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Original Article

Influence of Nitrogen Sources and Plant Growth-Promoting Rhizobacteria Inoculation on Growth, Crude Fiber and Nutrient Uptake in Squash (*Cucurbita moschata* Duchesne ex Poir.) Plants

Alice I. TCHIAZE[1], Victor D. TAFFOUO[1]*, Henri FANKEM[1], Martin KENNE[2], Régis BAZIRAMAKENGA[3], Georges E. EKODECK[4], Hani ANTOUN[3]

[1]*University of Douala, Faculty of Science, Department of Plant Biology, 24157 Douala, Cameroon; ifoueav@yahoo.fr; dtaffouo@univ-douala.com (*corresponding author); fankemhenri@yahoo.fr*

[2]*University of Douala, Faculty of Science, Department of Animal Biology, 24157 Douala, Cameroon; martin.kenne@univ-douala.com*

[3]*University of Laval, Horticulture Research Center, Department of Soil and Food Engineering, 2480, blvd Hochelaga Québec, G1V 0A6, Canada; regis.baziramakenga@fsaa.ulaval.ca; hani.antoun@fsaa.ulaval.ca*

[4]*University of Douala, Faculty of Science, Department of Earth Science, 24157 Douala, Cameroon; gemekodeck@hotmail.fr*

Abstract

Plant growth promoting rhizobacteria (PGPR, B) have immense potential application in sustainable agriculture as ecofriendly biofertilizers and biopesticides. In this study, the effects of three nitrogen (N) sources (NO_3^-, NH_4^+ and NO_3NH_4) and PGPR on growth, crude fiber and nutrient uptake were investigated in squash plants. Some growth parameters [root dry weight (RDW), shoot dry weight (SDW), total plant dry weight (PDW), number of leaves (NL), shoot length (SL), stem diameter (SD) and number of ramifications (NR)], crude fiber (cellulose content) and nutrient uptake (N, P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn) were determined. Application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation led to a significant increase in RDW, SDW, PDW, NL, SL, SD and NR. Na, Cu and Zn contents, on the contrary, decreased in inoculated treated plants while no significant differences were recorded in cellulose contents (CE) of leaves except in plants fed with NO_3^-. The leaf CE content ranged from 12.58 to 13.67%. The plants supplied with NO_3+B, NH_4+B and NO_3NH_4+B showed significantly higher plant biomass and accumulation of N, P, K and Mn concentrations in leaves compared to all other treatments. These results suggest that specific combinations of PGPR with NO_3^-, NH_4^+ or NO_3NH_4 fertilizers can be considered as efficient alternative biofertilizers to improve significantly the squash growth and nutrient uptake.

Keywords: cellulose, growth, nitrogen sources, nutrient uptake, PGPR

Abbreviations: Plant growth promoting rhizobacteria-PGPR, B; root dry weight-RDW; shoot dry weight-SDW; total plant dry weight-PDW; number of leaves-NL; shoot length-SL; stem diameter-SD; number of ramifications-NR; cellulose-CE; nitrogen-N; phosphorus-P; potassium-K; Nitrate-NO3-; crude fiber-CF; days after planting-DAP; weeks after sowing-WAS.

Introduction

Squash (*Cucurbita moschata* Duchesne ex Poir.) is a crop that has been used traditionally for human and animal food (Lira and Montes, 1992). Leaves of squash are the most valuable source of essential nutrients such as proteins, vitamins, minerals and fibers (Hunter and Fletcher, 2002; Sun *et al.*, 2002). They are also rich in antioxidant and phenolic components (Tang *et al.*, 2004; Wong *et al.*, 2006) that may provide specific protective effects against oxidative stress; the latter can lead to coronary heart disease and cancer (Kaur and Kapoor, 2002; Katalinic *et al.*, 2006).

