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Growing Hardier Crops for Better Health: Salinity Tolerance and the Nutritional Value of Broccoli

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To evaluate the variations in the nutritional components of a broccoli cultivar under saline stress, two different NaCl concentrations (40 and 80 mM) were assayed. Glucosinolates, phenolic compounds, and ascorbic and dehydroascorbic acids (vitamin C) were analyzed by HPLC, and mineral composition was determined by ICP spectrophotometry. Qualitative differences were observed for several bioactive compounds depending on the plant organ and the intensity of the salt stress. Glucosinolate content showed the most significant increase in the florets; phenolic compounds also increased in the florets, whereas no variation in the vitamin C content was observed as a result of the saline treatments. The mineral composition of the edible parts of the inflorescences remained within the range of the recommended values for human consumption. Overall, the nutritional quality of the edible florets of broccoli was improved under moderate saline stress.

KEYWORDS: *Brassica oleracea*; bioactive compounds; glucosinolates; phenolics; vitamin C; mineral nutrients; salinity

INTRODUCTION

Irrigation with saline water is one of the main factors that lead to a decrease in agricultural productivity. Generally, it is known that salt stress depresses a number of morphological, physiological, biochemical, and molecular characteristics in plants (*1*−*3*) and disrupts homeostasis in water potential (*4*). In these conditions plants undergo an osmotic adjustment, and the compatible solutes or osmoprotectants accumulated include different secondary metabolites (*5*). Glucosinolates has been observed to increase under salt stress, suggesting that under low water potential they could be involved in the osmotic adjustment (*6*).

A diet rich in Brassicaceae vegetables may help to decrease the risk of developing cancer (*7*, *8*), with broccoli being distinguished by the presence of numerous bioactive compounds with health-promoting properties, for example, glucosinolates, phenolics, vitamin C, and carotenoids (*8*). Broccoli has been considered to be a moderately sensitive crop to salinity, showing a biphasic response. During the first phase, there is a significant reduction in growth, more related to water stress, whereas in the second phase, the growth reduction could be explained by specific injury in the plant due to Na^+ or Cl^- accumulation, because osmotic adjustment was achieved and water relations were re-established (*9*).

Glucosinolates, a class of sulfur compounds prevalent in Brassicaceae, have been recognized as having a health-promoting effect depending on sulfate assimilation (*10*). It has been shown that some environmental factors can change the glucosinolate content, such as temperature and photoperiod (*11*), season (*12*), or sulfur fertilization (*13*). In addition, large differences in the levels of glucosinolates have been observed in *Brassica* plants, presumably due to the use of different varieties, analytical methods, and environmental conditions (*13*). However, more information is needed regarding the effect of external stress on glucosinolate accumulation in the plant. Other than glucosinolates, robust health-promoting properties have also been attributed to phenolic compounds (flavonoids and hydroxycinnaric acid derivates) and mineral nutrients, also found in broccoli (*8*), due to their antioxidant properties (*10*).

During recent years several campaigns have been designed to increase fruit and vegetable consumption as well as fat-free and low-fat dairy products, potassium, magnesium, calcium, fiber, and protein (*14*). It is known that mineral elements (Na, K, Ca, Mg, Cl, and P) are essential for human beings in amounts of >50 mg/day, whereas trace elements (Fe, Zn, Cu, Mn, I, F, Se, Cr, Mo, Co, and Ni) are essential in concentrations of <50 mg/day. These mineral nutrients have a wide range of functions, for example, as electrolytes, as enzymes constituents, and as building materials, for example, in bones and teeth. Linked to mineral composition special attention should be directed to nitrate and nitrite accumulation in vegetable crops, because they may be considered to have a health risk factor.

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Dietary vitamin C is important in an optimal diet, due to its antioxidant activity, which plays an important role in human nutrition. More than 85% of vitamin C in human diets is supplied by fruits and vegetables and, although the content of vitamin C varies significantly among *Brassica* vegetables, broccoli in particular is a rich source of this and other vitamins (*8*).

Several publications have compared different varieties of broccoli and various cultivation conditions (*12*); however, little information is available on the influence of salinity on plant composition. The amounts of bioactive compounds, as well as the quantity and types of antioxidant properties, have been observed to be highly variable within the same variety of broccoli. Thus, in the current study we focused on variations in bioactive compounds, such as glucosinolates, phenolic compounds, vitamin C, and mineral content under moderate and high saline stress, observing differences between the old and young leaves and inflorescences to establish the nutritive value and quality of the cultivar.

