

# Root adaptation and ion selectivity affects the nutritional value of salt-stressed hydroponically grown baby-leaf *Nasturtium officinale* and *Lactuca sativa*

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The response of watercress (*Nasturtium officinale* L.) to salinity has been scarcely addressed in literature despite its growing importance in the baby-leaf market and its wide cultivation in salt-affected agricultural regions. This work evaluates the effect of salinity (2.5, 5 and 10 dS m<sup>-1</sup>) on productive and quality features of watercress compared with another crop widely cultivated for the baby-leaf sector (lettuce, *Lactuca sativa*). In watercress, a linear relationship ( $R^2=0.75$ ) was observed between yield decrease and Cl<sup>-</sup> accumulation in leaves, whereas yield was not affected by salinity in lettuce. NaCl application increased Na<sup>+</sup> accumulation at the expense of Ca<sup>2+</sup> uptake in the leaf tissues of both crops, but also of K<sup>+</sup> in watercress. Health-related features were improved by salinity (e.g. increased phenolics and reduced nitrates), especially in watercress, with limited sensorial quality evaluation effects.

**Key words:** phenolics, nitrates, salinity, quality, ready-to-eat

## Introduction

Increased health consciousness and growing interest in the role of food for maintaining and improving human well-being have resulted in changes in dietary habits and are important factors in the increased diffusion of minimally processed or fresh-cut leafy vegetables (Soliva-Fortuny and Martín-Belloso 2003, Brecht et al. 2004). Leafy vegetables are widely considered as a fundamental source of vitamins, minerals, fibre, and other phytochemicals with health-promoting effects, such as carotenoids and polyphenols (Kimura et al. 2003). Nonetheless, their nutritional quality can be influenced by several factors during the growing season or as a result of post-harvest processes (Kader 2008), leading to the biosynthesis and accumulation of antinutritional and toxic principles such as nitrate, oxalate and saponins (Amr and Hadidi 2001). Plants exposed to environmental stresses have been shown to increase the levels of beneficial compounds they contain (e.g. antioxidants), and to enhance specific quality features, such as a reduction in the leaf nitrate content (Pérez-López et al. 2015). The use of soilless cultivation systems such as the floating system allows clean and safe vegetables to be obtained for the processing industry. Moreover, being relatively cheap, this technique can be easily implemented at commercial level and promotes efficient water use (Rodríguez-Hidalgo et al. 2009). Indeed, its application for growing fresh-cut vegetables has increased steadily in recent years (Tomasi et al. 2015), only inhibited by water quality and availability (Acosta et al. 2011). In recent years, an increase in the application of intensive agricultural practises (including the wide use of fertilizers) and the frequency of extreme droughts have increased salinization of the available irrigation water (Perez-Sirvent et al. 2003, Ekholm et al. 2007).

Watercress (*Nasturtium officinale* R. Br.), a member of the Brassicaceae family, is native of Europe, northern Africa and temperate and tropical parts of Asia and Europe, and it is naturalized elsewhere (GRIN 2012). It is normally consumed as a vegetable in fresh salads. Several studies have reported the beneficial effects to human health of consuming watercress; these include anticancer effects (Verhoeven et al. 1997), reduced lymphocyte DNA damage and alterations of the blood antioxidant status in healthy adults (Gill et al. 2007), as well a well-documented

diabetes prevention effect (Shahrokhi et al. 2009). However, knowledge of the tolerance to salt stress of this species is limited, although Kaddour et al. (2013) reported that at up to 100 mM NaCl, watercress responses to salt include maintained growth, osmotic adjustment via  $\text{Na}^+$  and  $\text{Cl}^-$ , and high  $\text{K}^+:\text{Na}^+$  selectivity. In the same way, natural antioxidant defence compounds (vitamin C, carotenoids and polyphenols) increased when plants were grown at up to 100 mM NaCl. However, despite its generally high concentrations in plant tissue, there are no reports on the potential effect of salinity for reducing the nitrate content of watercress (Pignata et al. 2016). This contrasts with the several studies that highlight decreases in the nitrate content of lettuce (*Lactuca sativa* L.) as salinity increases (Scuderi et al. 2011, Neocleus et al. 2014).

In view of the above, the aim of the present work was to compare the responses of one variety of watercress and one variety of lettuce to different salinity conditions in terms of plant growth performances, mineral composition and some aspects of nutritional quality.