The development of soil fertility management options in order to increase the productivity of stable food crops is a challenge in most parts of sub-Saharan Africa, where soils are constrained by N, P and K deficiencies (Christianson and Vlek, 1991; Manu *et al.*, 1991; Jemo *et al.*, 2010). Adequate soil supply

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of N is beneficial for carbohydrates and protein metabolism, and it promotes cell division and cell enlargement of plants (Shehu *et al.,* 2010). The availability of N is the primary limiting factor of productivity in most natural and managed soils (Berendse and Aerts, 1987; Aerts and Chapin, 2000). Although some plants rely on N organic form (Ohland and Nasholm, 2004), most N is supplied to plants through ammonification and nitrification (Haynes and Goh, 1978; Bloom, 1985; Chapin *et al.*, 1987). Nitrification plays a major role in cultivated soil. NO_3^- is mobile and circulates with the solution of the soil towards the roots of the plant (Mantelin and Touraine, 2004). Under certain conditions of temperature, ventilation, moisture and pH, ground micro-organisms change all shapes of N into NO_3^-, which is most mobile in the ground, so most accessible to the plants. Govindarajulu *et al.* (2005) reported that after uptake of mineral N (NO_3^- or NH_4^+), both forms are assimilated mainly into the amino acid arginine and then transferred in the form of NH_4^+ to the plant.

The substitution of chemical fertilizers by biological fertilizers made up of bacteria involved in N_2 fixation is one of the effective steps in sustainable agriculture. In fact, microbes are associated with key processes such as soil structure formation, decomposition of organic matter, toxin removal, and the cycling of elements - C, N, P, K, and S (Adesemoye and Egamberdieva, 2013). The rhizospheric soil contains diverse types of bacteria that actively colonize plant roots and enhance plant growth and yield via various plant growth promoting substances, as well as, biofertilizers when compared to synthetic fertilizers, insecticides and pesticides (Jay, 2013; Mathivanan *et al.,* 2014). PGPR include the strains in the genera *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium and Serratia* (Rodriguez and Fraga, 1999; Sturz and Nowak, 2000).

There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism: suppression of plant disease (bioprotectants), improved nutrients acquisition (biofertilizers), or phytohormone production (biostimulants) (Figueiredo *et al.,* 2010). Generally, PGPR facilitate the plant growth by two ways, either directly by contributing to essential minerals acquisition (solubilisation of P or K, uptake of N) and through the production of phytohormones such as indole-3-acetic acid, gibberellic acid, cytokines, zeatin and ethylene (Podile and Kishore, 2007; Herman *et al*., 2008; Raval and Desai, 2012) or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Ahemad and Kibret, 2014). Most of P in insoluble compounds is unavailable to plants. N_2-fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake by the crop plants, and playing a crucial role in biofertilization (Glick *et al.,* 1999). The plant biomass, nutrient uptake, and yield of wheat were increased by P solubilizing bacteria *Bacillus* strains (Chen *et al.,* 2006). In *Arabidopsis*, NO_3^- uptake measurement in response to PGPR, over time, can lead to conflicting results: NO_3^- influx was increased in seedlings, upon 24 h inoculation with *Phyllobacterium brassicacearum* STM196, while it was reduced 7 days later (Mantelin *et al.,* 2006). PGPR showed positive effects in plants, such as on germination rate, drought tolerance, shoots and roots dry weight, leaf area, total plant biomass and yield components (Kloepper *et al*., 2004; Mia *et al.,* 2010). According to Cummings (2009), the mechanisms by which PGPR seem to exert their most significant effect on crop growth is by enhanced nutrient uptake. Inoculation of PGPR led to the enhancement of defence related enzyme activity such as phenyl alanine ammonia lyase, peroxidase, chitinase and β-1,3 glucanase in tea leaves (Chakraborty *et al.,* 2013). According to Adesemoye *et al.* (2009), microbial inoculants can not be universal to all ecosystems and biofertilizer performance might be specific since effectiveness relies on plant type, soil type, and many other factors. PGPR can be very effective because they are susceptible to enrich soil fertility and improve agricultural yield (Jay, 2013).

CF is a term used to describe the fibrous food residue that is left over after it has been dissolved in the laboratory with certain harsh chemical solvents such as sulfuric acid and sodium hydroxide (Pomeranz and Meloan, 1994). Numerous workers (Naumann, 1940; Stallcup, 1958) have shown that CF is of variable composition, consisting mainly of celluloses, lignin, hemicelluloses, pentosans, and small amounts of crude protein and ash. CE of crude fiber is a polysaccharide that has a structural role in animals and plants. In plants, CE is the compound that gives rigidity to the plant stems, leaves and branches (Klemm *et al.,* 2005). It appears in different ratios in various species and is also influenced by the stage of maturity of the plant (Armstrong *et al.,* 1958). CE is also characterized by low or no nutritional value; however, based on its effect on the digestive system, it could play a role in the treatment of diabetes and high levels of blood cholesterol (Pamplona, 2011). New frontiers, including environmentally friendly cellulose fiber technologies, bacterial cellulose biomaterials, and in-vitro syntheses of cellulose are highlighted together with future aims, strategies, and perspectives of cellulose research and its applications (Klemm *et al.,* 2005).

