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EFFECTS OF OXYFERTIGATION AND PLANT GROWTH PROMOTING RHIZOBACTERIA ON GREENHOUSE LETTUCE GROWN IN PERLITE

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ABSTRACT

This study was conducted in order to determine the effects of oxygen enrichment of nutrient solution coupled with plant growth promoting rhizobacteria on soilless grown iceberg lettuce (cv. 'Papiro') production. Seeds were treated with *Bacillus subtilis*, *Pseudomonas putida*, *P. fluorescens*, *P. punonensis* and combined application of *B. subtilis* + *P. fluorescens* and were sown into vermicompost : peat (1 : 1.5, v/v) mixture on January 14th, 2015. After germination in growth chamber, seedlings were moved to a greenhouse for seedling growing till they were ready for planting. Seedlings were transplanted to the polyethylene greenhouse 35 days after sowing. Perlite as growing medium was used in open-system soilless culture. Nutrient solution was aerated with an air compressor and applied to plants 2 days after planting with drip irrigation. To diffuse oxygen into nutrient solution in large bubbles, a circular air-stone commonly used in fisheries was used. The nutrient solution without oxyfertigation and plants not treated with bacteria constituted the control treatment. Experiments were conducted in randomized plots design with 2 factors and 3 replications. Heads were harvested 2 months after transplanting. Yield and head quality parameters of head were determined. It was concluded that oxygen enrichment of nutrient solution through a compressor (aeration) provided increases in yield and plant growth. Especially root development, head size and leaf number were higher in plants grown with aerated nutrient solution. Among the tested bacteria, *B. subtilis*, *P. fluorescens* and *B. subtilis* + *P. fluorescens* were found promising due to the their higher performance under aerated conditions on greenhouse lettuce grown in perlite.

Key words: iceberg, oxygen, *Bacillus subtilis*, *Pseudomonas* spp., yield, nitrate

INTRODUCTION

In nutrient solution recipes of soilless culture C, H and O fertilization do not exist since they are assumed to be supplied from the ambient air in a gas form [Gül 2008]. However, plants need oxygen for respiration. Plants use dissolved oxygen or gas form of atmospheric oxygen. Oxygen should be in dissolved form to be up taken by the roots [Morard and Silvestre 1996]. Under normal conditions (20°C, 1 atm), dissolved oxygen concentration is maximum 9 ppm. This value decreases with increasing temperature and salinity. In this case, "hypoxia" (oxygen insufficiency) is experienced when oxygen is not supplied to rhizosphere [Drew 1997] and hypoxia resulted in morphological, structural and physiological-metabolic changes in plants [Morard and Silvestre 1996, Drew 1997]. It was also considered that hypoxia in root region made plants more sensitive to some root diseases caused by fungal pathogens, especially those belonging to the genera of *Pythium* and *Phytophthora* [Cherif et al. 1997].

Most of growing media used in soilless culture are porous and thus contain optimum amounts of oxygen. Previous studies indicated that oxygen content of

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drainage solution in soilless culture was greater than 3 mg l^{-1} for nutrient solution [Gilerød and Kempton 1983]. However in summer time when the temperature is high in greenhouse and the water's oxygen transport capacity is low, oxygen deficiency problem could be encountered due to high root and microorganism respiration rate, inadequate and frequent water application [Raviv et al. 2004, Bonachela et al. 2005]. There are several methods developed for oxygen enrichment of nutrient solution -oxyfertigation- such as pure and pressurized oxygen gas treatments, mechanic mixing or aeration through bubbling, ozone treatments and chemical (K_2O_2) treatments [Vanachter et al. 1988, Bhattarai et al. 2005, Marfa et al. 2005, Urrestarazu et al. 2005]. Among this methods, aeration with pump or mechanic mixing are quite practical, cheap, no chemicals interact with other elements of nutrient solutions and not required fertigation equipment. Therefore, enrichment of nutrient solution in oxygen is a significant issue for optimum plant culture. It was reported in previous studies that aeration of nutrient solutions improved plant and root development in soilless culture [Goto et al. 1996, Gislerod et al. 1997, Öztekin 2017].

