4.29b	4.08c	4.18bc
Glutamate	1.74	1.75	1.68	1.72	1.81	1.68	1.74
Asparagine	16.23a	14.33d	15.31bc	14.29d	15.57b	14.78cd	15.58b
Serine	6.40 a	6.35a	6.28ab	6.11c	6.18bc	6.32a	6.07c
Glutamine	5.17a	5.07b	5.04bc	4.87d	5.08ab	4.95cd	5.13ab
Histidine	2.65ab	2.59bc	2.72a	2.58bc	2.51c	2.57bc	2.72a
Glycine	3.18ab	3.26a	2.92c	2.73d	3.10b	3.21ab	2.92c
Threonine	3.86a	3.83ab	3.66c	3.52d	3.88a	3.80bc	3.71bc
Arginine	9.56a	9.61a	8.96b	7.70d	8.44c	9.36ab	9.01b
Alanine	8.38b	7.65d	9.01a	5.54e	8.33b	7.70d	8.13c
Tyrocine	1.09a	1.12a	0.96b	0.95b	0.92bc	0.89c	0.93bc
Cystine	1.35b	1.24c	1.42a	1.30bc	1.13d	1.00e	1.07d
Valin	0.80bc	0.95a	0.81b	0.76c	0.68d	0.91a	0.69d
Methionine	1.67a	1.57b	1.42cd	1.69a	1.40d	1.74a	1.49c
Tryptophan	2.05b	1.93c	1.76d	1.59e	1.78d	2.23a	2.06b
Phenylalanine	1.78b	1.80b	1.94a	1.89a	1.67c	1.92a	1.70c
Isoleucine	2.03a	1.89b	1.92b	1.80c	1.81c	1.65d	1.92b
Leucine	2.54 a	2.27c	2.49ab	2.40bc	2.09de	2.13d	1.96e
Lysine	3.29a	3.40a	3.06bc	2.91d	3.00cd	3.11bc	3.15b
Hydoxyprolin	1.37ab	1.37ab	1.36ab	1.28bc	1.41a	1.31ab	1.19c
Sarcosine	6.22b	7.19a	6.04d	6.16bc	5.99e	6.00de	6.08cd
Proline	0.094a	0.089b	0.080d	0.084c	0.090b	0.089b	0.095a

Means within rows not followed by the same letter differ significantly by DMRT at 5%

All growth parameters investigated with the exception of dry shoot weight, plant length, stem diameter, SPAD chlorophyll reading value and leaf area were significantly affected by PGPR treatments. Highest fresh shoot (33.67 g) and dry root weight (0.64 g) of cabbage transplants were obtained from application of *B. megaterium* KBA-10 and root diameter (3.49 mm), root length (22.32 cm) and fresh root weight (9.34 g) were obtained from application of *B. megaterium* TV-91C. Although, parameters of dry shoot weight, plant length, stem diameter, SPAD chlorophyll reading was non-significant, PGPR applications have increased in value of this parameters compared to control (tab. 5).

Table 5. Effects of PGPR applications on growth parameters in cauliflower transplant after 40 days

	Control	*B. megaterium* TV-3D	*B. megaterium* TV-91C	*P agglomerans* RK-92	*B. subtilis* TV-17C	*B. megaterium* TV-87A	*B. megaterium* KBA-10
Fresh shoot w. (g)	25.53c	28.20bc	30.42b	28.49bc	27.04bc	28.83bc	33.67a
Dry shoot w. (g)	2.49	2.90	3.42	3.27	2.96	3.25	3.62
Root diam. (mm)	3.01b	3.07b	3.49a	3.27ab	3.17b	3.08b	3.19b
Root lenght (cm)	18.82e	19.65de	22.32a	21.67ab	20.87bc	20.54cd	19.95cd
Fresh root w. (g)	6.30b	6.27b	9.34a	7.51b	6.62b	7.14 b	7.94ab
Dry root w. (g)	0.41c	0.44bc	0.49b	0.48b	0.41c	0.46 bc	0.64a
Plant length (cm)	14.02	14.83	13.70	14.20	14.60	14.63	15.32
Stem diam. (mm)	3.33	3.75	3.71	3.55	3.73	3.57	3.77
Chl. reading value	38.63	43.21	42.10	42.13	42.21	42.68	42.40
Leaf area	21.57	27.16	24.19	25.05	25.27	23.25	23.72

Means within rows not followed by the same letter differ significantly by DMRT at 5%

Table 6. Effects of PGPR applications on mineral content in cauliflower transplant after 40 days (mg kg^{-1})