Due to the negative environmental impact of chemical fertilizers and their increasing costs, the use of beneficial soil microorganisms such as PGPR for sustainable and safe agriculture has increased globally during the last couple of decades (Podile and Kishore, 2007). Biofertilizers such as PGPR are recognized as efficient soil microbes for sustainable agriculture and hold great promise in the improvement of agriculture yield (Jay, 2013). Application of biofertilizers and different mineral N sources to improve soil structure, fertility and, consequently, development and growth of squash plants has received little attention. The availability of K and P in arid saline soils is limited (Adesemoye and Egamberdieva, 2013). In such soils, the presence of bacterial strains that are able to solubilize unavailable forms of K and P-bearing minerals to bring the K and P into solution is an important approach to enhance crop growth and yield.

The use of PGPR in combination with different mineral N sources may be an approach to substitute chemical fertilizers and pesticides for sustainable cultivation of vegetable crops. It was hypothesized that the PGPR inoculation in combination with different mineral N sources can act as efficient bioinoculants, which may colonize the rhizosphere of squash roots and increase plant growth and nutrient uptake. Therefore, this study was undertaken to evaluate the effects of different mineral N sources and PGPR inoculation on the growth, crude fiber and nutrient uptake of squash plants.

Materials and Methods

Plant and bacterial materials

Squash (*Cucurbita moschata*) is generally more tolerant of hot, humid weather than *C. maxima* or *C. pepo*. It also displays a greater resistance to disease and insects, especially to the squash vine borer. Plants of this species are vigorous in their growth and have a higher leaves/fruits ratio in comparison to *C. maxima* or *C. pepo*, and have higher protein, vitamin, mineral and fiber contents. Their leaves are also rich in antioxidant and phenolic components. Seeds of squash plants were provided by the breeding program of the Agronomic Institute for Research and Development of Cameroon. The bacterial inoculum used was a commercially available PGPR (Bionutrients AG 8-1-9; provided by Growth Products; USA) containing four beneficial mixture strains of *Bacillus subtilis*; *B. amyloliquefaciens*; *B. pumilus*; *B. licheniformis* and *Saccharomyces cerevisiae* at 7.5×10^7, 7.5×10^7, 7.5×10^7, 2.5×10^8 and 4.5×10^9 colony forming units per gram (cfu g^{-1}), respectively.

Plant growth conditions and inoculation of plants

The trial was conducted under greenhouse conditions, located at the Research Center of Horticulture of Laval University, Canada, from October 2012 to May 2013. Seeds of squash plants were surface sterilized with 70% (v/v) ethanol solution for 15 min, then rinsed four times with deionized water. Five days after germination, when primordial leaves were fully established, seedlings were transferred to 7 L plastic pots (Teku Container MCC 31; Germany) filling with 5 kg of a 3:1 (w/v) mixture of heat pasteurized (70 °C for 24 h) dry soil substrate (promix) and sterilized sand. The experiment was carried out in a randomized complete block design with seven treatments and eight replicates for a total of 56 pots. 0.1 g of bacterial inoculum was added to the planting hole of each seedling treated with different N fertilisation sources singly or in combination. One plant was grown in the middle of each pot. All plants were fertilized with a nutrient solution containing (in g L^{-1}): 0.01 g KH_2PO_4, 0.62 g K_2SO_4, 0.15 g MgSO4, 0.20 g FeNaEDTA, 0.20 g $MnCl_2.4H_20$, 0.20 g $ZnCl_2$, 0.20 g $CuCl_2.2H_2O$ and 0.20 g H_3BO_3. In addition, three different mineral N sources were supplied as $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ alone or in combination at a rate of 50 mL pot^{-1} every 10 DAP. Untreated plants (without rhizobacteria inoculation and N fertilisation sources) were used as control. Plants were watered with deionized water every morning. The daily amounts of water added to the pots were the same for all treatments. Throughout the growth period, average day/night temperatures in the greenhouse were 22.1 °C/18 °C and the relative air humidity averaged 51%.