Like oxygen enrichment, Plant Growth-Promoting Rhizobacteria (PGPR) that can actively colonize plant roots, also promote plant growth through increase plant vigor [Kohler et al. 2010, Kalita et al. 2014] with produce plant growth-promoting compounds, including phytohormones (i.e. auxins, cytokinins, gibberellins) and siderophores. It has been reported that PGPRs improve yield [Yildirim et al. 2011], water and nutrient uptake [Şahin et al. 2015], biotic and abiotic stress tolerance [Hoffmann-Hergarten et al. 1998, Kohler et al. 2010]. Soil-borne pathogens and other deleterious microorganisms can be suppresed with PGPRs [Zehnder et al. 2000]. With the increasing problems associated with the use of synthetic chemicals in agriculture, there has been a rising interest in the use of beneficial microorganisms to improve plant health and productivity [Avis et al. 2008].

In present study the effects of aeration and PGPRs are combined in order to test interaction effects of treatments. Thus, the study was conducted to investigate the effects of oxygen enrichments of nutrient solution through aeration (with a compressor) combined with PGPRs on plant growth, yield and head quality of lettuce grown in greenhouses.

MATHERIAL AND METHODS

This research was conducted in winter-early spring season of 2015 in the polyethylene greenhouse of Department of Horticulture, Faculty of Agriculture, Ege University (EUFA). Iceberg lettuce cv. 'Papiro' (AG Tohum, Antalya/Turkey) was used as plant material.

Tested bacteria were *Bacillus subtilis* (Stain 63/3, Epiphytic), *Pseudomonas putida* (Stain 18/1K, Epiphytic), *Pseudomonas fluorescens* (Stain 112, Endophytic), *Psedomonas punonensis* (Stain 56, Endophytic) and *B. subtilis* + *P. fluorescens*. The bacterial strains were obtained from the culture collection unit of the Department of Plant Protection of EUFA. *B. subtilis* and *P. putida* had been isolated from tomato, *P. fluorescens* and *P. punonensis* had been isolated from cucumber rhizospheres in the Aegean region of Turkey. PGPR inoculation took place before sowing as seed coating. Bacterial inoculants were suspended with carboxyl methyl cellulose (CMC, 1.5%) [Özaktan et al. 2015]. In control treatment, seeds were treated only CMC (1.5%) suspension. It was determined that isolates of bacteria colonized seeds at the rate of 10^6 CFU seed g^{-1} after bacterization [Callan et al. 1990].

Seeds were sown on the January 14th, 2015 into 60% local peat and 40% vermicompost mixture [Tüzel et al. 2014]. After sowing, seedling trays having 210 cells in each were left in a germination room for three days (18/18°C day/night, 80% RH, dark) and then moved to adaptation greenhouse (mean 22°C) until transplanting. Seedlings were fertilized with a commercial liquid composted farmyard manure (Botanica, Camli Yem Besicilik, Izmir, Turkey; 30 l ha^{-1}) every day with boom irrigation system.

Seedlings were transferred to the greenhouse on February 20th, 2015 with a plant density of 3.48 plant m^{-2}. Perlite was used as growing medium in open-system soilless culture. A total of 18 l growth medium (6 l per plant) was placed in horizontal plastic pots which contained 3 plants. Nutrient solution was applied to plants 2 days after planting. The recipe specified by Gül [2008] was used in plant nutrition (mg l^{-1}): N 150, P 50, K 150, Ca 150, Mg 50, Fe 5.0, Mn 0.50, Zn 0.05, B 0.50, Cu 0.03, Mo 0.02. The pH of prepared nutrient solution was adjusted to be between 5.5–6.5 and electrical conductivity (EC) between 1.8–2.0 dS m^{-1}. Drip irrigation was used to apply nutrient

solution to plant root zones. Drain holes were provided at the bottom of the pots to drain excess nutrient solution. Samples were taken from the main and drainage tanks three times in a week and pH and EC of samples were measured. EC values of drainage solution in control and aerated treatments were 2.86 and 2.74 dS m^{-1} with an average. Time of nutrient solution application was determined based on cumulative solar radiation value of 1 MJ m^{-2} within the greenhouse. Amount of applied solution was determined based on observations made at drainage outlets, the ratio of drained/applied nutrient solution was adjusted about 20–40% daily.

