Scientia Horticulturae 195 (2015) 154–162

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress

Gabriela Fasciglione[a,c], Elda M. Casanovas[a], Victoria Quillehauquy[a], Alejandra K. Yommi[a], María G. Goñi[b,c], Sara I. Roura[b,c,1], Carlos A. Barassi[a,*]

[a] Unidad Integrada Balcarce (Fac. de Ciencias Agrarias, UNMdP—INTA EEA, Balcarce, CC 276, Zip Code 7620 Balcarce, Argentina
[b] Fac de Ingeniería, UNMdP, Juan B. Justo 4302, Zip Code 7600 Mar del Plata, Argentina
[c] Consejo Nac. de Inv. Científicas y Técnicas (CONICET), Av. Rivadavia 1917, Zip Code 1033 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form 8 September 2015
Accepted 10 September 2015
Available online 24 September 2015

Keywords:
Azospirillum
Lettuce
Saline stress
Quality
Harvest
Posharvest

ABSTRACT

Recent results have shown that *Azospirillum*-inoculated lettuce seeds yield a higher number of transplanted plants with superior traits than non-inoculated controls grown with and without saline stress. However, little available information on the possibility that *Azospirillum* could improve nutritional and quality market parameters on plants grown under saline conditions and extend life storage has prompted us to explore this issue. In this work, seed inoculation with *Azospirillum* not only promoted higher biomass but also improved lettuce quality when plants were grown under salt-stress conditions. Compared to non-inoculated controls, higher ascorbic acid content was accompanied by a lower oxidation rate in *Azospirillum*-inoculated plants. In addition, better overall visual quality was linked to higher chlorophyll content, hue, Chroma, L and lower browning intensity. While the beneficial effects of *Azospirillum* inoculation were more evident at harvest, some of these continued thereafter. Compared to non-inoculated controls, higher aerial biomass, chlorophyll and ascorbic acid content, better overall visual quality, hue, Chroma and antioxidant capacity, and a lower browning intensity were observed after storage in *Azospirillum*-inoculated plants grown under salt stress. These results suggest that seed inoculation with *Azospirillum* not only could improve freshly product quality but also extend storage life in lettuce grown under salt stress.

1. Introduction

Food intake by humans in the last decades shows a tendency towards an increased consumption of fruits and vegetables of high nutritional quality (Lester, 2006). This tendency could have been triggered by a better knowledge regarding the role fresh fruits and vegetables plays in preventing a number of human diseases (Subhasree et al., 2009). Indeed, several diseases are usually associated with high fat and sugar contents and poor proportion of vitamins, minerals and fiber in the diet. All these negative factors could be minimized with a diet rich in leafy vegetables (Martín-Diana et al., 2007). Amongst these, lettuce (*Lactuca sativa*) is one of the most popular ingredients used worldwide in fresh salads (Esparza Rivera et al., 2006). The main nutrients provided by its ingestion are insoluble fiber, folates, pyridoxine, riboflavin, thiamin, vitamins A and C, calcium, potassium, iron, manganese and phosphorus (USDA, 2014). In addition to nutrition deficiencies, both the accelerated rhythm of life and the lack of physical activity, inherent to urban population, can be connected to a limited food preparation time at home and the demands of low-calorie diets.

While agriculture intensification has greatly increased the productive capacity of agroecosystems, it has had unintended environmental consequences including degradation of soil and water resources and alteration of biogeochemical cycles (Yamaguchi and Blumwald, 2005). In addition to these anthropogenic factors, and considering that water and soil salinity are the major abiotic stresses agricultural plants suffer, any increase in one or both would undoubtedly impair worldwide food production (Yamaguchi and Blumwald, 2005). In particular, it has been estimated that approximately 50% of the cropland and 20% of the irrigated land in the world are already affected by high salinity, a problem that could be aggravated by natural causes (Kohler et al., 2010).

Leaf expansion, plant growth, and carbon metabolism of many plants could be negatively affected by nutritional imbalance, osmotic stress, water deficiency, and/or oxidative stress, factors

* Corresponding author.
E-mail address: cbarassi@mdp.edu.ar (C.A. Barassi).
[1] Deceased.

http://dx.doi.org/10.1016/j.scienta.2015.09.015
0304-4238/

that could be a consequence of a high salt stress (Bashan and de-Bashan, 2010). In this regard, which ever the way horticultural crops are exposed to salt, this exposure could cause growth alterations, low absorption and/or low distribution of nutrients to the plant, affecting the quality of the product. Furthermore, salt and other abiotic stresses could trigger an oxidative stress that plants could counteract by producing antioxidants, many of them being considered beneficial to human health (Subhasree et al., 2009). However, strategies based upon growing vegetables under abiotic stresses in order to enhance synthesis of nutraceutical compounds could impair yield at harvest, and better taste and visual characteristics and nutritional quality of the product.

Therefore, two important targets in modern horticulture are the improvement of plant growth and nutritional quality when plants grow under salt-stress conditions. A new promising strategy is the use of plant-growth promoting microorganisms (PGPM) inoculants to improve crop production under abiotic stresses (Grover et al., 2011; Coleman-Derr and Tringe, 2014). In particular, PGPM are known to increase plant growth by improving phosphorus absorption, fixing nitrogen, catching traces of iron and/or producing phytohormones (Bashan and de-Bashan, 2010; Nadeem et al., 2014). Within PGPM, *Azospirillum* spp. is the most studied microorganism because it is able to colonize a wide range of vegetable species and improve general plant performance under normal and/or stressing growth conditions (Bashan and de-Bashan, 2010).

Even though some lettuce cultivars could be considered relatively tolerant to salt, this species is generally included on the list of sensitive vegetables (Shannon, 1997). Moreover, lettuce yield in plants grown under salt stress is higher in those germinated from *Azospirillum*-inoculated seeds than in non-inoculated controls (Fasciglione et al., 2012). Little is known, however, about the possibility of extending this advantage in order to obtain a product with better taste and visual characteristics, nutritional quality, and superior post-harvest behaviour.

Within this framework, we hypothesize that *A. brasilense* Sp 245 inoculation improves both yield and quality at harvest in lettuce grown under continous salt stress, and extends product quality upon storage.

2. Materials and methods

2.1. Seed inoculation

Azospirillum brasilense Sp245 was cultured as described before (Barassi et al., 2006), modified as follows: late exponential cells were centrifuged for 10 min at 8142 × *g* in a Sorvall SS43 rotor and resuspended in phosphate buffer 66 mol m^{-3} (pH 7) to obtain 10^9 cell inocula per seed. Butterhead *L. sativa* L. **s**eed inoculation proceeded as described before (Fasciglione et al., 2012), modified as follows: both total liquid volume and time needed for a complete seed imbibition were calculated from time-course experiments performed with cv. Elisa seeds (Sakata Seed Sudamerica Ltd., Brasil) during a 24-h period (data not shown). Seeds were surface disinfected in 1 g kg^{-1} NaOCl for 1 min, washed three times with sterile distilled water, and inoculated by immersion for 90 min in a total imbibition volume of phosphate buffer (Control) or in the bacterial inocula indicated above (Inoculated).