Plant growth parameters determination

Plants were harvested 56 DAP. SL, SD, NL and NB were recorded. Leaves, stems and roots were separately dried at 62 °C for 72 h and their dry weights determined.

Nutrient content determination

Subsamples (300 mg) of ground leaves (including leaves lost over the growth period), were extracted by 0.5 M $NaHCO_3$ buffered at pH 8.5 for 30 min. P concentration in the filtrate was analysed colorimetrically with a spectrophotometer (EPOS 5060, Eppendorf, Hamburg, Germany) at 420 nm wavelength, after staining with phospho-molybdate solution (Watanabe and Olsen, 1965). For determination of K, Na, Ca, Mg and Mn, 0.3 g of dried ground leaves was dry ashed at 550 °C for 4 h and thoroughly mixed with 250 mL of deionized water. The filtrate was analysed with an atomic absorption spectrophotometer (EPOS 5060, Eppendorf, Darmstadt, Germany). As the quantification of N concentration in plant material is concerned, 0.1 g of dried ground leaf samples (including leaves lost over the growth period) were analysed in an elemental analyser (Elementar vario EL, Hanau, Germany). Powders previously got from the leaves were analysed for Zn, Fe and Cu concentration determination. For the extraction of these three elements, five samples of dried and ground leaves of 0.5 g each were thoroughly mixed with 20 mL of HCL 1/10 for 24 h, and their concentrations were determined by the atomic absorption spectrophotometer (EPOS 5060, Eppendorf, Darmstadt, Germany) method (Pauwels *et al.,* 1992).

Cellulose content determination

CE content was determined by the method of Crampton and Mainard (1938). For the quantitative estimation of CE, 2 g of plant material (leaves) was taken and the sample was freed of non cellulose, organic constituents by digestion with an alcohol-nitric acid reagent. The treatment involved boiling the sample with the reagent for 2 h.

Statistical analysis

All data were statistically analysed using Statistica (version 9, Tulsa, OK, USA) and first subjected to analyses of variance (ANOVA). Statistical differences between treatment means were established using the Fisher LSD test at $p < 0.05$. Multi-factorial ANOVA was used to estimate whether N fertilization sources, PGPR inoculation, alone or in interaction had a significant influence on the measured parameters.

Results

Plant growth

Squash growth was estimated by measuring RDW, SDW, PDW, SL, SD, NL and NR of inoculated (PGPR) and non-inoculated plants under different N fertilization sources at vegetative stage (8 WAS). Under greenhouse conditions, different N fertilization sources supply singly or in combination had significant effects on plant growth (Table 1). Application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation led to a significant increase in RDW, SDW, PDW, SL, SD, NL and NR compared to untreated plants (Table 1, Fig. 1). The combination of PGPR inoculation with NO_3^-, NH_4^+ or NO_3NH_4 fertilizers showed significantly higher PDW as compared to the plants fed with NO_3^-, NH_4^+ or NO_3NH_4 singly and untreated controls (Table 1). A significant two-way interaction between the factors N fertilization sources and PGPR inoculation was observed for SDW and PDW (Table 1).

Leaf nutrient contents

The nutrient contents in leaves of *C. moshata* were affected by different N fertilization sources and bacterial inoculation (Fig. 2). Application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation had a positive effect on leaf N, K, P and Mn concentrations (Fig. 2A and C). The highest

Table 1. Effect of N fertilization sources and PGPR inoculation (B) on growth in squash plants at the vegetative stage (8 WAS)

Parameters	Treatments							Two-way ANOVA Results		
	Control	NO_3^-	NH_4^+	NO_3NH_4	NO_3^-+ B	NH_4^++ B	NO_3NH_4+ B	N sources	PGPR	Interactions N X B
RDW ($g plant^{-1}$)	1.12 ± 0.06b	4.54 ± 0.04a	3.61 ± 0.02a	4.41 ± 0.05a	4.80 ± 0.03a	4.82 ± 0.04a	4.65 ± 0.06a	83.14*	0.54	5.68
SDW ($g plant^{-1}$)	3.88 ± 0.30d	28.56 ± 0.13b	23.74 ± 0.09c	28.75 ± 0.28b	29.84 ± 1.18ab	30.52 ± 0.55a	32.33 ± 0.35a	245.89**	78.43*	87.86*
PDW ($g plant^{-1}$)	5.00 ± 0.05d	33.10 ± 0.09b	27.35 ± 0.07c	33.16 ± 0.04b	34.64 ± 0.09a	35.34 ± 0.06a	36.98 ± 0.07a	376.77**	18.75*	81.76*

Values shown are means (n=8) ± SD; within rows, means followed by different letter are significantly different ($p < 0.05$). **, * significant at 1 and 5% probability levels, respectively.