## Materials and methods

### Plant material and growth conditions

The experiments were conducted at the “Tomás Ferro” Experimental Agro-Food Station, Technical University of Cartagena (UPCT; lat. 37° 41' N; long. 0° 57' W). The commercial cultivar of watercress (*Nasturtium officinale* R. Br.) “Large leaf” (Tozer Seeds Co., Cobham, U.K.) and of baby leaf lettuce (*Lactuca sativa*) cv. Ganeria (green batavia) (Rijk Zwaan, De Lier, Netherlands) were cultivated in a floating system in an unheated greenhouse covered with a thermal low density polyethylene film of 200  $\mu$  thick. Two crop cycles (winter and spring) were carried out with sowings on 20 February 2012 (lettuce) and 27 February 2012 (watercress) and in 23 April 2012 (lettuce) and 30 April 2012 (watercress). Sowing was carried out manually into “styrofloat” trays containing peat, which were then transferred to flotation beds, floating on fresh tap water with an electrical conductivity (EC) of 1.1  $\text{dS}\cdot\text{m}^{-1}$  and a pH of 7.8 (Niñirola et al. 2014). Aeration was provided using a blow pump connected to a perforated pipe trellis positioned at the bottom of each flotation bed. Seven days after sowing (DAS) the watercress and lettuce plants were thinned, leaving 12 (2040 plants  $\text{m}^{-2}$ ) and 10 (1700 plants  $\text{m}^{-2}$ ) plants per hole, respectively. At the same time, the tap water in the beds was replaced with a nutrient solution containing, in mM:  $\text{NO}_3^-$ , 7.2;  $\text{NH}_4^+$ , 4.8;  $\text{H}_2\text{PO}_4^-$ , 2.0;  $\text{Ca}^{2+}$ , 3.2;  $\text{K}^+$ , 6.0 and  $\text{Mg}^{2+}$ , 4.0, plus a commercial solution of microelements Nutromix 10, Biagro (2  $\text{mg}\cdot\text{l}^{-1}$ ) and Sequestrene (an Iron chelate, 1.5  $\text{mg}\cdot\text{l}^{-1}$ ). The nutrient solution was adjusted to EC 2.5  $\text{dS}\cdot\text{m}^{-1}$  and pH 5.6.

Sodium chloride was added to the nutrient solution for 5 days starting on DAS 13 to reach total concentrations of 9 mM, 41 mM and 91 mM NaCl, thus providing three different levels of salinity (2.5, 5 and 10  $\text{dS}\cdot\text{m}^{-1}$ ). Each treatment level for all the experiments was carried out in 135 cm x 125 cm x 20 cm beds located in three places inside a greenhouse. Each bed had four floating trays of 60 cm x 41 cm. The EC and temperature of the nutrient solution were monitored during the growing cycles using Campbell CS547 sensors (Campbell Scientific Inc., Logan, USA) in each flotation bed.

Harvesting was carried out at the same phenological stage for both cycles, that is, when three to four leaves in lettuce and seven to eight leaves in watercress had formed on each plant. Forty eight plants in watercress and forty plants in lettuce from four fissures randomly chosen from each tray were harvested for each treatment. The plants were divided randomly into two lots, one for harvest analysis and one for postharvest analysis.

### Analyses and determinations

Fresh and dry matter of the aerial part and root growth were measured. Total root length and diameter were determined only in winter grown plants with a Winrhizo LA 1600 root counter (Regent Inc., Quebec, Canada). The dry matter contents were determined by drying in an oven at 50 °C until constant weight was reached. In addition, the total production (yield) was calculated. Moreover, the nitrate, potassium, calcium, sodium and chloride contents were extracted in triplicate by using 0.2 g of shoot dry samples per each treatment and quantified by ion chromatography (Lara et al. 2011). The total phenolic content was determined by the Folin-Ciocalteu colorimetric method (Tarazona-Díaz et al. 2011).

### Sensory panel test

In the spring cycle, harvested plants were disinfected, packed in thermosealed polypropylene (PP) baskets of 1 l capacity and stored at 5 °C for 7 days, according to Niñirola et al. (2014). The sensory quality was evaluated in a tasting room after 7 d of cold storage by a test-trained panel consisting of 11 people (6 females and 5 males).

Visual quality factors (overall visual quality and global quality) were scored on a 9-point hedonic scale (1 = extremely poor, 3 = poor, 5 = acceptable and limit of usability, 7 = good, and 9 = excellent). Disorders (browning, visual dehydration, off-odours, off-colour, and off-flavours) were scored according to the following scale of damage incidence and severity: 1 = none, 2 = slight, 3 = moderate (limit of usability), 4 = severe, 5 = extreme (Tomás-Callejas et al. 2011).

### Statistical analysis

A randomized complete block design with three replicates per level of salinity was used in the greenhouse in both growing seasons. Data were analysed using ANOVA and when significant differences ( $p \leq 0.05$ ) were observed, means were compared by Least Significant Difference (LSD).

## Results

### Environmental conditions during the experiments

The winter growing cycle for lettuce lasted 28 days, during which the temperatures ranged between 15.5 °C and 23.2 °C (Table 1).