2.2. Growth and postharvest storage conditions

Control and inoculated seeds were individually sown in 66-plug trays (33.0 cm^3 each) containing Dynamics 3 commercial substrate (Dynamics, Argentina), based on processed peat moss (*Musgo sphagnum*) and perlite enriched with N, P, K, Mg and micronutrients (Radigen, Germany). To allow irrigation by capillarity, each tray was put into a larger tray containing 1.0 L of distilled water or 40 mol m^{-3} NaCl solution, both of them changed every 7 days. Daily controls of salt concentration were performed by determining electrical conductivities. To avoid NaCl concentration by evaporation, distilled water was added when required. No additional nutrients were provided during the pre-transplanting period. At 45 days after sowing, both control and inoculated plants growing at 0 and 40 mol m^{-3} were individually transplanted to corresponding 5 L pots containing the same commercial substrate cited above. To continue with capillarity irrigation, pots were put into a large tray containing 2.5 L of distilled water or 40 mol m^{-3} NaCl solution supplemented with 100 ppm of N, P, K, Ca and Mg (1:1:1:1:1). Daily controls of salt concentration were performed as described and solutions changed every 7 days. A completely randomized design was used with a factorial combination of two levels of salinity and two levels of inoculum, with six plants per combination. Pots were kept in a greenhouse under natural light (approximate 17 mol m^{-2} d^{-1} PAR) at INTA Balcarce (Lat 38°S, Long 58°W) and cultivated during fall-winter season. Temperature was 16 ± 2 °C during daytime and 12 ± 2 °C at night. Relative humidity inside the greenhouse during the experiment was 60 ± 15%. Harvested plants were cooled, individually placed into polyethylene bags and stored in a chamber at 4 °C and 98% relative humidity for 20 days.

2.3. Growth analysis

Immediately after transplanting and at harvest, plant samples were analyzed for total fresh weight (FW), dry weight (DW) and total leaf area. Ten plants per treatment were used for these measurements. In addition, relative growth rate and net assimilation rate were calculated. The relative growth rate was expressed in g g^{-1} d^{-1} units, calculated according to the equation (Hunt et al., 2002):

$$\text{Relative growth rate} = \frac{(1\text{nDW}_2 - 1\text{nDW}_1)}{(t_2 - t_1)},$$

where t is sampling time.

The physiological net assimilation rate component was calculated from relative growth rate factorization including total leaf area (A), according to the equation (Hunt et al., 2002):

$$\text{Net assimilation rate} = \left[\frac{(\text{DW}_2 - \text{DW}_1)}{(t_2 - t_1)}\right] \times \left[\frac{(1\text{nA}_2 - 1\text{nA}_2)}{(\text{A}_2 - \text{A}_2)}\right],$$

and expressed in $gm^{-2}day^{-1}$.

Dry weight and total leaf area were determined in the period elapsed between 25 days after transplant (t_1) and harvest (t_2), that is, during the last 25 days of active growth.

2.4. Physiological status indexes during postharvest storage

Plant samples were taken at harvest, 10 and 20 days after harvest and analyzed for DW, FW, total chlorophyll content, its components chlorophylls *a* and *b*, and relative water content. Triplicate samples involving four plants per treatment were analyzed. Total aerial FW and DW, relative water content (Esparza Rivera et al., 2006) and chlorophyll concentrations (Agüero et al., 2008) were taken into account as plant physiological status indicators. Aerial biomass was expressed on the basis of FW and DW. Total chlorophyll content and its components chlorophyll *a* and chlorophyll *b* were determined according to Agüero et al. (2008), and expressed on the basis of DW. Rectangular portions from two leaves characterizing the middle part of plants were cut and weighed to obtain FW. Afterwards, each piece was immersed in distilled water and kept at the dark for 20 h to obtain turgid weight (TW), followed by oven drying at 60 °C to

determine DW. Relative water content was calculated according to the equation:

$$\text{Relative water content} = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100.$$

2.5. Nutritional and market quality changes during postharvest storage

Plant samples were taken at harvest, 10 and 20 days after harvest and analyzed for, ascorbic acid content, oxidation rate ratio, overall visual quality, and three color attributes: hue, Chrome and L. Hue represents the attribute by which human distinguish different colors, Chroma refers to chromatic intensity (saturation) and L degree of lightness (brightness or darkness). In addition, browning intensity and antioxidant capacity were determined at harvest and up to 20 days after. Triplicate samples involving four plants per treatment were analyzed. Ascorbic acid was determined as described before (Fasciglione et al., 2012) and reported as mg kg^{-1} FW. Determinations were performed in triplicates.

2.5.1. Antioxidant activity and oxidative status

Oxidation rate ratio and antioxidant activity parameters were determined according to the β-carotene bleaching method (Velioglu et al., 1998), modified as follow. Twenty μL linoleic acid plus 200 μL Tween 20 were added to 2.0 mL ß-carotene solution (0.2 mg mL^{-1}) in Cl_3CH. After Cl_3CH removal in a rotavapor during 10 min at 35 °C, 100 mL DW were added and mixed vigorously to form an emulsion. Leaf samples of 0.5 g FW each were ground in mortar and pestle with liquid N to obtain a paste, transferred to centrifuge tubes with 10 mL 80% methanol (v/v) in distilled water (extracting solution) and incubated for 2 h at 50 °C in a water bath. After centrifugation at 13000 × *g* during 10 min at 4 °C, 200 μL supernatants were transferred to test tubes containing 5 mL of a fresh ß-carotene emulsion and absorbance at 470 nm (A_{470}) determined at 0, 30, and 60 min thereafter. Tubes lacking β-carotene emulsion were used as blanks. Tubes containing only extracting solution were used as controls. Triplicates were used in each ß-carotene bleaching assay.

Oxidation rate ratio was calculated as R_{sample} /$R_{Control}$, where bleaching rates of control ($R_{Control}$) and extract (R_{sample}) samples were the absolute slope values obtained by plotting A_{470} vs. time in each case.

$$\text{Antioxident activity was expressed as \% inhabitation} = \left[\frac{(AS_{t60} - AC_{t60})}{(AS_{t0} - AC_{t0})}\right] \times 100$$

where $As_{(t60)}$ and $Ac_{(t60)}$ were the respective absorbances of sample and control, obtained at $t = 60$ min. Similar expressions were used for A_{470} values taken at $t = 0$ min.