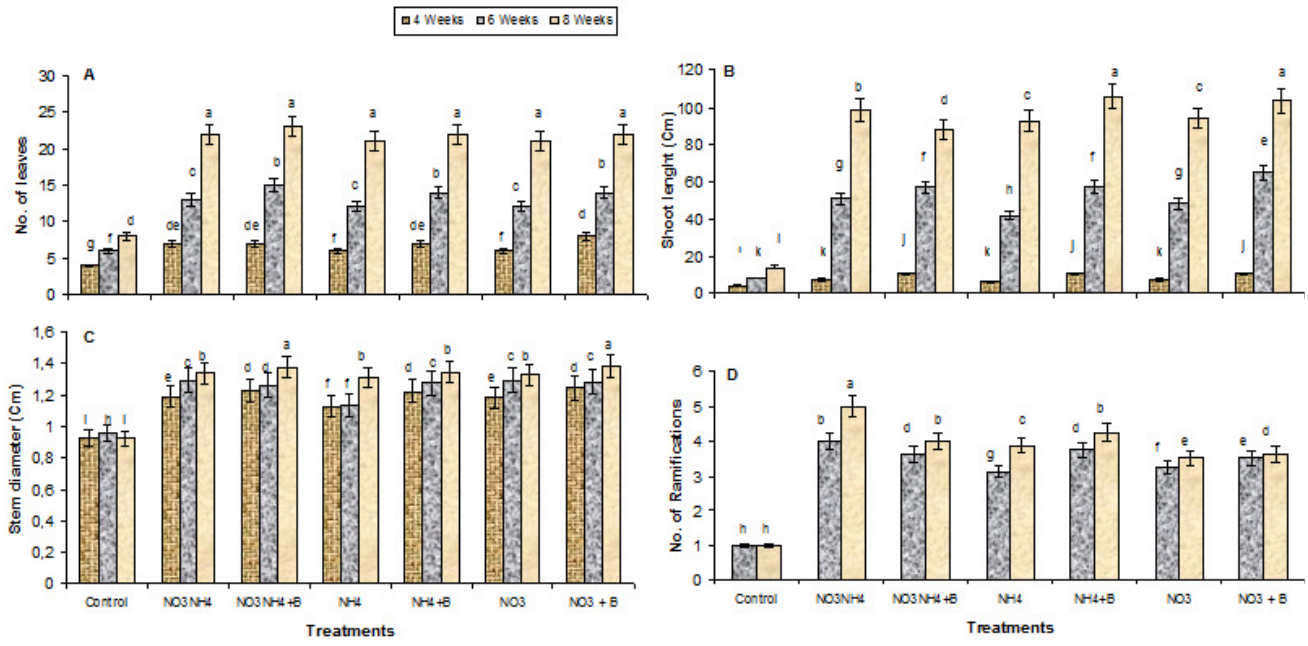

Fig. 1. Effect of N fertilization sources and PGPR inoculation on growth in squash plants at vegetative stage (8 WAS). Number of leaves (A), Shoot length (B), Stem diameter (C) and Number of ramifications (D). Bars are means (n=5) ± SD. Means followed by different letter are significantly different ($p < 0.05$)

increase of leaf K content was found in plants supplied with NH_4^+ compared to all other treatments (Fig. 2A). Application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR, on the contrary, decreased Mg, Na, Cu and Zn concentrations in inoculated plants except the plants fed with NO_3^- and NO_3NH_4+B (Fig. 2B and C). The analysis of Fe content data in *C. moshata* leaves indicated that this micronutrient was positively affected only by NH_4^+ (Fig. 2C).

Cellulose content

The analysis of CE data in leaves of *C. moshata* indicated that the CE was affected only by the application of NO_3^- (Fig. 3). The levels of CE content in squash plants ranged from 12.58 to 13.67% (Fig. 3).