Experiments were conducted in randomized plots design with 2 factors (oxyfertigation and bacteria) with 3 replications. Oxygen sources were composed of "Aeration". The nutrient solution without oxyfertigation (unaerated) and plants without bacteria constituted the "Control" treatment.

An air compressor (HAILEA brand, ACO-009 model, 102 W power, 110 l min^{-1} output, >0.035 Mpa pressure) was used to supply oxygen in bubbles to nutrient solution. Compressor was also equipped with a timer able to adjust oxygen supply durations. To diffuse oxygen into nutrient solution in large bubbles, a circular air-stone (20 cm in diameter and 20 l min^{-1} capacity) commonly used in fisheries was used. To adjust oxygen supply quantities, an oxygen meter (OXYGUARD, Oxy C-DO II model) were used. Oxygen meter probe was continuously kept inside the nutrient solution.

The oxygen supply was controlled with a timer (THEBEN brand, Eltimo 020 S DCF). The system was started operation 30 min before irrigations and oxygen and air supplied to nutrient solution based on relevant treatment. The system was stopped automatically 5 min after irrigations. Based on input set values (7–15 mg l^{-1}), oxygen flow was supplied from the compressor when the oxygen level went below the set value. Oxygen content of nutrient solution varied between 3.5–5.4 (average value of 3.7) mg l^{-1} in unaerated nutrient solution; between 8.6–10.4 (average value of 9.3) mg l^{-1} in aerated nutrient solution.

Greenhouse indoor temperature, relative humidity and solar radiation values were measured throughout the experiments with Delta T brand RHT2 (temperature + humidity) and GS (global radiation) sensors. In growing period average temperatures, relative humidity and solar radiation were 24.7°C, 68.9% and 45.9 MJ m^{-2} day^{-1}, respectively.

Iceberg heads were harvested on the April 21th, 2015 when they were ready to harvest. Weight of heads from each treatment was measured and total yield was calculated. Five heads were sampled in order to measure head sizes (length and width), leaf number, color and fresh (FW) and dry (DW) weights. Head sizes were measured by ruler from root collar to top of the head as length, from middle (radius) of head as width. Leaf color was assessed with a colorimeter (Minolta, CR-300, Japan) with 8 mm diameter viewing area [McGuire 1992]. Heads were weighed for fresh weight (g $head^{-1}$) and dry weight (g $head^{-1}$) was determined by drying at 65°C until a constant weight using a thermo ventilated oven. Nitrate content was determined according to Cataldo et al. [1975].

Resultant data were subjected to variance analysis with JMP (version 15.0) software. Tukey test was used to compare treatment means. F test results were presented as *ns* (nonsignificant at 5% level, $P \geq 0.05$), * (significant at 5% level, $P \leq 0.05$) and ** (significant at 1% level, $P \leq 0.01$ or $P \leq 0.001$). Results in figures were presented as mean ± standard errors.

RESULTS AND DISCUSSION

Plant growth

Main and interaction effects of oxyfertigation and bacteria on head length (mean 15.7 cm) and width (mean 13.5 cm) were found nonsignificant. Leaf number changed between 28.0 and 39.3 (mean 32.7) and effects of treatments and their interactions on leaf number were found significant. Oxyfertigation and bacteria applications increased leaf number of heads. The highest leaf number was obtained from plants treated with *B. subtilis* + *P. fluorescens* irrigated with aerated nutrient solution followed by aerated × *P. fluorescens* treatment. Plants that were not treated bacteria and aeration gave the lowest number. Number of waste leaves varied between 3.8 (unaerated × *P. fluorescens*) and 6.7 (unaerated × *B. subtilis* + *P. fluorescens)*. Main effects of treatments did not affect waste leaf numbers (Tab. 1).