2.5.2. Overall visual quality index

At each storage time, individual lettuce plants were taken from storage, codified and immediately subjected to a sensory panel. The panel was composed of 6 judges highly qualified in sensorial evaluation of lettuce. Five samples were distributed at random amongst the judges, who made independent evaluations of middle leaf zones. The judges were asked to evaluate overall visual quality on the basis of the subjective leaf parameters color (shade and intensity), brightness, texture, and absence of defects as browning and mechanical fragility (small ruptures in the external leaf edge). Each parameter was qualified by a number, in which 9 stood for excellent individual characteristic and 1 for very poor quality. The average was expressed as the overall visual quality index, where the limit of acceptance was 5 (Agüero et al., 2008). Even though this sensorial examination could be standardized by a panel of experts where independent observations were qualified by numbers, its reliability was highly dependent on the panel composition.

2.5.3. Color characterization

At harvest and every 10 days after, triplicates of the apical zone were taken from the middle part of plants and analysed with a chromameter (Minolta CR-300, Osaka, Japan) to determine color characteristics on the basis of spectral reflectance properties. The instrument has a built-in control unit and a measuring probe that emits an intense white light covering the entire visible spectrum for illuminating an area 8 mm in diameter. The color of the reflected light is analyzed by 3 high-sensitivity silicone photocells that are filtered to match the CIE (Commission Internationale de l'Eclairage) standard observer curves. The instrument was calibrated with a standard white plate before sampling lettuce leaves. The obtained data were expressed in hue, Chrome and *L* values. Hue was expressed in degrees, where pure colors correspond to: 0° (red), 90° (yellow), 180° (green), 270° (blue) (McGuire, 1992). Chroma indicates color saturation: higher values indicate greater color intensity. The *L* value gives the relative brightness (or luminance), ranging from total black (0) to total white (100) (Gazula et al., 2007).

2.5.4. Browning intensity

Browning intensity was determined according to Couture et al. (1993) modified as follows. Stem cuttings were weighed, homogenized in DW, filtered through cheesecloth and centrifuged at 25000 × *g* for 15 min. The supernatant was measured immediately for absorbance at 320 nm (A_{320}) with a UV–vis spectrophotometer (UV 1601 PC Shimadzu Corporation, Kyoto, Japan) to estimate the degree of tissue browning. Determinations were performed in triplicate.

2.6. Statistical analysis

Data were subjected to a variance analysis (ANOVA) using a R-Commander (version 2.9.1) statistical program. Both salt stress and inoculation levels and their interactions were considered. In the situation of a significant interaction ($P < 0.05$), means within each stress and inoculum level were compared by least significant difference (LSD) test ($\alpha = 0.05$). In non-significant interactions means from both salt stress and inoculation effects were independently compared by LSD test.

3. Results

3.1. Salt stress and inoculation with A. brasilense in lettuce growth. Impact on plant growth indexes

Figs. 1 and 2 show relative growth and net assimilation rates from transplant to harvest, in *Azospirillum*-inoculated and non-inoculated control lettuce plants grown at 0 and at 40 mol m^{-3} NaCl levels. The variance of both parameters indicated a significant interaction ($P < 0.05$) between salinity and inoculation factor levels. Even though salt stress decreased relative growth rate in controls, this parameter did not change in *Azospirillum*-inoculated plants (Fig. 1).

While no effects on relative growth rate due to inoculation were observed at 0 mol m^{-3} NaCl, the negative effect of 40 mol m^{-3} NaCl was reverted in *Azospirillum*-inoculated plants (Fig. 1). Regarding net assimilation rate analysis, while a non-significant change due to stress was observed in controls, a significant rise was observed in inoculated plants at 40 mol m^{-3} NaCl (Fig. 2). Similarly to relative growth rate results, while no effects on net assimilation rate were observed at 0 mol m^{-3} NaCl, the negative effect of 40 mol m^{-3} NaCl was reverted in *Azospirillum*-inoculated plants (Fig. 2). In brief,

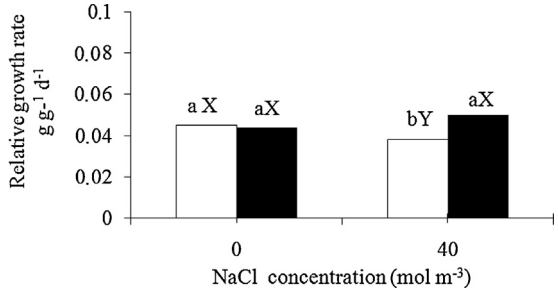

Fig. 1. Relative growth rate from 25 days after transplant to harvest in non-inoculated controls (□) and *A. brasilense*-inoculated plants (■), in lettuce grown at 0 or 40 mol m^{-3} NaCl. Different small letters on top of bars indicate significant differences ($P < 0.05$) between control and inoculated plants at each salt stress level. Different capital letters indicate significant differences ($P < 0.05$) between 0 and 40 mol m^{-3} NaCl plants at each inoculum level.

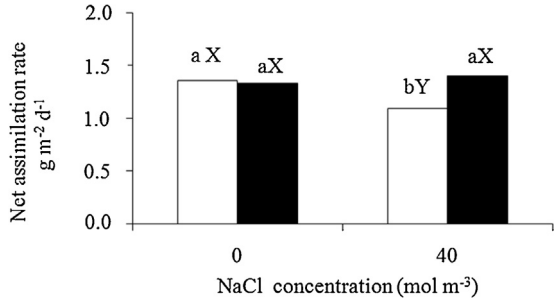

Fig. 2. Net assimilation rate from 25 days after transplant to harvest, in non-inoculated controls (□) and *A. brasilense*-inoculated plants (■), in lettuce grown at 0 or 40 mol m^{-3} NaCl. Different small letters on top of bars indicate significant differences ($P < 0.05$) between control and inoculated plants at each salt stress level. Different capital letters indicate significant differences ($P < 0.05$) between 0 and 40 mol m^{-3} NaCl plants at each inoculum level.

Azospirillum inoculation promotes both higher relative growth and net assimilation rates in lettuce plants exposed to salt stress.