Discussion

A great number of studies underlined that the growth-promoting ability of some bacteria might be specific to certain plant species, cultivars and genotypes (Bashan, 1998; Lucy *et al.,* 2004). The main objective was to look for any specificity of the single form of N sources or in combination with these PGPR which advocate the maximum benefits associated to additive or individual effect. In the present study, application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation led to a significant increase in RDW, SDW, PDW, SL, SD, NL and NR compared to untreated control plants (Table 1, Fig. 1). The maximum amount of N supply in sunflower resulted in higher total dry matter production per plant and the effect was prominent from 34 DAP (Wajid and Asghari, 2012). N deficiencies induce modification of many morphological and physiological parameters such as limitation of growth, leaf area and leaf number (Mia *et al.,* 2010).

Numerous studies have reported that PGPR inoculation increased growth and yield by enhancing solubilization of P or K and N uptake (Podile and Kishore, 2007; Herman *et al.*, 2008). Growth attributes such as leaf area, chlorophyll content, and consequently the total biomass were also increased due to PGPR inoculation (Mia *et al.,* 2010). Application of PGPR strains, especially *Bacillus subtilis* was reported to inhibit the

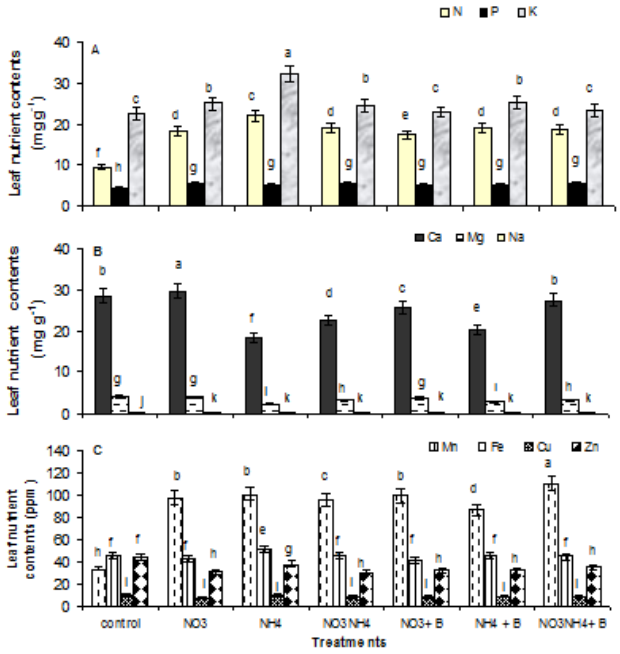

Fig. 2. Effect of N fertilization sources and PGPR inoculation (B) on nutrient contents in leaves of squash plants at vegetative stage (8 WAS). Bars are means (n=5) ± SD. Means followed by different letter are significantly different ($p < 0.05$)

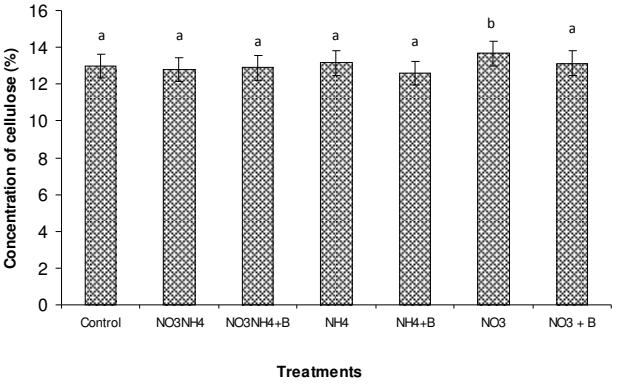

Fig. 3. Effect of N sources and PGPR inoculation on cellulose content in leaves of squash plants at vegetative stage (8 WAS). Bars are means (n=5) ± SD. Means followed by different letter are significantly different ($p < 0.05$)

growth of stem blight pathogen *Corynespora casiicola* in groundnut and pigeon pea (Jay, 2013). PGPR stimulated plant growth either directly by improving nutrient acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Verma *et al.,* 2010; Ahemad and Kibret, 2014). According to Tsigie (2001), these rhizobacteria are endophytic and produce indolacetic acid (IAA), uptake of P from phosphate mineral solubilizing bacteria (*B. subtilis*) and inhibit some soil-borne pathogenic fungi. Production of IAA enhances the formation of root hair (Pacôme *et al.,* 2013). Chen *et al.* (2006) and Tsigie *et al.* (2011) also reported an enhancement of nodules number in lentil and soybean, plant biomass, nutrient uptake, and yield of wheat in response to *Bacillus* strains. A significant effect of PGPR inoculation was found on SDW and PDW (Table 1). Similarly RDW, SDW and PDW increased significantly with PGPR inoculation in chickpea (Verma *et al.,* 2010; Pacôme *et al.,* 2013).