Table 1. Environmental conditions during the experiments

		Days	Temperature			Daily light integral (mol·m <sup>-2</sup> ·s <sup>-1</sup> )
			Min (°C)	Avg (°C)	Max (°C)	
Lettuce	Winter	28	15.5	18.3	23.2	9.6
	Spring	23	17.8	21.4	24.4	9.6
Watercress	Winter	27	12.1	18.0	23.2	7.3
	Spring	27	17.8	23.3	26.0	8.4

The spring cycle was shorter at 23 days, with an average temperature of 21.4 °C. The mean amount of photosynthetically active radiation was 9.6 mol m<sup>-2</sup> s<sup>-1</sup> in both cycles. In the case of watercress, the winter and spring growing cycles had the same duration of 27 days. Differences in the light integral were evident in watercress, being higher in spring (8.4 mol m<sup>-2</sup> s<sup>-1</sup>) than in winter (7.3 mol m<sup>-2</sup> s<sup>-1</sup>) (Table 1). During the spring cycle, the average temperature of 23.3 °C was higher than in the winter cycle (Table 1).

### Yield and plant composition in response to salinity and growing season

Root morphology was observed to be modified as a result of salinity stress, with a general reduction in the percentage of thicker roots (> 0.5 mm) in both lettuce and watercress exposed to 5 dS m<sup>-1</sup> compared with plants grown in control conditions (Fig. 1). However, when salinity was increased to 10 dS m<sup>-1</sup>, the percentage of thicker watercress roots was reduced further, while in lettuce a reduction in the percentage of thinner roots (< 0.5 mm) resulted in non-significant decrease in the relative quantity of thicker roots compared with those in control conditions, restoring control mean root diameter (Fig. 1).

Lettuce yield was not affected by salinity in either growing season: mean of, 2.4 kg m<sup>-2</sup> and 2.3 kg m<sup>-2</sup> for the three salinity treatments in winter and spring, respectively (Fig. 2A). In watercress grown in the winter cycle, the yield was not reduced at 5 dS m<sup>-1</sup> salinity (compared with the control), whereas a 37% decrease was measured at 10 dS m<sup>-1</sup>. The greatest yield reduction was observed in watercress in spring causing a 28% and 46% decrease at 5 and 10 dS m<sup>-1</sup>, respectively (Fig. 2D).

Saline stress resulted in changes in ion concentrations. The Cl<sup>-</sup> concentration, for example, increased in both lettuce and watercress as salinity increased (Figs. 2B and 2E), although to a greater extent in watercress exposed to 10 dS m<sup>-1</sup> salinity in spring, when it reached values of 4866 mg kg<sup>-1</sup> FW (Fig. 2E). The absence of any significant yield depletion in response to salinity resulted in a low regression coefficient between relative yield and Cl<sup>-</sup> concentration in lettuce (Fig. 2C), whereas high regression coefficients were observed in watercress (R<sup>2</sup> = 0.80 and R<sup>2</sup> = 0.87 in winter and spring cycles, respectively) (Fig. 2F), pointing to an inverse linear relationship between Cl<sup>-</sup> accumulation and yield.

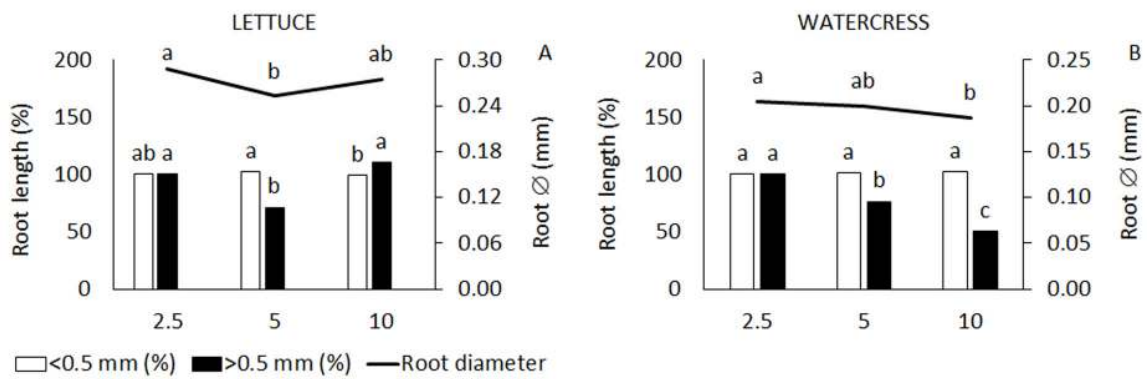


Fig. 1. Effect of salinity (2.5, 5, and 10, dS m<sup>-1</sup>) on length of roots (% of control value at 2.5 dS m<sup>-1</sup>) of lettuce (A) and watercress (B) plants grown in winter, according to their diameter below 0.5 mm (white) or above 0.5 mm (black), and their mean diameter ( $\phi$ ) in mm. Different letters indicate significant differences at  $p \leq 0.05$ .