Oxygen adding to nutrient solution affected root, shoot fresh and dry weights significantly, and with

Table 1. Effects of oxyfertigation and bacteria on lettuce head morphology

Oxyfertigation	Bacteria	Head lenght (cm)	Head width (cm)	Total leaf number (no)	Wastes leaves (no)
Unaerated		15.5	13.3	30.1 b	5.6
Aerated		16.0	13.7	35.2 a	5.4
	B. subtilis	16.1	13.6	32.2 ab	5.7
	P. putida	15.2	13.3	32.5 ab	5.5
	P. fluorescens	15.9	13.7	36.5 a	5.0
	P. punonensis	16.0	13.5	31.3 ab	5.7
	B. subtilis + *P. fluorescens*	16.1	13.9	33.3 ab	5.6
	$Control_{(without\ bacteria)}$	15.0	13.0	30.0 b	5.5
Unaerated	*B. subtilis*	15.9	13.6	28.7 b	6.3 a
	P. putida	14.8	13.4	34.0 ab	5.0 abc
	P. fluorescens	16.1	13.3	34.0 ab	3.8 c
	P. punonensis	15.9	13.7	28.3 b	5.7 ab
	B. subtilis + *P. fluorescens*	15.3	13.3	27.3 b	6.7 a
	$Control_{(without\ bacteria)}$	14.7	12.4	28.0 b	5.9 ab
Aerated	*B. subtilis*	16.3	13.6	35.7 ab	5.2 abc
	P. putida	15.6	13.1	31.0 ab	5.9 ab
	P. fluorescens	15.6	14.1	39.0 a	6.2 ab
	P. punonensis	16.1	13.2	34.3 ab	5.8 ab
	B. subtilis + *P. fluorescens*	16.9	14.4	39.3 a	4.4 bc
	$Control_{(without\ bacteria)}$	15.3	13.6	32.0 ab	5.1 abc
Analysis of variance	oxyfertigation	ns	ns	**	ns
	bacteria	ns	ns	*	ns
	oxyfertigation × bacteria	ns	ns	*	*

Different letters in the same row indicate a significant difference according to Tukey test ($P < 0.05$)

aeration shoot fresh and dry weight increased 5.8 and 27.2%, the increase was 22.2 and 47.1% in root fresh and dry weights, respectively. Main effect of bacteria was found significant only on shoot fresh and root dry weights. Among the tested bacteria *P. fluorescens* showed higher values in both parameters. *B. subtilis* + *P. fluorescens* and *B. subtilis* were followed *P. fluorescens* on shoot fresh weight. Similarly, interaction effects of oxyfertigation and bacteria was found significant only on shoot fresh and root dry weights which were found higher on aerated × *B. subtilis* + *P. fluorescens* (Tab. 2).

Plant growth and development was better in aerated treatments. It was reported in previous studies that aeration of nutrient solutions (oxyfertigation) improved plant and root development in soilless culture [Goto et al. 1996, Gislerod et al. 1997, Öztekin 2017]. In another study carried out in hydroponic culture, three different dissolved oxygen doses were applied to lettuce plants and high doses (7.7–8.6 mg l^{-1}) had positive impacts on plant growth and yielded longer roots, longer and more number of leaves [Gülsoylu 2012]. Present findings comply with all those earlier findings.