	Control	*B. megaterium* TV-3D	*B. megaterium* TV-91C	*P. agglomerans* RK-92	*B.subtilis* TV-17C	*B. megaterium* TV-87A	*B. megaterium* KBA-10
N (%)	0.70d	0.76b	0.83a	0.72cd	0.75bc	0.86a	0.83a
Na	131.33ab	122.33cd	118.67d	137.33a	127.00bc	133.33a	126.33bc
K	4417.67e	4537.67d	4536.67d	4763.00b	4654.33c	4948.33a	4831.00b
Ca	3740.33d	3856.67bc	3934.00b	3937.67b	4057.67a	3904.00bc	3822.33cd
Mg	151.00d	156.33cd	167.67b	178.33a	164.33bc	180.00a	164.67bc
P	621.00d	652.33bc	673.67a	635.33cd	653.67b	639.33bc	638.67bc
Fe	26.38e	31.12bc	29.33cd	28.20de	31.51bc	34.74a	32.21b
Cu	2.91d	3.29ab	2.31e	3.34a	3.15bc	2.95d	3.12c
Mn	4.17e	4.44d	4.75b	5.01a	4.59c	4.17e	4.50cd
Zn	2.59c	2.76ab	2.85a	2.83a	2.80a	2.80a	2.65bc

Means within rows not followed by the same letter differ significantly by DMRT at 5%

The concentrations of macro and micro plant nutrient content in cauliflower transplants were significantly affected by PGPR treatments (tab. 5). PGPR inoculations increased the plant nutrient element content with the exception of Na. The highest content of P and Zn was obtained from *B. megaterium* TV-91C bacterial application, the highest content of Na, Cu and Mn was obtained from *P. agglomerans* RK-92 bacterial application, the highest content of Ca was obtained from *B. subtilis* TV-17C bacterial application, the highest content of N, K, Mg and Fe was obtained from *B. megaterium* TV-87A bacterial application. The lowest value of mineral content of cauliflower transplant was determined in control plant with the exception Na.

DISCUSSION

The applications of *B. megaterium* TV-3D, *B. megaterium* TV-91C, *P. agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10 showed high levels of amino and organic acid content and promoted significantly transplant growth and content of hormones, organic acids, organic acids and mineral content of cauliflower transplant.

Except for abscisic acid, the gibberellic acid, salicylic acid, indole acetic acid contents were increased by ratio of 23.64, 89.54% and 25.63%, respectively in compared to the control. Thus, various PGPRs having the ability to produce the IAA, cytokinine and other plant hormones which play an important function in plant growth and yield. It has been reported that the bacterial strain of *Bacillus* were capable of producing IAA, cytokinine, N2-fixiting and phosphate solubilizing capacity. It is known that PGPR can promote plant growth by producing ACC deaminase, which reduces ethylene levels in the roots of developing plants, and by producing plant growth regulators such as indole acetic acid (IAA), gibberellic acid, and cytokinines which can stimulate plant cell elongation, cell division etc. [Cakmakci et al. 2001, Patten and Glick 2002, Bashan and Bashan 2005, Karlidağ et al. 2007, Bi et al. 2008, Pırlak and Kose 2009]. This situation has had an effect on development of cauliflower transplant.

In present study, we have found that bacterial applications significantly affected organic acids and amino acids in cauliflower transplant. The amount of organic acids with PGPR applications have increased at a ranging ratio from 9.63 to 186.02%. Although, the amounts of amino acids not change much compared to the control in generally, the applications have increased in varying proportions (2.61, 2.48, 7.45, 2.38, 5.20, 18.80, 4.46, 8.92, 8.91, 3.51, 3.24 and 5.50% respectively) content of histidine, glycine, alanine, tyrocine, cystine, valine, methionine, tryptophan, phenylalanine, lysine, hydoxyproline and sarcosine of transplant. Malonic, acetic, oxalic, glycolic, and formic acids play a vital role in the acquisition of phosphorus, calcium, iron, zinc and manganese by plants growing in low available nutrient soils. The release of these acids in response to nutrient starvation differs between plant species. For example, fumaric, malic and citric acids can also chelate Fe and Mn in iron and manganese oxides, thus making them available for uptake by the plant [Ohwaki and Hirata 1992, Marschner 1995]. Much of fertilizer sources associated with hormonal, organic or amino acid contents, promote plant growth. These ingredients render insoluble forms of plant nutrients into soluble forms through acidification, chelation and exchange reactions. These processes compensate for the higher cost of manufacturing fertilizers in industry and mobilize the fertilizers added to soil. These amino and organic acid productions by PGPR changes in the chemical properties of the rhizosphere and might stimulate growth, yield and nutrient uptake from soil in different plant species under stressful growth conditions. For example, proline, glycine, and alanine inhibited stomatal opening, while histidine and methionine promoted stomatal opening of *Vicia faba*, histidine and proline promoted calcium uptake in phaseolus seedlings, while proline relieved salt toxicity in barley plants by changing the salt transport from root to shoot [Kumar and Sharma 1989, Rana and Rai 1996].