MATERIALS AND METHODS

Growth Conditions and Experimental Design. Broccoli seeds (*Brassica oleracea* L. var. *italica* cv. Marathon) were prehydrated with aerated, deionized water for 12 h and germinated in vermiculite, at 28 °C in an incubator, for 2 days. They were then transferred to a controlled-environment chamber with a 16 h light−8 h dark cycle and air temperatures of 25 and 20 °C, respectively. The relative humidity (RH) was 60% (day) and 80% (night), and photosynthetically active radiation (PAR) was 400 μmol m^{-2} s^{-1}, provided by a combination of 44 fluorescent tubes (Philips TLD 36 W/83 and Sylvania F36 W/GRO) per 2 metal halide lamps (Osram HQI.T 400 W). Environmental temperature and humidity were controlled during the experiment. After 5 days, the seedlings were placed in 15 L containers and were supplied with a complete, modified Hoagland nutrient solution (*9*). The solution was replaced completely every week. After 15 days, plants were transplanted to perlite containers (one plant per container) and modified Hoagland nutrient solution irrigation was used (*9*). The perlite containers were placed then into a greenhouse without controlled-environment conditions.

The experimental design consisted of two salinity levels, 40 and 80 mM NaCl, respectively, beginning applications one day after transplanting and reaching 40 and 80 mM NaCl gradually over a period of 3 days by adding the appropriate amount of NaCl to the nonsaline Hoagland nutrient solution. Each treatment had 10 replicates. The aerial parts were collected 11 weeks after transplanting. At this age, broccoli plant growth was about 55 cm tall for control plants, 45 cm for 40 mM NaCl treated plants, and 35 cm for 80 mM NaCl treated plants. All of them exhibited a head in the center of the leaves, the size of which decreased as salinity increased. The leaves chosen for the analysis were in the initial and intermediate−final position of the plant. Leaves that appeared after 2 weeks of transplantation were designated old leaves, whereas leaves that appeared after 9 weeks of transplantation were designated young leaves.

The fresh weight (W_f) was directly recorded with a portable balance (Scout Pro 400 g, Ohaus Corp.).

Extraction and Determination of Intact Glucosinolates. The multipurpose phytochemical method procedure described by Martínez-Sánchez (*15*), with modifications, was used for extraction. Analysis was done by HPLC-DAD. Freeze-dried powder of the inflorescences and young and old fully expanded leaves (60 mg) was extracted with 1.5 mL of 70% MeOH; samples were placed in a sonicator bath for 10 min to improve the methanolic extraction. Then, the mixture was heated at 70 °C for 30 min in a heating bath, shaken every 5 min with a vortex stirrer, followed by centrifugation (30 min, 17500*g*, 4 °C) to pellet insoluble material. The supernatants were collected, and methanol was removed using a rotary evaporator; the dried residue was redissolved in ultrapure water, giving the same initial volume of the supernatant, and filtered through a 0.45 μm polyethersulfone filter (Millipore). Each sample (20 μL) was analyzed in a Waters HPLC system (Waters Cromatografía S.A., Barcelona, Spain) consisting of a W600E multisolvent delivery system, in-line degasser, W717plus autosampler, and W2996 photodiode array detector at 227 nm, using a LiChrospher 100 RP18 column (25 cm × 0.4 cm, 5 μm particle size; Merck KGaA, Darmstadt, Germany) with a LiChroCART 4-4 guard column. The mobile phase was a mixture of water/formic acid (99:1, v/v) (A) and acetonitrile (B). Glucosinolates were eluted off the column in 35 min. The flow rate was 1 mL min^{-1} in a linear gradient, starting with 1% B and reaching 20% B in 30 min and 1% B at 40 min. Samples were then identified using the previously described intact glucosinolate LC-MS method and quantified by HPLC-DAD using sinigrin (sinigrin monohydrate from horseradish; Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) as standard (*15*). Chromatograms were recorded at 227 nm. The glucosinolate content was expressed as milligrams per 100 g of fresh weight.

Extraction and Determination of Phenolic Compounds. Phenolic compounds were analyzed according to the procedure of Vallejo et al. (*16*). Freeze-dried powders of the floret edible part and young and old fully expanded leaves (1 g) were homogenized in 25 mL of 70% methanol, three times, and the homogenates were filtered through cheesecloth. Samples were kept in ice during the whole procedure. Next, the homogenates were centrifuged (4000*g*, 5 min, 4 °C) and the supernatants evaporated under vacuum at 30 °C to approximately 1 mL and then rediluted up to 2 mL with ultrapure water. The samples were then filtered through a 0.45 μm polyethersulfone filter (Millex-HV, Bedford, MA). The extracted samples (20 μL) were analyzed on a Merck-Hitachi liquid chromatograph equipped with a pump (model L-6200) and a UV−vis detector (model L-7420). Separations were achieved on a LiChroCART column (Merck) (ODS-18, 25 cm × 0.4 cm, 5 μm particle size). The mobile phase was a mixture of water/formic acid (95:5, v/v) (A) and methanol (B). The flow rate was 1 mL min^{-1} in a linear gradient, starting with 10% B and reaching 15% B at 5 min, 30% B at 20 min, 50% B at 35 min, and 90% B at 40 min. Chromatograms were recorded at 280, 320, and 360 nm. Caffeoylquinic acid derivatives were quantified as chlorogenic acid ($C_{16}H_{18}O_9$, Sigma, St. Louis, MO), flavonoids as quercetin-3-rutinoside (Sigma), and sinapic and ferulic acid derivatives as sinapinic acid (Sigma). The contents of flavonoids and hydroxycinnamic acid derivatives were expressed as milligrams per 100 g of fresh weight.