In our study the tested PGPRs did not show any significant difference in head length, width, waste leaves, shoot dry weight and root fresh weight. *P. fluorescens, B. subtilis* and their combination had the highest biomass in general. Some studies have found a positive effect of PGPR application on shoot growth in a hydroponic culture [Yasufumi and Kaneaki 2003,

Table 2. Effects of treatments on lettuce root and shoot fresh (FW) and dry (DW) weight

Oxyfertigation	Bacteria	Shoot		Root	
		FW (g)	DW (g)	FW (g)	DW (g)
Unaerated		715.2 b	21.7 b	44.6 b	4.54 b
Aerated		756.5 a	27.6 a	54.5 a	6.68 a
	B. subtilis	734.7 ab	26.8	51.1	6.55 ab
	P. putida	711.3 b	22.7	45.7	5.59 ab
	P. fluorescens	796.2 a	27.0	56.7	7.08 a
	P. punonensis	711.3 b	24.8	46.9	5.48 ab
	B. subtilis + *P. fluorescens*	767.8 ab	23.8	51.3	5.17 ab
	$Control_{(without\ bacteria)}$	693.8 b	22.7	45.4	3.78 b
Unaerated	*B. subtilis*	716.8 abc	21.9	42.7	5.1 abc
	P. putida	692.3 bc	23.9	41.7	4.9 bc
	P. fluorescens	771.7 abc	22.3	52.3	5.6 abc
	P. punonensis	698.5 bc	22.0	43.5	4.9 bc
	B. subtilis + *P. fluorescens*	737.3 abc	21.3	42.5	3.3 c
	$Control_{(without\ bacteria)}$	674.7 c	18.6	44.8	3.5 c
Aerated	*B. subtilis*	752.5 abc	31.6	59.4	8.0 ab
	P. putida	730.3 abc	21.6	49.8	6.3 abc
	P. fluorescens	820.7 a	31.7	61.2	8.6 a
	P. punonensis	724.1 abc	27.6	50.4	6.0 abc
	B. subtilis + *P. fluorescens*	798.3 ab	26.2	60.2	7.0 abc
	$Control_{(without\ bacteria)}$	713.0 abc	26.7	45.9	4.1 bc
Analysis of variance	oxyfertigation	*	**	*	**
	bacteria	*	ns	ns	*
	oxyfertigation × bacteria	*	ns	ns	**

Different letters in the same row indicate a significant difference according to Tukey test ($P < 0.05$)

Malkoclu et al. 2017]. However, efficiencies of PGPR strains depend upon the host plant and soil environment besides their inherent capabilities [Nadeem et al. 2014] and climatic variations [Ahemad and Kibret 2014]. Thus, aeration combined with bacteria gave the highest results.

Yield

Mean plant weight was found nonsignificant under tested treatments and changed 675 g (unaerated × control) and 825 g (aerated × *P. fluorescens)* per head (unpublished data). Main and interaction effects of oxyfertigation and bacteria on total and marketable yields were found significant ($0.01 < P \leq 0.05$). Total and marketable yield was 2.49 and 2.23 kg m^{-2} on plants with unaerated nutrient solution whereas they were 2.64 and 2.40 kg m^{-2} with 6.1 and 5.5% increases with aeration. In terms of tested bacteria, total yield changed between 2.41 and 2.77 kg m^{-2}, marketable yield was between 2.12 and 2.53 kg m^{-2}. Lowest yields were obtained from control plants followed by *P. punonensis*. Plants treated with *P. fluorescens* and *B. subtilis* + *P. fluorescens* gave the highest yields. Under oxyfertigation and bacteria interaction total and marketables yields were found higher in the treatments of aeration × *B. subtilis* + *P. fluorescens* followed by aeration × *P. fluorescens*; while the lower ones was unaerated × control treatment (Fig. 1).

It was reported in previous studies carried out in perlite culture that oxygen enrichment increased yield