3.2. *Salt stress and inoculation with* A. brasilense *in lettuce plants at harvest and postharvest: impact on physiological status indexes and nutritional and market quality*

The variance analysis indicated a non-significant interaction ($\alpha = 0.05$) between salt stress and inoculum factor levels and significant effects ($P < 0.05$) of both salt stress and inoculum on aerial biomass, relative water content and chlorophylls were obtained at harvest and up to 20 days lettuce storage (Table 1). Salt stress decreased aerial biomass both at harvest and up to 20 days storage. Aside from the stress effect, *Azospirillum*-inoculated lettuce plants displayed a significant increase in aerial biomass both at harvest and post-harvest periods (Table 1). Regarding relative water content, a significant increase due to inoculation both at harvest and 10 days after, was observed (Table 1). At harvest, while salt stress diminished root biomass from 1.37 ± 0.46 to 0.78 ± 0.16 g plant^{-1} DW, *Azospirillum* rose this parameter from 0.83 ± 0.21 to 1.31 ± 0.41 g plant^{-1} ($P < 0.05$).

A drop in chlorophyll *b* concentration due to salt stress was responsible for a total chlorophyll content decrease at harvest and after 10 days storage (Table 1). However, no changes in total chlorophyll content and its components *a* and *b* were evident at the end of the storage period (Table 1). Aside from the stress effect, *Azospirillum*-inoculated lettuce displayed a significant increase in total chlorophyll content at harvest and post-harvest periods (Table 1). However, while both chlorophylls *a* and *b* rose after 10 days storage, only chlorophyll *b* was responsible for the

Table 1
Salt stress and *Azospirillum* effects at harvest and post harvest periods, in lettuce grown under saline conditions: physiological parameters.

Harvest and post-harvest periods.												
	Harvest				10 days after harvest				20 days after harvest			
	Stress effects*		*Azospirillum* effects**		Stress effects*		*Azospirillum* effects**		Stress effects*		*Azospirillum* effects**	
Physiological parameter	0 NaCl mol m^{-3}	40 NaCl mol m^{-3}	Control	Inoculated	0 NaCl mol m^{-3}	40 NaCl mol m^{-3}	Control	Inoculated	0 NaCl mol m^{-3}	40 NaCl mol m^{-3}	Control	Inoculated
Aerial biomass (g plant^{-1} FW)	217.2a	109.8b	149.6b	177.4a	209.6a	110.9b	142.2b	178.3a	218.1a	103.0b	141.7b	179.31a
Aerial biomass (g plant^{-1} DW)	10.8a	6.1b	7.47b	9.49a	8.9a	6.1b	6.9b	8.1a	13.5a	5.39b	8.2b	9.7a
Relative water content (%)	74.7a	70.9b	70.4b	75.2a	72.8b	77.9a	73.9b	76.8a	77.6a	71.3b	71.0a	70.8a
Total chlorophyll (mg kg^{-1} DW)	85.3b	78.6a	76.9b	86.9a	72.0a	64.8b	63.0b	73.4a	62.4a	61.9a	56.3b	67.9 a
Chlorophyll a (mg kg^{-1} DW)	29.4a	27.8a	27.5b	29.9a	26.94a	24.14a	24.6b	26.2a	26.9a	24.1a	24.8a	26.2a
Chlorophyll b (mg kg^{-1} DW)	55.9a	50.8b	49.4b	57.3a	45.06a	40.66b	38.6b	47.2a	35.5a	37.8a	31.5b	41.7a

FW: fresh weight, DW: dry weight.
* Different letters indicate significant differences between stress levels regardless of inoculum level ($P < 0.05$).
** Different letters indicate significant differences between inoculum levels regardless of stress level ($P < 0.05$).

total chlorophyll content increase at the end of the storage period (Table 1).

In addition to aerial plant biomass, relative water content and chlorophylls, it is important to find out if other plausible changes due to *Azospirillum* inoculation could help to improve lettuce quality at harvest and after storage. The variance analysis indicated a non-significant interaction ($\alpha = 0.05$) between salt stress and inoculum factor levels and significant effects ($P < 0.05$) of both salt stress and inoculum on ascorbic acid, oxidation rate ratio, overall visual quality, hue, chroma and *L* values were obtained at harvest and up to 20 days lettuce storage. Table 2 shows how several nutritional and quality market parameters were affected by salt and *Azospirillum* inoculation, in lettuce grown under saline conditions. Either salt stress or *Azospirillum* inoculation triggered an ascorbic acid content increase at harvest, accompanied by a lowered oxidation rate ratio parameter (Table 2). However, while ascorbic acid decreased after 20 days storage by effect of salinity, its content remained higher than controls in *Azospirillum*-inoculated plants. On the other hand, no differences on oxidation rate ratio due to salt stress or *Azospirillum* inoculation were observed at the end of the storage period (Table 2).

According to sensorial overall visual quality data, while salt stress deteriorated lettuce appearance at harvest and up to 10 days after, *Azospirillum* inoculation improved this parameter at harvest and at all postharvest periods (Table 2). Objective data on color characteristics as hue, Chroma and *L* values were provided by a chromatometer. While salt stress diminished hue at harvest, it remained constant in *Azospirillum*-inoculated plants grown either under normal or stressed conditions. After 20 days storage, *Azospirillum*-inoculated plants had higher hue values than non-inoculated plants grown at 40 mol m^{-3} NaCl (Table 2). Under salt stress conditions, while Chroma decreased (less saturated color) at harvest and up to 10 days after, L remained constant at harvest but decreased (less brightness color) thereafter (Table 2). Independently of salt concentration, Chroma remained constant but a higher *L* in *Azospirillum*-inoculated plants than in controls was observed at harvest (Table 2). Moreover, after 20 days of storage a higher Chroma and a constant *L* in *Azospirillum*-inoculated plants was observed (Table 2).

The antioxidant activity of lettuce extracts can be calculated from data obtained with the β-carotene bleaching test (Kulisic et al., 2004). The variance of this parameter indicated a significant interaction ($P < 0.05$) between salinity and inoculation factor levels. Figure 3 shows the antioxidant activity (as percentage of oxidant inhibition) at harvest and 10 days after in control and *Azospirillum*-inoculated lettuce plants grown at 0 and at 40 mol m^{-3} NaCl. The antioxidant activity of lettuce leaves did not change at harvest due to the effect of salt stress both in control and *Azospirillum*-inoculated plants (Fig. 3). However, *Azospirillum* inoculation raised this parameter in plants grown at 0 mol m^{-3} NaCl (Fig. 3). After 10 days storage, the antioxidant activity did not change due to the effect of salt stress in control plants but experienced a significant increase in *Azospirillum*-inoculated plants exposed to salt stress (Fig. 3).

Another important characteristic taken into account in lettuce is tissue browning. The variance of this parameter indicated a significant interaction ($P < 0.05$) between salinity and inoculation factor levels. Salt stress increased browning intensity in non-inoculated plants both at harvest and postharvest periods. However, salt stress increased browning intensity in inoculated plants only 20 days after harvest (Table 3). Moreover, at 0 mol m^{-3} NaCl *Azospirillum* did not change browning intensity in any of the periods considered in this study. However, at 40 mol m^{-3} NaCl *Azospirillum* inoculation diminished browning intensity at all periods (Table 3).