The highest fresh shoot and dry root weight of cabbage transplants were obtained from application of *B. megaterium* KBA-10 and root diameter, root length and fresh root weight were obtained from application of *B. megaterium* TV-91C in our study. Similar findings were reported in previous studies. It was reported that the use of PGPR increased stem diameter and root weight of transplant and plants were more resistant to stress conditions such as drought, disease [Vavrina 1999a, Kokalis-Burelle et al. 2003]. Kokalis-Burelle et al. [2003] determined that PGPR treatments increased shoot weight, shoot length and stem diameter of muskmelon and watermelon transplant. Also, the researchers indicated that root weight of transplant was increased by PGPR. In another study, PGPR isolates increased shoot length, root length and dry matter production of shoot and root of *Cicer arietinum* transplant [Misra et al. 2010].

In this study, bacterial inoculations increased the plant nutrient element content. While the highest value of mineral content was determined bacterial applications, the lowest value of mineral content of cauliflower transplant was determined in control plant with the exception Na. The increase of mineral content may explained by organic acids plant growth hormones and amino acids production by plant and bacterial inoculations. These findings in our study were similar with previous studies [Karlidağ et al. 2007, Bi et al. 2008, Walia et al. 2013].

We have identified that different bacterial inoculations increased plant growth parameters such as fresh shoot weight, dry shoot weight, root diameter, root length, fresh root weight, dry root weight, plant height, stem diameter, leaf area and chlorophyll reading value of cauliflower transplant (ratio of 31.88, 45.38, 15.95, 23.91, 48.25, 56.10, 9.27, 13.21, 11.86 and 25.92% respectively). Similarly, PGPR used as biofertilizer in a study increased growth parameters (plant height, stem width, root length, internode length) of tomato transplant compared to control [Ibiene et al. 2012]. In a different study, Garcia et al. [2003] have determined that three PGPR strains improved growth of transplants of tomato and pepper in different media. The strain of *B. subtilis* increased in seed germination (35.08%), shoot length (5.22%), root length (21.12%), shoot dry weight (63.50%) and root dry weight (54.08%) of tomato transplants compared to the control [Walia et al. 2013]. It was reported that seed inoculation with PGPR enhanced seed germination and transplant vigour of maize. Researchers determined that leaf and shoot dry weight and leaf surface area were increased by bacterial inoculation [Nezarat and Gholami 2009]. The bacterial application with manure increased plant height and stem diameter of cucumber and tomato transplant compared with control [Bi et al. 2008]. The reason of increase in transplant growth may be due to increasing nutrient uptake, providing plant growth hormones, improving chlorophyll content and organic acids with bacterial applications.

In conclusion, the present study shows that the tested PGPR strains such as *B. megaterium* TV-3D, *B. megaterium* TV-91C, *P. agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10 might be used in sustainable and organic agriculture to increase transplant growth and content as a substitute for costly mineral fertilizers. They may decrease the input cost of vegetable production without harming on plant and environment. In the future study, bio fertilization could be use an alternative fertilizer source for transplant performance in different plants.

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WPŁYW RYZOBAKTERII STYMULUJĄCYCH ROZWÓJ NA WZROST, ZAWARTOŚĆ SUBSTANCJI ODŻYWCZYCH, KWASÓW ORGANICZNYCH, AMINOKWASÓW I HORMONÓW U ROZSADY KALAFIORA (*Brassica oleracea* L. var. *botrytis*)

Streszczenie. Badanie przeprowadzono w celu określenia wpływu różnych szczepów ryzobakterii stymulujących wzrost roślin (PGPR) na wzrost i jakość rozsady kalafiora w warunkach szklarniowych. Wykorzystano szczepy *Bacillus megaterium* TV-3D,

B. megaterium TV-91C, *Pantoea agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10. Z badań wynika, że różne bakteryjne inokulacje zwiększają takie parametry wzrostu roślin, jak świeża masa pędów, sucha masa pędów, średnica korzenia, długość korzenie, świeża masa korzenia, sucha masa korzenia, średnica łodygi, powierzchnia liścia oraz zawartość chlorofili w rozsadzie kalafiora. Z wyjątkiem kwasu abscysynowego (ABA), zawartości kwasu giberelinowego (GA), kwasu salicylowego (SA) oraz kwasu indolilooctowego (IAA) – dzięki aplikacji *B. megaterium* KBA-10 oraz *P. agglomerans* RK-92 – zwiększyła się odpowiednio o 23,64, 89,54 i 25,63% w porównaniu z kontrolą. Ilość kwasów organicznych przy aplikacji *B. subtilis* TV-17C PGPR zwiększała się zmiennie, z 9,63 do 186,02%. Szczepienia PGPR zwiększały też zawartość makro- i mikroelementów w rozsadzie kalafiora. W rezultacie, zabiegi z użyciem bakterii mogą być środkiem polepszania wzrostu i jakości kalafiora.

Słowa kluczowe: rozsada kapustowatych, PGPR, warunki szklarniowe, produkcja fitohormonów, parametry wzrostu roślin

Accepted for print: 18.09.2014