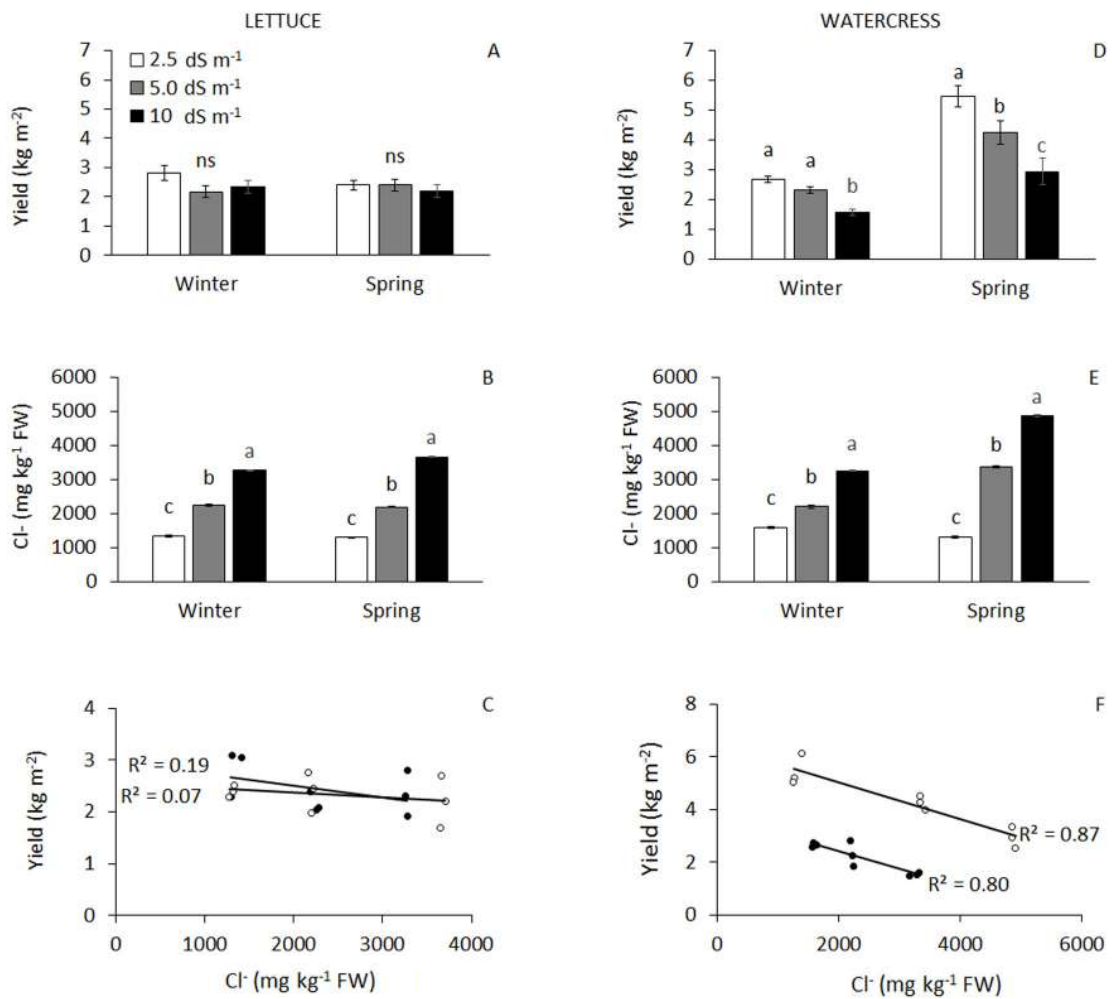


Fig. 2. Effect of salinity (2.5, 5 and 10 dS m<sup>-1</sup>) on yield (kg m<sup>-2</sup>), Cl<sup>-</sup> content in leaves (mg kg<sup>-1</sup> FW) and their relationship (winter, closed symbols, and spring, open symbols) in lettuce (A, B, C) and watercress (D, E, F). Mean values  $\pm$  SE. Different letters indicate significant differences at  $p \leq 0.05$ , according to LSD test; ns = no significant differences.

A significant increase in  $\text{Na}^+$  accumulation in leaf tissues of both species was associated with salinity stress (Figs. 3A and 3D). The highest  $\text{Na}^+$  accumulation was observed in winter cultivated watercress ( $3028 \text{ mg kg}^{-1} \text{ FW}$ ) subject to  $10 \text{ dS m}^{-1}$  (Fig. 3D) while the lowest  $\text{Na}^+$  accumulation was observed in lettuce (Fig. 3A), independently of the growing season. Both the  $\text{Na}^+:\text{K}^+$  and  $\text{Na}^+:\text{Ca}^{2+}$  ratios increased with salinity in both lettuce and watercress (Figs. 3B, 3C, 3E and 3F). The highest  $\text{Na}^+:\text{K}^+$  ratio was associated with the winter cultivation of watercress (Fig. 3E) while the  $\text{Na}^+:\text{Ca}^{2+}$  ratio increased most in winter cultivated lettuce (Fig. 3C).