Table 2
Salt stress and *Azospirillum* effects at harvest and post harvest periods, in lettuce grown under saline conditions: quality parameters.

Harvest and post-harvest periods.												
	Harvest				10 days after harvest				20 days after harvest			
	Stress effects*		*Azospirillum* effects**		Stress effects*		*Azospirillum* effects**		Stress effects*		*Azospirillum* effects**	
Parameter	0 NaCl mol m^{-3}	40 NaCl mol m^{-3}	Control	Inoculated	0 NaCl mol m^{-3}	40 NaCl mol m^{-3}	Control	Inoculated	0 NaCl mol m^{-3}	40 NaCl mol m^{-3}	Control	Inoculated
Ascorbic acid (mg kg FW^{-1})	20.9b	25.9a	17.8b	28.7a	15.7a	18.7a	13.9b	20.5a	15.6a	7.4b	9.1b	13.8a
Oxidation rate ratio	11.4a	9.6b	11.3a	9.7b	12.1a	8.3b	10.3a	10.1a	17.1a	16.5a	16.4a	17.3a
Overall visual quality	8.5a	7.7b	7.9b	8.4a	7.6a	6.0b	6.6b	7.0 a	6.4b	4.7b	4.9b	6.1a
Hue (angle degree)	125a	121b	122b	124a	124a	120b	121b	123a	119a	120a	118b	122a
Chroma	38.0a	34.1b	39.7a	41.9a	35.9a	33.3b	39.1a	40.6a	35.0a	34.4a	37.1b	40.4a
L	54.0a	53.1a	54.8b	57.4a	52.7a	51.0b	53.3a	53.2a	54.1a	52.4b	52.2a	51.5a

Hue, Chroma, and *L* values were representatives of color class, chromatic intensity, and degree of lightness, respectively.
* Different letters indicate significant differences between stress levels regardless of inoculum level ($P < 0.05$).
** Different letters indicate significant differences between inoculum levels regardless of stress level ($P < 0.05$).

Table 3
Browning intensity in lettuce grown under saline conditions: stress and *Azospirillum* effects.

Harvest and postharvest periods				
Treatment		Harvest	10 days after harvest	20 days after harvest
0 mol m^{-3} NaCl	Control	0.025aY	0.177aY	0.225aY
	Inoculated	0.037aX	0.176aX	0.196aY
40 mol m^{-3} NaCl	Control	0.063aX	0.261aX	0.459aX
	Inoculated	0.039bX	0.173bX	0.251bX

Different small letters indicate differences ($P < 0.05$) between Control and Inoculated plants at each salt stress level. Different capital; letters indicate differences ($P < 0.05$) between 0 and 40 NaCl mol m^{-3} treated plants at each inoculum level.

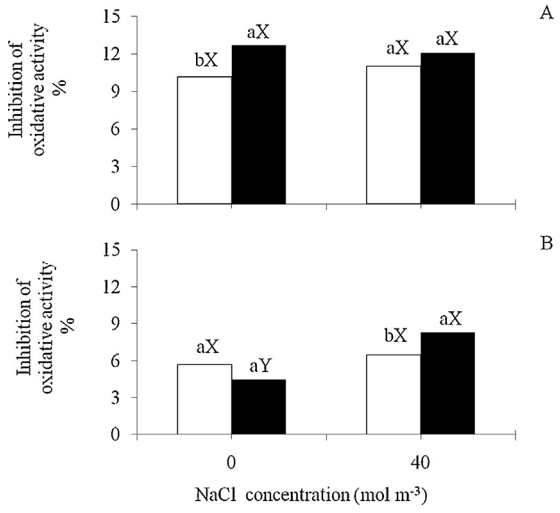

Fig. 3. Antioxidant activity expressed as inhibition of oxidative activity (%) in non-inoculated controls (□) and *A. brasilense*-inoculated plants (■), in lettuce grown at 0 or 40 mol m^{-3} NaCl at harvest (A) and 10 days after harvest (B). Different small letters indicate significant differences between control and *Azospirillum*-inoculated plants at each salt stress level, and different capital letters indicate significant differences between 0 and 40 mol m^{-3} NaCl plants at each inoculum level ($P < 0.05$).

4. Discussion

Even though we initially have taken 10^6 *Azospirillum* cells $seed^{-1}$ into account as the optimum inoculum concentration on wheat (Bashan, 1986), a calculated 10^9 *Azospirillum* cellsseed-1 was previously determined as the one required to obtain a significant effect on promoting early *L. sativa* L. cv. Elisa seeds germination exposed to both 0 and 40 mol m^{-3} NaCl (Fasciglione et al., 2012).

Considering that the edible part of lettuce is the aerial section, our research was focused on the study of this portion of the plant. While relative growth rate describes growth as the change in biomass per unit of initial biomass per unit time, net assimilation rate is largely the net result of carbon gain (photosynthesis) and carbon losses (respiration, exudation, volatilization) expressed per unit leaf area (Poorter and Remkes, 1990). Both parameters provide useful data concerning salt stress and/or inoculation effects on plant growth, foliar expansion and photosynthesis. The analysis of relative growth and net assimilation rates as components of plant growth showed the relevance of the application of *A. brasilense* and *Pantoea dispersa* to sweet pepper in order to mitigate the effect of salt stress (del Amor and Cuadra-Crespo, 2012). The growth promotion effect exerted by *Azospirillum* in lettuce exposed to salt (Fig. 1) could be explained by a concomitant higher net assimilation rate (Fig. 2). In comparison to our study, a salinity level of 40 mol m^{-3} NaCl was shown to reduce the net assimilation rate of non-inoculated sweet pepper while remained unaffected in *A. brasilense*-inoculated plants (del Amor and Cuadra-Crespo, 2012). It would be interesting to see if these effects could be accompanied by other physiological changes.

According to previous results, both biomass and chlorophyll content rose at harvest in *Azospirillum*-inoculated lettuce plants (Fasciglione et al., 2012). However, no information on changes in these parameters after product storage has been reported yet.