Application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation had a positive effect on leaf N, P, K and Mn concentrations while those supplied with NH_4^+ showed significant increase of leaf K concentration (Fig. 2A and C). Numerous studies have reported that PGPR inoculation increased growth, yield, solubilization of P or K, uptake of N and some other mineral elements (Podile and Kishore, 2007; Herman *et al.*, 2008). Although some plants rely on organic form of N (Ohland and Nasholm, 2004), most N is supplied to plants through ammonification and nitrification (Haynes and Goh, 1978; Bloom, 1985; Chapin *et al.,* 1987). The microbes are involved in key processes such as soil structure formation, decomposition of organic matter, toxin removal, and the cycling of elements-C, N, P, K, and S (Adesemoye and Egamberdieva, 2013). In such soils, the presence of bacterial strains that are able to solubilize unavailable forms of K and P-bearing minerals to bring the K and P into solution is an important approach to enhance crop growth and yield (Rodriguez and Fraga, 1999; Jay, 2013). Modification of root system architecture by PGPR implies the production of phytohormones and other signals that lead, mostly, to the increase of lateral root branching and development of root hairs (Pacôme *et al.,* 2013). PGPR also modify root functioning, improve plant nutrition and have an impact on the physiology of the whole plant (Vacheron *et al.,* 2013).

The impact of PGPR on plant nutrition may result from effects on plant nutrient uptake and plant growth rate (Mantelin and Touraine, 2004). The inoculation of PGPR can directly increase nutrient supply in the rhizosphere and/or stimulate ion transport systems in root (Adesemoye and Kloepper, 2009; Vacheron *et al.,* 2013); phosphate solubilisation is one key effect of PGPR on plant nutrition. Soils generally contain a large amount of P, which accumulates in the wake of regular fertilizer applications, but only a small proportion of the latter is available for plants. Plants are able to absorb by their own means mono and dibasic phosphate; organic or insoluble forms of phosphate need to be mineralized or solubilized by microorganisms, respectively (Ramaekers *et al.,* 2010). Many PGPR such as *Pseudomonas*, *Bacillus*, *Rhizobium* are able to dissolve insoluble forms of phosphate (Richardson *et al.,* 2009). Inoculation of canola with *Achromobacter* sp. strain U80417 resulted in an increase of both NO_3^- and K^+ net influx rates per root surface area unit (Bertrand *et al.,* 2000).

In this study, the levels of CE content of crude fiber in squash plants ranged from 12.58 to 13.67% (Fig. 3). The findings by Armstrong *et al.* (1958) on the alfalfa hays corroborate the results obtained in our study. The CE content of leaves (12.58-13.67%) recorded in this study was lower than those (26.22-32.62%) obtained by Stallcup (1958). In an experiment conducted by Yodkraisri and Bhat (2012), a potato chip had relatively lower CE content of about 4%. Numerous researchers (Naumann 1940; Stallcup 1958) have shown that crude fiber has a variable composition, mainly made up of CE, lignin, hemicelluloses, pentosans, and small amounts of crude protein and ash. According to Armstrong *et al.* (1958), the amount of CE is

specific to species and is influenced by the stage of maturity of the plant or the quality of the soil used as substrate. CE is an important parameter in food and feed analyses, specifically in poultry and stock feeds, succulence of fresh fruits and vegetables (Trowel *et al.,* 1976).

Conclusions

The specific combinations of PGPR mixture strains with NO_3^-, NH_4^+ or NO_3NH_4 fertilizers can exert beneficial effects at the early stage of squash seedlings growth. Application of NO_3^-, NH_4^+ or NO_3NH_4 singly or in combination with PGPR inoculation led to a significant increase in RDW, SDW, PDW, NL, SL, SD and NR. Improvements in N, P, K and Mn accumulation in squash leaves corroborate beneficial effects of PGPR inoculation and N fertilization sources interactions. Therefore, the specific combinations of bacteria with NO_3^-, NH_4^+ or NO_3NH_4 fertilizers can be considered as efficient alternative biofertilizers to improve significantly the squash growth and N, P, K and Mn contents of leaves.

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