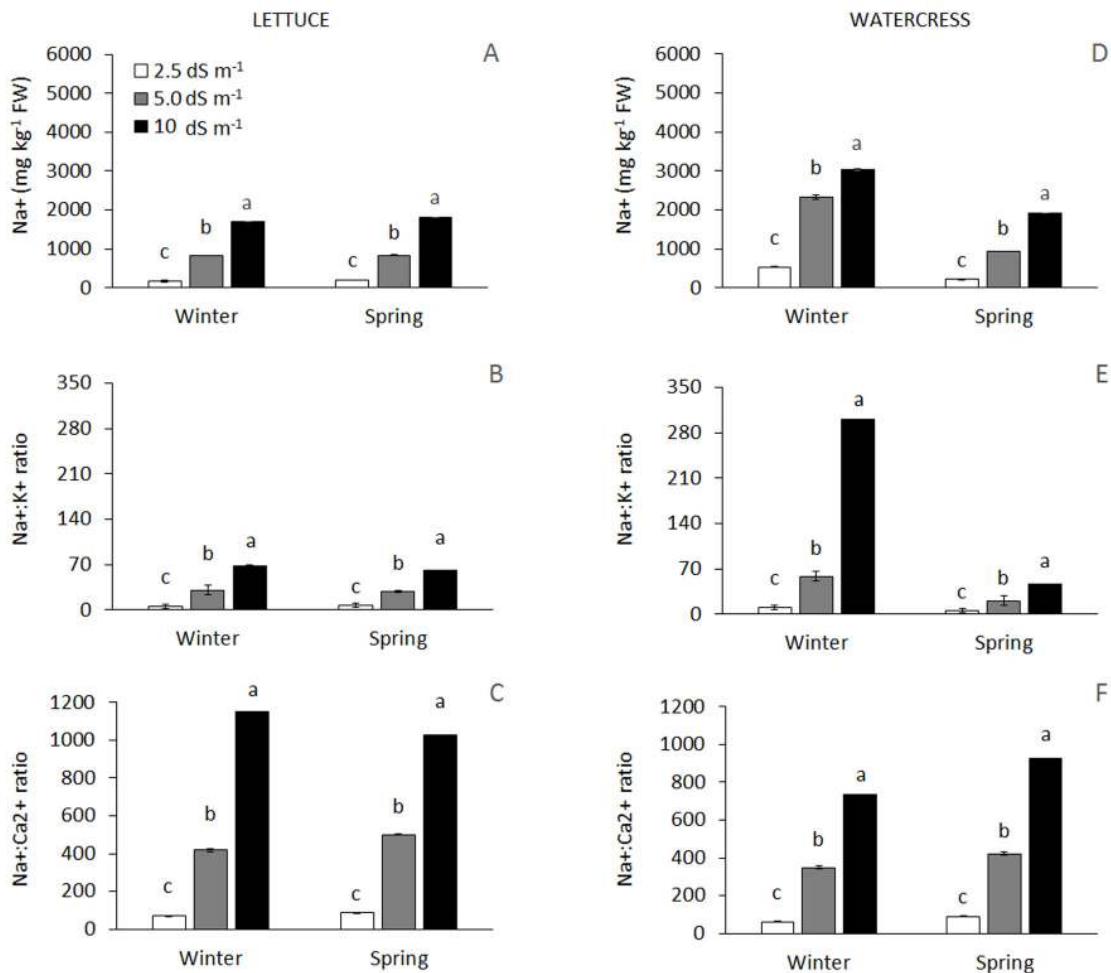


Fig. 3. Effect of salinity (2.5, 5, and 10  $\text{dS m}^{-1}$ ) on  $\text{Na}^+$  ( $\text{mg kg}^{-1} \text{ FW}$ ),  $\text{Na}^+:\text{K}^+$  ratio and  $\text{Na}^+:\text{Ca}^{2+}$  ratio of lettuce (A, B, C) and watercress (D, E, F). Mean values  $\pm$  SE. Different letters indicate significant differences at  $p \leq 0.05$ , according to LSD test.

Nitrate accumulation decreased as the level of salinity increased in both species (Figs. 4A and 4C). In lettuce, nitrate levels were highest in spring, with a mean value of  $1728 \text{ mg kg}^{-1} \text{ FW}$  for 2.5 and 5  $\text{dS m}^{-1}$  of salinity, but  $1583 \text{ mg kg}^{-1} \text{ FW}$  in the 10  $\text{dS m}^{-1}$  treatment (Fig. 4A). In watercress, nitrate accumulation was about 4-folds higher when the plant was grown in the winter cycle ( $6860 \text{ mg kg}^{-1} \text{ FW}$ , average for 2.5 and 5  $\text{dS m}^{-1}$ ) as compared with plants grown in spring under 10  $\text{dS m}^{-1}$  (Fig. 4C). High regression coefficients were observed between leaf chloride and nitrate concentrations in both lettuce and watercress plants in both growing seasons (Figs. 4B and 4D).

The total phenolic content of lettuce was not affected by salinity in winter, while in spring salinity stress resulted in a 33% increase in phenolic biosynthesis (mean value of 5 and 10  $\text{dS m}^{-1}$ , Fig. 5A). In watercress grown in winter no statistical difference was found in total phenolics between 2.5 and 5  $\text{dS m}^{-1}$  salinity levels (mean value  $34 \text{ mg CAE kg}^{-1} \text{ FW}$ ) while 10  $\text{dS m}^{-1}$  resulted in a 2-fold increase in the phenolic concentration in control conditions. In spring, watercress reached its highest level of total phenolics ( $134 \text{ mg CAE kg}^{-1} \text{ FW}$ ) at the highest level of salinity stress (Fig. 5B).