Salt stress decreased aerial biomass both at harvest and after 20 days storage (Table 1). Aside from the stress effect, *Azospirillum*-inoculated lettuce plants displayed a significant increase in aerial biomass both at harvest and post-harvest periods (Table 1). It is well known that *Azospirillum* inoculation promotes higher root biomass and as a consequence, better water absorption and plant water status (Bashan and Dubrovsky, 1996; Creus et al., 2004). Moreover, higher relative water content in *Azospirillum*-inoculated wheat than in controls was observed in plants germinated under salt stress (Creus et al., 1997). A significant higher root biomass found in *Azospirillum*-inoculated lettuce plants grown under salt stress could account for better relative water content and higher aerial FW and DW at harvest (Table 1). However, a higher aerial DW in *Azospirillum*-inoculated plants (Table 1) could imply not only more efficient water absorption but also nutrients. The effect of *Azospirillum* spp. inoculation in the partitioning of dry matter (both carbon compounds and minerals) at the whole plant level has been already reported in grasses, where shoot-to-root ratio was clearly enhanced (Bashan and Dubrovsky, 1996). Also in whole plants, more recent results showed significantly greater plant height in *Azospirillum lipoferum*-inoculated plants than the ones without inoculation, in wheat seedlings continously irrigated with high NaCl concentration (Bacilio et al., 2004).

Salt stress decreased relative water content in non-inoculated lettuce plants at harvest and 20 days after. However, this parameter experienced a significant increase after 10 days storage (Table 1). As suggested by Agüero et al. (2008) in lettuce grown under normal conditions, this transient increase could be the result of an adaptive response of lettuce tissues to product confinement within a preservative water-saturated environment as the one used here. Independently of salt stress effects, *Azospirillum* inoculation promoted higher relative water content both at harvest and after 10 days storage (Table 1).

On the other hand, under normal growth conditions *A. brasilense* Cd has been reported to enhance the production of chlorophylls *a* and *b* and other auxiliary photoprotective photosynthetic pigments that usually increase in wheat grown under stress conditions (Bashan et al., 2006). In our situation, a total chlorophyll content fall in salt stressed lettuce resulting from a chlorophyll *b* decrease was observed at harvest and after 10 days storage (Table 1). On the contrary, total chlorophyll content as well as its chlorophylls *a* and *b* components displayed a significant increase at harvest and after 10 days storage, in *Azospirillum*-inoculated lettuce plants (Table 1). These results extend Bashan et al.. (2006) hypothesis to salt stressing conditions, that inoculation with *Azospirillum* enhanced production of auxiliary photoprotective pigments that are advantageous for plant growth. Further research to explain the bacterial molecular mechanism of action on promoting higher chlorophyll content is needed.

High aerial biomass and chlorophyll content and a turgid product accompanied by a prolonged shelf life are desirable traits but they represent insufficient qualities when the lettuce arrives at the market. Product quality is referred to us as a sum of desirable nutritional and sensorial parameters, including chlorophyll and ascorbic acid contents and color characteristics determined by a sensory panel and chromameter measurements (Tables 1 and 2). In particular, ascorbic acid is an important nutritional element. It participates in the biosynthesis of collagen, neurotransmitters and hormones, enhances intestinal iron absorption and modulates cell growth and differentiation. Epidemiological evidence has also associated antioxidant properties of vitamin C-containing fruit and vegetable consumption with lower risks of cardiovascular diseases (Hacisevki, 2009). Either salt stress or *Azospirillum* inoculation triggered an ascorbic acid increase in lettuce at harvest. However, while its content decreased after 20 days storage in non-inoculated controls, it rosed in *Azospirillum*-inoculated plants (Table 2). Both higher chlorophyll and ascorbic acid contents in lettuce mean higher nutritional quality, parameters that were increased by *Azospirillum* inoculation in plants grown under salt stress (Tables 1 and 2).

Apart from these nutritional traits, the quality of lettuce at the market is usually determined by its visual aspect. A sensorial examination of the product can be standardized by establishing a panel of experts where independent observations are qualified by numbers. This kind of evaluation indicated an overall visual quality loss due to salt stress in fresh-harvested lettuce plants. As a counterpart, a net quality improvement in *Azospirillum*-inoculated plants was observed (Table 2). However, the perception of color by the human eye is highly subjective. This limitation could be surmounted by determining hue, Chroma and L parameters in a chromameter. An optimum *L. sativa* cv. Elisa color quality determined by the sensorial panel has been associated to hue 121 ± 2, Chroma 38.0 ± 2.4 and L 54.0 ± 1.7 chromameter values (data not shown). Hue angles determined in *L. sativa* cv. Lores ranged between 111 and 124, representing hues between yellow and green (Leon et al., 2007). As indicated by hue, chroma and *L* values determined at harvest and postharvest periods, a more deteriorated color was found in lettuce leaves grown under salt stress (Table 1). Similar results have already been reported (Kim et al., 2008). However, *Azospirillum* inoculation contributed to higher color retention in salt-stressed lettuce both at harvest and up to 20 days storage (Table 2), which in turn could be partially due to chlorophyll increase (Table 1). These results agree with the less objective overall visual quality data determined by the sensory panel (Table 2). Though no color tolerance system is perfect, the method used here has a 75% agreement with the visual perception of product (McGuire, 1992). The results indicated that most of the negative changes experienced by the sensorial overall visual quality under salt stress or *Azospirillum* inoculation are accompanied with similar tendencies in hue, Chroma and L.

At harvest, a lower oxidation rate ratio in both control and inoculated plants could be related to higher ascorbic acid content caused by a continous salt stress during lettuce growth (Table 2). It has been reported that several antioxidants including ascorbic acid are involved in environmental adaptation and stress tolerance in lettuce (Oh et al., 2009). On the other hand, salt stress did not change antioxidant activity either in control or inoculated plants (Fig. 3). It has been stressed that no single assay accurately reflects the mechanism of action of all antioxidants present in lettuce plants (Viacaba et al., 2014). In this regard, the β–carotene bleaching test used here could be insufficient to explain a lower oxidation rate ratio and a constant antioxidant activity due to salt stress. However, the presence of other antioxidants as phenolic compounds could also be contributing to reduce oxidation rate ratio (Proteggente et al., 2002).

After 10 days storage, while ascorbic acid remained constant, a low oxidation rate ratio was maintained in non-inoculated plants exposed to salt stress (Table 2). In this regard, the participation of other antioxidants as chlorophylls to maintain low oxidation rate ratio could not be discarded. In effect, recent investigations have shown that chlorophyll and its derivatives act as antioxidants to prevent oxidative DNA damage and lipid peroxidation both by chelating reactive ions and by scavenging free radicals (Hsu et al., 2013). Our results show higher chlorophyll content both at harvest and storage, in *Azospirillum*-inoculated lettuce grown under salt stress than in non-inoculated controls (Table 1). In contrast, a constant oxidation rate and a higher ascorbic acid content was observed in *Azospirillum*-inoculated plants (Table 2). Moreover, while a constant antioxidant activity was observed in salt-stressed control, it was enhanced in the inoculated plants (Fig. 3).