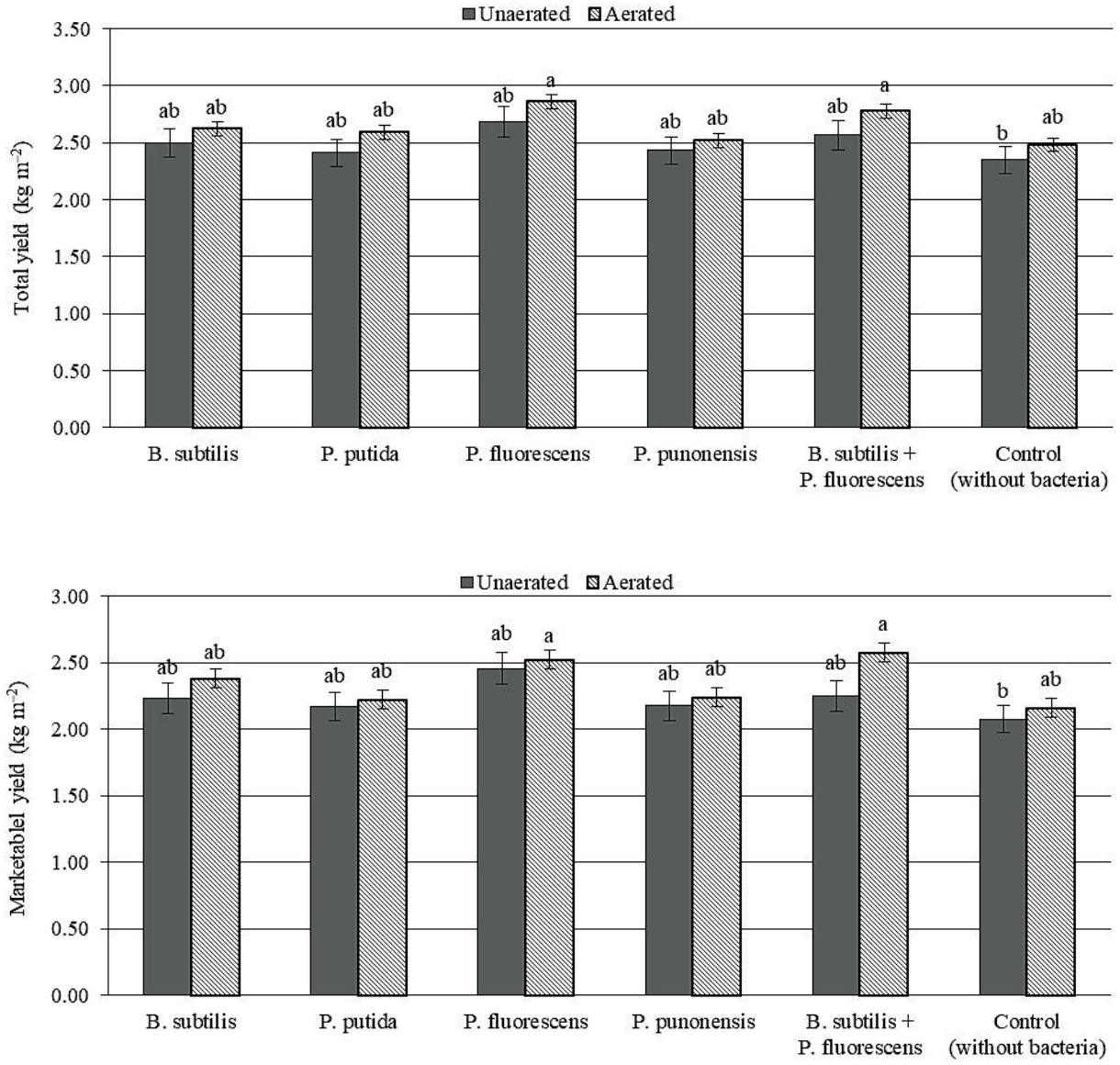

Fig. 1. Total and marketable yields under oxyfertigation × bacteria interaction

levels and number of fruits in peppers [Marfa et al. 2005, Urrestarazu and Mazuela 2005], tomato [Öztekin 2017], melon [Urrestarazu and Mazuela 2005] as compared to untreated control plants. Present findings revealed that supplied oxygen to nutrient solution with an compressor oxygen treatments had higher yield levels than the control (unaerated) treatments.

Yield data did change significantly by tested PGPRs. This could be caused prominent mechanisms of PGPRs directly contributing plant growth are phytohormone production and enhancement of plant nutrition [Podile and Kishore 2006]. In this research the performance of *P. fluorescens* and *B. subtilis* +*P. fluorescens* on total and marketable yields were found the

highest and their effect increased with aeration due to the important role of oxygen in microorganism growth and its metabolism.