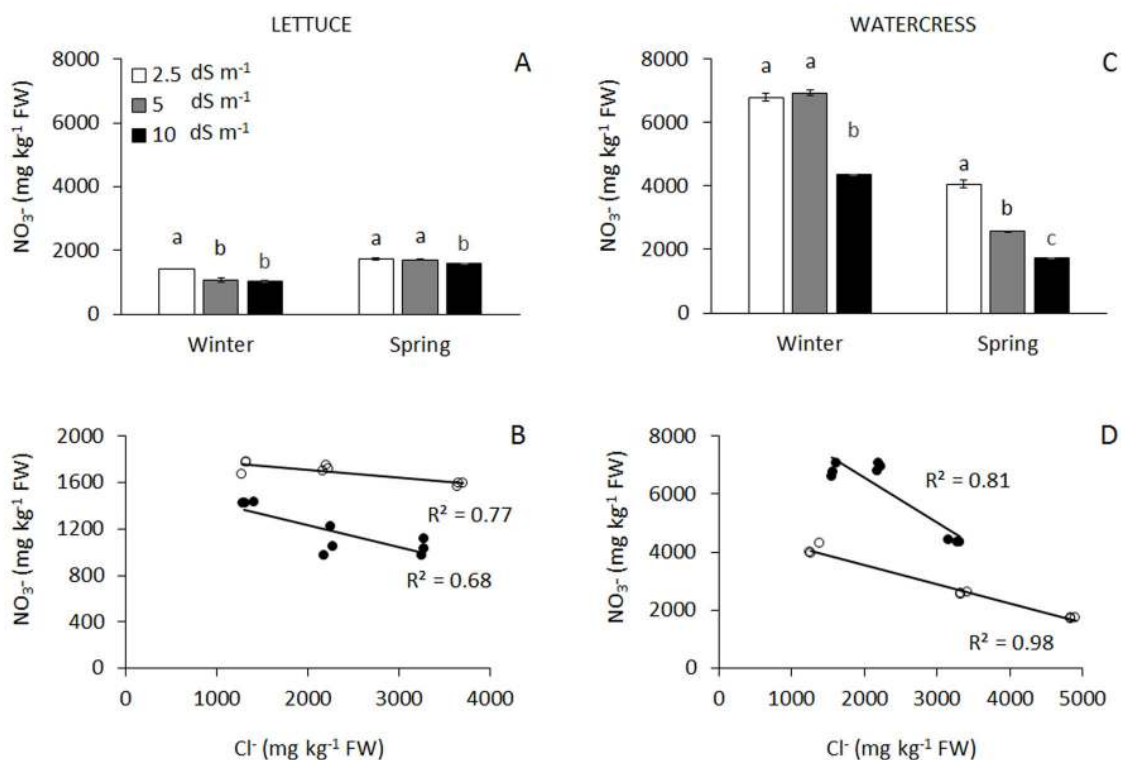


Fig. 4. Effect of salinity (2.5, 5 and 10 dS m<sup>-1</sup>) on leaf nitrate content (mg kg<sup>-1</sup> FW, left) and linear relationships between leaf nitrate and chloride concentration in winter (closed symbols) and spring (open symbols) grown lettuce (A, B) and watercress (C, D). Mean values ± SE. Different letters indicate significant differences at *p* ≤ 0.05, according to LSD test.

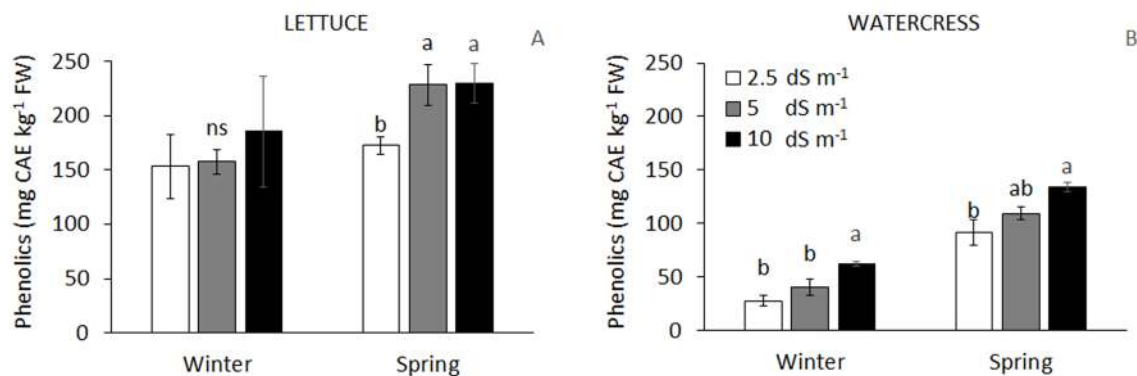


Fig. 5. Effect of salinity (2.5, 5 and 10 dS m<sup>-1</sup>) on leaf phenolics (mg CAE kg<sup>-1</sup> FW) of lettuce (A) and watercress (B). Mean values ± SE. Different letters indicate significant differences at *p* ≤ 0.05, according to LSD test.