Another important lettuce characteristic taken into account by consumers is tissue browning, whose appearance and progress are directly related to shelf life (Saltveit and Qin, 2008). Wound associated with harvesting cut induces changes in phenolic metabolism and causes stem browning (butt discoloration), process that is accompanied by additional organoleptic and biochemical changes that alter the product quality (Kim et al., 2008). Quality loss has been associated to the action of the enzyme polyphenol oxidase that catalyzes the oxidation of phenolic compounds to *o*-quinones (Viacaba et al., 2014). The possibility of preventing enzymatic browning in fresh-cut products through the application of exogenous ASA suggests the possible involvement of this antioxidant compound in the occurrence of this disorder in wounded tissues (Degl'Innocenti et al., 2007). Our results open the need to study the biochemical mechanisms associated to *Azospirillum* effects in ameliorating lettuce browning at harvest and postharvest periods, in plants grown under salt stress (Table 3).

Even though we do not provide data on the mode of action of *Azospirillum* on enhancing quality at harvest and upon storage in lettuce grown under salt stress, different plausible mechanisms could contribute to the bacterial effects. A higher relative water content in *Azospirillum*-inoculated lettuce plants (Table 1) indicates a better plant water status. This implies a higher cell wall elasticity (Creus et al., 2004), which in turn could explain a higher lettuce texture and a lower mechanical fragility, both parameters included into the overall visual quality assessment (Tables 1 and 2).

On the other hand, even though the chlorophyll biosynthesis pathway is well known, the knowledge on its fine regulation and control of degradation remains elusive. Recent data indicate that phytochrome B mediates the regulation of chlorophyll synthesis through transcriptional regulation of *ChlH* and *GUN4* genes whose products exert their action at the branching point of the chlorophyll biosynthesis pathway (Inagaki et al., 2015). In addition, nitric oxide (NO) regulates many physiological and biochemical processes in plants, including chlorophyll biosynthesis (Liao and Yu, 2014) and breakdown during leaf senescence (Liu and Guo, 2013). On the other hand, it has been shown that NO produced by *Azospirillum* (Creus et al., 2005) induces physiological changes conducting to root branching in inoculated plants (Molina-Favero et al., 2008). The possibility that *Azospirillum* could contribute in this way to improve lettuce quality parameters (Tables 1 and 2) as those related to chlorophyll synthesis and/or its breakwdown, should not be excluded.

It is also well known that salt stress triggers an oxidative stress. Increased accumulation of ascorbic acid and also antioxidant enzyme activities are involved in order to overcome NaCl-induced oxidative stress in lettuce (Eraslan et al., 2007). A key gene as galactose dehydrogenase (*L-GalDH*), involved in the biosynthesis of ascorbic acid, was rapidly and consistently activated in lettuce in response to different abiotic stresses (Oh et al., 2009). On the other hand, the significance of plant growth-promoting rhizobac-

teria mediated increase in antioxidant potential in vegetables is yet unknown (Nautiyal et al., 2008). However, the same authors have shown induction of antioxidant enzymes, namely, polyphenol oxidase, ascorbate peroxidase, catalase, and superoxidase dismutase, in edible parts of *L. sativa,* after inoculation with *Bacillus lentimorbus* B-30488. Thus, the possibility that *Azospirillum* inoculation could enhance L-GalDH and the expression of other genes involved in antioxidant activity in lettuce grown under saline (Table 2 and Fig. 3), should not be discarded.

The above constitute mere speculations on some plausible mechanisms *Azospirillum* could use to improve product quality in lettuce grown under salt stress. An excellent review on this topic provides extense information on the multiple molecular tools this versatile bacteria could use to promote plant growth (Bashan and de-Bashan 2010). The authors emphasize that no unique mechanism had been established to explain the benefical effects. Instead, the most accepted hypothesis postulates that a sum of events accounts for the general plant growth promotion (Bashan and de-Bashan 2010).

5. Conclusion

In this study, *Azospirillum* inoculation not only improved yield and nutritional value but also extended storage life and product quality in lettuce grown under salt stress. The results presented here encourage more studies to explore the possibility of establishing nurseries and/or growing vegetables in world regions where the salt limits the production.

Acknowledgements

This work was supported by Universidad Nacional de Mar del Plata (UNMdP, project FCA AGR369/12) and Instituto Nacional de Tecnología Agropecuaria (INTA, project PNHFA1106082), Argentina. The present paper constitutes part of the PhD thesis of Gabriela Fasciglione at the UNMdP. Thanks are due to Carlos Pereyra for his help at the greenhouse and to Lisa Bradford for correcting the English text.

References

Agüero, M.V., Barg, M.V., Yommi, A., Camelo, A., Roura, S.I., 2008. Postharvest changes in water status and chlorophyll content of lettuce (*Lactuca sativa* L.) and their relationship with overall visual quality. J. Food Sci. 73, S47–S55.

Bacilio, M., Rodriguez, H., Moreno, M., Hernandez, J.P., Bashan, Y., 2004. Mitigation of salt stress in wheat seedlings by a *gfp*-tagged *Azospirillum lipoferum*. Biol. Fertil. Soils 40, 188–193.

Barassi, C.A., Ayrault, G., Creus, C.M., Sueldo, R.J., Sobrero, M.T., 2006. Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. Sci. Hortic. 109, 8–14.

Bashan, Y., 1986. Significance of timing and inoculation with rhizosphere wheat plants. Soil Biol. Biochem. 18, 297–301.

Bashan, Y., Dubrovsky, J.G., 1996. *Azospirillum* spp. participation in dry matter partitioning in grasses at the whole plant level. Biol. Fertil. Soils 23, 435–440.

Bashan, Y., Bustillos, J.J., Leyva, L.A., Hernandez, J.-P., Bacilio, M., 2006. Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. Biol. Fertil. Soils 42, 279–285.

Bashan, Y., de-Bashan, L.E., 2010. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. Adv. Agron. 108, 77–136.

Coleman-Derr, D., Tringe, S.G., 2014. Building the crops of tomorrow: advantages of simbiont-based approaches to improving abiotic stress tolerance. Front. Microbiol. 5, 1–6.

Couture, R., Cantwell, M.I., Ke, D., Saltveit Jr., M.E., 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. HortScience 28 (7), 723–725.

Creus, C.M., Sueldo, R.J., Barassi, C.A., 1997. Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. Plant Physiol. Biochem. 35, 939–944.

Creus, C.M., Sueldo, R.J., Barassi, C.A., 2004. Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. Can. J. Bot. 82, 273–281.

Creus, C.M., Graziano, M., Casanovas, E.M., Pereyra, M.A., Simontacchi, M., Puntarulo, S., Barassi, C.A., Lamattina, L., 2005. Nitric oxide is involved in the *Azospirillum brasilense*-induced lateral root formation in tomato. Planta 221, 297–303.