Quality

Oxyfertigation, bacteria and interaction effects of them were found significant in only leaf a (-a: green) color but b color (+b: yellow) was affected only by oxyfertigation significantly. Lightness (L) and a/b ratio were found nonsignificant. Although a color was high in control plants, a/b ratio was found almost similar in all plants under both treatments. Thus there were no big differences in leaf color (Tab. 3).

Leaf nitrate content varied between 1210.6 and 1480.9 mg kg^{-1} and did not affected from oxyfertigation, bacteria and their interaction. Although there were no significant differences between unaerated and aerated treatments, leaf nitrate content decreased with aeration of nutrient solution (Tab. 3).

Our findings revealed that oxygen sources did not have significant effects on head quality. Bacteria application did not change the a/b ratio (which is an important colour criteria) or nitrate content. Also, oxygen enrichment did not create significant differences in quality parameters of watermelons grown in perlite culture [Bonachela et al. 2005]; Liberoxy (K_2O_2) supplementation to nutrient solution did not result in significant differences in fruit quality of pepper (grown in perlite culture), melon and cucumber (grown in stone wool culture) [Urrestarazu and Mazuela 2005]. Our results in lettuce were found in a harmony with previous works done with different vegetable crops.

Table 3. Effects of treatments on some lettuce head qualities

Oxyfertigation	Bacteria	Colour				NO_3 (mg kg^{-1})
		L	a	b	a/b	
Unaerated		50.64	–15.4 b	25.4 a	–0.61	1367.2
Aerated		47.95	–14.5 a	23.5 b	–0.62	1262.8
	B. subtilis	48.38	–14.4 a	23.7	–0.61	1354.8
	P. putida	51.78	–15.2 ab	26.0	–0.59	1319.1
	P. fluorescens	50.80	–15.6 b	26.1	–0.60	1296.8
	P. punonensis	47.88	–14.4 a	22.7	–0.63	1342.0
	B. subtilis + *P. fluorescens*	49.41	–15.3 ab	24.9	–0.62	1255.3
	$Control_{(without\ bacteria)}$	47.52	–14.7 ab	23.4	–0.63	1321.8
Unaerated	*B. subtilis*	51.95	–15.0 abcd	25.9	–0.58	1480.9
	P. putida	51.80	–15.7 cd	25.9	–0.61	1351.1
	P. fluorescens	55.20	–16.2 d	28.4	–0.57	1312.8
	P. punonensis	46.97	–14.1 abc	22.2	–0.64	1391.5
	B. subtilis + *P. fluorescens*	49.17	–15.7 bcd	25.7	–0.61	1300.0
	$Control_{(without\ bacteria)}$	48.76	–15.3 abcd	24.5	–0.63	1367.0
Aerated	*B. subtilis*	44.80	–13.8 a	21.6	–0.64	1228.7
	P. putida	51.76	–14.7 abcd	26.1	–0.57	1287.2
	P. fluorescens	46.40	–15.0 abcd	23.7	–0.63	1280.9
	P. punonensis	48.80	–14.6 abcd	23.2	–0.63	1292.6
	B. subtilis + *P. fluorescens*	49.65	–15.0 abcd	24.1	–0.63	1210.6
	$Control_{(without\ bacteria)}$	46.27	–13.9 ab	22.2	–0.63	1276.6
Analysis of variance	oxyfertigation	ns	*	*	ns	ns
	bacteria	ns	*	ns	ns	ns
	oxyfertigation × bacteria	ns	*	ns	ns	ns

Different letters in the same row indicate a significant difference according to Tukey test ($P < 0.05$)

CONCLUSION

It was concluded that among the tested bacteria, *B. subtilis*, *P. fluorescens* and *B. subtilis* + *P. fluorescens* were found more promising in terms of their effects on plant growth and yield. However, these bacteria could display even more their abilities under aerated conditions on greenhouse lettuce grown in perlite.

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