No differences were observed among most of the parameters examined in the sensory evaluation (Table 2), except “strange taste”, which was significantly higher in watercress grown under 10 dS m<sup>-1</sup> than in plants grown in control conditions in the spring cycle.



Table 2. Sensory quality evaluation of lettuce and watercress grown in floating system in spring cycle as a consequence of nutrient solution salinity (2.5, 5 and 10 dS m<sup>-1</sup>) and stored for 7 days at 5 °C, as reported by 11 independent panellists. Different letters indicate significant differences at  $p \leq 0.05$ .

		Strange colour	Browning	Dehydration	Strange aromas	Strange taste	Visual appearance	Global quality
Lettuce	2.5	1.71	2.00	2.57	1.14	1.57	6.42	6.71
	5	2.14	1.85	2.57	1.57	1.85	6.42	6.57
	10	2.57	2.14	2.00	1.28	2.00	5.71	5.85
Watercress	2.5	1.16	1.00	1.66	1.16	1.16	7.33	7.66
	5	1.00	1.20	1.60	1.00	1.20	7.00	7.20
	10	1.33	1.16	1.66	1.16	2.33	7.00	6.50

## Discussion

### Plant growth and mineral uptake

The decrease in root diameter as a response to salt conditions has previously been reported in cotton (Zhang et al. 2014). However, the activation of such a response was not always evident in mildly salt-stressed lettuce plants (Lucini et al. 2015), as it is generally dependent on the magnitude of the stress perceived by the crop. On the other hand, a Cl<sup>-</sup> toxicity effect in leaves was assumed in watercress exposed to salinity (Figs. 2E and 2F). This was especially true in spring when, due to the higher temperatures (Table 1), plant growth was higher (Fig. 2D), resulting in greater water uptake and a consequent increase in Cl<sup>-</sup> accumulation (Fig. 2E). Unexpectedly, the lettuce yield response to salinity (Fig. 2A) differed from previous results reported in the literature (Ünlükara et al. 2008), where increasing salinity in a similar range (0.7 to 7 dS m<sup>-1</sup>) linearly reduced yield (about 12% reduction for each additional dS m<sup>-1</sup> in the nutrient solution). In the 1990s, the salinity threshold beyond which yield is reduced in lettuce was set at 2.7 dS m<sup>-1</sup> (De Pascale and Barbieri 1995), although this applies to standard growing conditions and may vary in response to the peculiar environmental features in which plants are grown. In the same study, however, lettuce was described as a relatively tolerant species compared with other winter vegetables (e.g. fennel), with a yield decrease slope of about 5.8% per dS m<sup>-1</sup>.

The increase in Na<sup>+</sup> and Cl<sup>-</sup> concentrations as the result of NaCl-salinity is a common and expected response, which has been reported for both lettuce (Eraslan et al. 2010) and watercress (Kaddour et al. 2013). Nonetheless, beyond the toxicity effect of chloride on the cell structure, plant Na<sup>+</sup> accumulation in shoots may result in reduced growth due to the impaired absorption of important nutritional ions (e.g. K<sup>+</sup> or Ca<sup>2+</sup>). In the studied species, differences in ion selectivity under salinity were evident. Lettuce mainly showed a reduced Ca<sup>2+</sup> content (Fig. 3C), (Neocleous et al. 2014) but unchanged K<sup>+</sup> uptake, resulting in maintained growth rates independently of salinity. The capacity of this species to preserve K<sup>+</sup> uptake in the face of salinity was previously documented (Kohler et al. 2009) and may explain the absence of a visible salt-induced yield reduction. As regards Ca<sup>2+</sup>, salinity not only reduced the accumulation of this ion in leaf tissue as a result of the accumulation of Na<sup>+</sup>, but also as a consequence of its reduced mobility and transport to shoots due to the lower water uptake (Coskun et al. 2013, Osakabe et al. 2014). While reduced water uptake impaired watercress growth in saline conditions (Fig. 3D), the Ca<sup>2+</sup> reduction was linearly correlated with Na<sup>+</sup> accumulation in shoots (Fig. 3F). Nonetheless, a consistent 5-fold reduction in K<sup>+</sup> uptake in 10 dS m<sup>-1</sup> treated plants (data not shown) resulted in a dramatic increase in the Na<sup>+</sup>:K<sup>+</sup> ratio in winter grown watercress (Fig. 3E), suggesting that in milder climatic conditions the plant counterbalanced a reduced Ca<sup>2+</sup> uptake by modulating the accumulation of K<sup>+</sup>. Similar responses have previously been observed in other Brassicaceae, such as cabbage (Lee et al. 2013) and canola (Tunçtürk et al. 2013), but also *Arabidopsis thaliana* (Kaddour et al. 2013), where at least 35 genes were confirmed as modulating K<sup>+</sup> uptake and transport in response to salinity (Qi and Spalding 2004). Any changes in the status of this cation (particularly strong K<sup>+</sup> deficiency) will affect growth by limiting cell expansion and inhibiting photosynthetic processes (Lebaudy et al. 2007). In previous studies, watercress was shown to efficiently maintain a fairly constant Na<sup>+</sup>:K<sup>+</sup> ratio up to the application of 100 mM NaCl (Kaddour et al. 2013), while any further increase in salinity would cause a rapid growth in the index comparable to that observed in our results obtained during the winter cycle.