Degl'Innocenti, E., Pardossi, A., Tognoni Guidi, L., 2007. Physiological basis of sensitivity to enzymatic browning in 'lettuce', 'escarole' and 'rocket salad' when stored as fresh-cut products. Food Chem. 104, 209–215.

del Amor, F., Cuadra-Crespo, P., 2012. Plant growth-promoting bacteria as a tool to improve salinity tolerance in sweet pepper. Funct. Plant Biol. 39, 82–90.

Eraslan, F., Inal, A., Savasturk, O., Gunes, A., 2007. Changes in antioxidative system and membrane damage of lettuce in response to salinity and boron toxicity. Sci. Hortic. 114, 5–10.

Esparza Rivera, J.R., Stone, M.B., Stushnoff, C., Pilon-Smits, E., Kendall, P.A., 2006. Effects of ascorbic acid applied by two hydrocooling methods on physical and chemical properties of green leaf lettuce stored at 5 °C. J. Food Sci. 71, S270–S276.

Fasciglione, G., Casanovas, E.M., Yommi, A., Sueldo, R.J., Barassi, C.A., 2012. *Azospirillum* improves lettuce growth and transplant under saline conditions. J. Sci. Food Agric. 92, 2518–2523.

Gazula, A., Kleinhenz, M.D., Scheerens, J.C., Ling, P.P., 2007. Anthocyanin levels in nine lettuce (*Lactuca sativa*) cultivars: influence of planting date and relations among analytic, instrumented, and visual assessment of color. HortScience 42 (2), 232–238.

Grover, M., Ali Sk, Z., Sandhya, V., Rasul, A., Venkateswarlu, B., 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J. Microbiol. Biotechnol. 27, 1231–1240.

Hacisevki, A., 2009. An overview of ascorbic acid biochemistry. J. Fac. Pharm. Ankara 38 (3), 233–255.

Hsu, C.-Y., Chao, P.-Y., Hu, S.-P., Yang, C.-M., 2013. The antioxidant and free radical scavenging activities of chlorophylls and pheophytins. Food Nutr. Sci. 4, 1–8.

Hunt, R., Causton, D.R., Shipley, B., Askew, A.P., 2002. A modern tool for classical plant growth analysis. Ann. Bot. 90, 485–488.

Inagaki, N., Kinoshita, K., Kagawa, T., Tanaka, A., Ueno, O., Shimada, H., Takano, M., 2015. Phytochrome B mediates the regulation of chlorophyll biosynthesis through transcriptional regulation of *ChlH* and *GUN4* in rice seedlings. PLoS One 10 (8), e0135408, http://dx.doi.org/10.1371/journal.pone.0135408.

Kim, H.J., Fonseca, J.M., Choi, J.-H., Kubota, C., Kwon, D.Y., 2008. Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). J. Agric. Food Chem. 56, 3772–3776.

Kohler, J., Caravaca, F., Roldán, A., 2010. An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*. Soil Biol. Biochem. 42, 429–434.

Kulisic, T., Radonic, A., Katalinic, V., Milosa, M., 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. 85, 633–640.

Leon, A.P., Viña, S.Z., Frezza, D., Chaves, A., Chiesa, A., 2007. Estimation of chlorophyll contents by correlations between SPAD-502 meter and chroma meter in butterhead lettuce. Commun. Soil Sci. Plant Anal. 20, 2877–2885.

Lester, G.E., 2006. Environmental regulation of human health nutrients (ascorbic acid, β-carotene, and folic acid) in fruits and vegetables. HortScience 41 (1), 59–64.

Liao, W.-B., Yu, J.-H., 2014. Nitric oxide and other signaling molecules: a cross-talk in response to abiotic stress. In: Khan, M.N., Mobin, M., Mohammad, F., Corpas, F.J. (Eds.), Nitric Oxide in Plants: Metabolism and Role in Stress Physiology. Springer, pp. 185–198, Ch. 11.

Liu, F., Guo, F.-Q., 2013. Nitric oxide deficiency accelerates chlorophyll breakdown and stability loss of thylakoid membranes during dark-induced leaf senescence in arabidopsis. PLoS One 8 (2), e56345, http://dx.doi.org/10.1371/journal.pone.0056345.

Martín-Diana, A.B., Rico, D., Barry-Ryan, C., Frías, J.M., Henehan, G.T.M., Barat, J.M., 2007. Efficacy of steamer jet-injection as alternative to chlorine in fresh-cut lettuce. Postharvest Biol. Technol. 45, 97–107.

McGuire, R.G., 1992. Reporting of objective color measurements. HortScience 27 (12), 1254–1255.

Molina-Favero, C., Creus, C.M., Simontacchi, M., Puntarulo, S., Lamattina, L., 2008. Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato. Mol. Plant Microbe Interact. 7, 1001–1009.

Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A., Ashraf, M., 2014. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol. Adv. 32, 429–448.

Nautiyal, C.S., Govindarajan, R., Lavania, M., Pushpangadan, P., 2008. Novel mechanism of modulating natural antioxidants in functional foods: involvement of plant growth promoting rhizobacteria NRRL B-30488. J. Agric. Food Chem. 56, 4474–4481.

Oh, M.-M., Trick, H.N., Rajashekar, C.B., 2009. Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. J. Plant Physiol. 166, 180–191.

Poorter, H., Remkes, C., 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83, 553–559.

Proteggente, A., Pannala, A., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van de Put, F., Dacombe, C., Rice-Evans, C., 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radical Res. 36, 217–233.

Saltveit, M.E., Qin, L., 2008. Heating the ends of leaves cut during coring of whole heads of lettuce reduces subsequent phenolic accumulation and tissue browning. Postharvest Biol. Technol. 47, 255–259.

Shannon, M.C., 1997. Adaptation of plants to salinity. Adv. Agron. 60, 75–120.
Subhasree, B., Baskar, R., LaxmiKeerthana, R., Lijina, S.R., Rajasekaran, P., 2009. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chem. 115, 1213–1220.
U.S. Department of Agriculture, Agricultural Research Service. 2014. USDA National Nutrient Database for Standard Reference, Release 27. Nutrient Data Laboratory Home Page, Available on line (10 October 2014) at http://www.ars.usda.gov/ba/bhnrc/ndl.
Viacaba, G.E., Gonzales-Aguilar, G., Roura, S.I., 2014. Determination of phytochemicals and antioxidant activity in butterhead lettuce related to leaf age and position. J. Food Biochem. 38, 352–362.
Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113–4117.
Yamaguchi, T., Blumwald, E., 2005. Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci. 10, 615–620.