## Quality of baby-leaf vegetables exposed to salt stress

Salinity caused a rise in the leaf concentrations of sodium of up to 1800 and 3000 mg kg<sup>-1</sup> in lettuce and watercress, respectively (Fig. 3). Salt recognition thresholds are strongly influenced by consumption habits (Hayes et al. 2010) and have been shown to range between around 600 (Hatae et al. 2009) and 1300 (Lucas et al. 2011, Mitchell et al. 2012) mg kg<sup>-1</sup>, being generally lower in female subjects, who are typically used to a lower dietary salt intake. Accordingly, the highest salinity level tested could result in an appreciable salty taste of the product, as was suggested by the sensory panel test analysis in watercress, which mentioned a “strange” taste (Table 2). Nonetheless, comparative studies between salt-rich and salt-poor foods found that no significant effect on consumer acceptability and purchase intent scores are generally related to product salinity (Mitchell et al. 2012).

The observed phenolics concentrations were in line with previous values reported in the literature (Conesa et al. 2015). The increased phenolics concentration in salinized crops in winter (watercress only) and spring (both crops) (Fig. 5) is consistent with the activation of a biochemical response to salinity as previously documented in both lettuce (Pérez-López et al. 2015) and watercress (Kaddour et al. 2013). Although the ion increase in the leaf tissue could also be associated with negative health effects (e.g. hypertension risk associated with salt intake — He and MacGregor 2009, Soetan et al. 2010), the proper management of salt concentration in the nutrient solution has been proven to provide an effective tool for improving leafy vegetable quality (Gruda 2009). More specifically, the proper management of salts in the nutrient solution in floating systems could be considered an effective tool to improve quality and nutritional aspects (Colla et al. 2013).

In spring, the nitrate concentration in lettuce plants was higher than the values observed in winter. Nitrate accumulation in plants is generally associated with a direct relationship with nitrogen availability (Kerbirou et al. 2013), and with an inverse relationship with water chloride concentration (Neocleous et al. 2014) and nitrate-reductase activity (Pinto et al. 2014). The enzymatic activity of nitrate reductase is known to be modulated by light access (Pinto et al. 2014), which, in lettuce, presented similar values in winter and spring cycles (9.6 mol m<sup>-2</sup> s<sup>-1</sup>, Table 1). Consistently, while no seasonal effect could be observed in the overall nitrate concentration in lettuce, Cl<sup>-</sup> salinity was responsible for the decreased nitrate content compared with measurements made in control conditions in both growing cycles (Fig. 4B). The same was observed in watercress, although the detected differences in the overall light integral (7.3 vs. 8.4 mol m<sup>-2</sup> s<sup>-1</sup> in winter vs. spring, respectively, Table 1) resulted in a much greater nitrate content in winter compared with spring-grown plants (Fig. 4C). Furthermore, the difference in both average (+5 °C) and maximum (+3 °C) temperatures was higher (Table 1) in spring than in winter. It has recently been shown how genes responsible for modulating nitrate reductase activity in *Arabidopsis* are promoted when temperatures increases from 22 to 28 °C (Wang et al. 2013). The lower nitrate values consistently, observed in the nitrate content in spring may be a result of both light access and increased temperatures, with the subsequent activation of nitrate reductase (Fig. 4). Given that differences in nitrate accumulation in response to salinity have been also shown to depend on a range of other factors (e.g. cultivar—Escobar-Gutierrez et al. 2002, Burns et al. 2011—; nitrogen quantity and form—Santamaria et al. 1998, Konstantopoulou et al. 2010, M'hamdi et al. 2014 or harvest time—Orsini and De Pascale 2006), further experiments should address complementary strategies for reducing nitrates in leafy vegetables.

## Conclusions

The present work provides a comprehensive vision of the elements involved in the salt stress response of watercress in different growing seasons. Overall, despite the observed yield reduction in the presence of salinity, the nutritional quality of the harvested product was improved both in terms of nutritional (increased phenolics) and anti-nutritional (reduced nitrate) compounds. These effects were maximized in the winter cycle, when detrimental effects on yield were also lower. No differences in the sensory evaluation were associated with salinity in the studied species, except in watercress grown at 10 dS m<sup>-1</sup> when a “strange taste” was detected by panellist. Accordingly, moderately saline water may be used to obtain watercress and lettuce plants with improved nutraceutical value for the baby-leaf market sector.

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