Extraction and Determination of Vitamin C (Ascorbic and Dehydroascorbic Acid). Extraction and determination of ascorbic acid was performed as fully described in Moreno et al. (*8*). Freeze-dried powder of the inflorescences and young and old fully expanded leaves (0.2 g) was homogenized in a vortex stirrer for 20 s with 10 mL of extraction solution: MeOH/H_2O (5:95) plus 2.1% citric acid, 0.05% EDTA and 0.01% NaF (all reagents r.a. grade from Sigma Aldrich Química S.A., Tres Cantos, Madrid, Spain). The homogenate was filtered through cheesecloth and the pH adjusted to 2.2−2.4 by the addition of 6 N HCl. The extract was centrifuged (3600*g* for 15 min, at 4 °C), and the supernatant was recovered, filtered through a C_{18} Sep-Pak cartridge (Waters, Milford, MA), previously activated with 10 mL of methanol followed by the same volume of water and then the same volume of air, and filtered through a 0.45 μm Millex-HV filter. HPLC-HV analysis of ascorbate (AA + DHAA) was achieved after conversion of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo(3,4-*b*)quinoxaline-1-one (DFQ) with freshly prepared 1,2-orthophenylenediamine (OPDA). OPDA solution was added to the water-soluble fraction eluted from a Sep-Pak C_{18} solid-phase extraction cartridge (1:3, v/v). The samples were incubated for 37 min at room temperature, in the dark. Samples (20 μL) were analyzed with a Merck-Hitachi (Tokyo, Japan) HPLC equipped with an L-4000 UV detector and an L-6000 pump. Separations of DFQ and AA were achieved on a Kromasil 100 C_{18} column (25 × 0.4 cm; 5 μm particle size; Tecnokroma, Barcelona, Spain). The mobile phase was methanol/water (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 0.9 mL/min; the detector wavelength was initially set at 348 nm and, after elution of DFQ, was shifted manually to 261 nm for AA detection. The total vitamin C content was expressed as milligrams per 100 g of fresh weight.

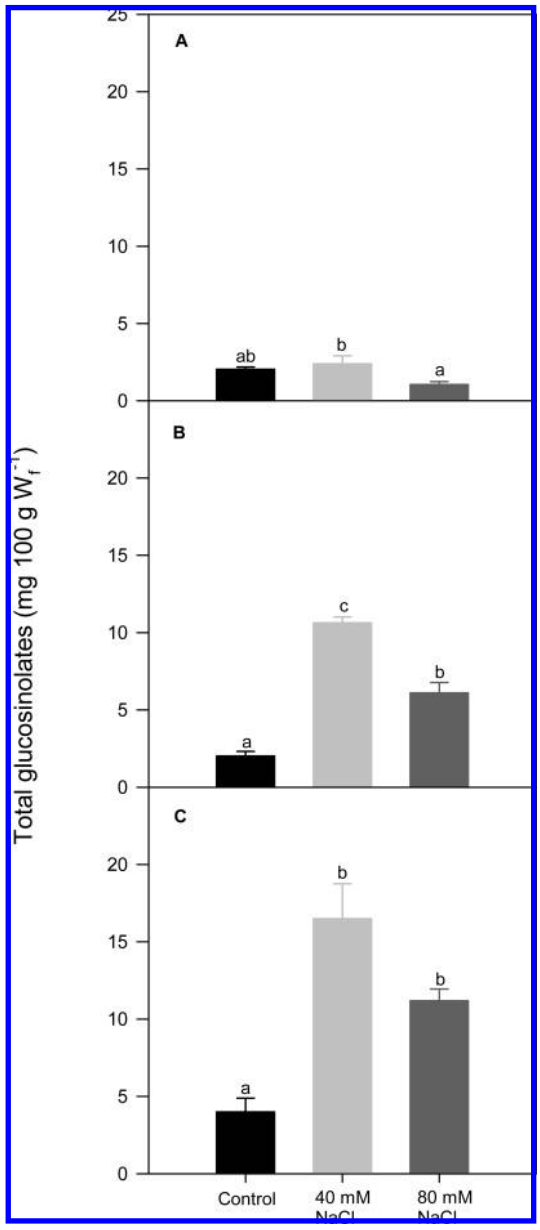

Figure 1. Total glucosinolate content (mg 100 g^{-1} of fresh weight) in the old leaves (**A**), young leaves (**B**), and florets (**C**) of broccoli plants grown under different saline treatments (control, 40 mM NaCl, 80 mM NaCl). Means ($n = 5$; $P < 0.001$) with the same letter are not significantly different at $P < 0.05$ according to Tukey's test.

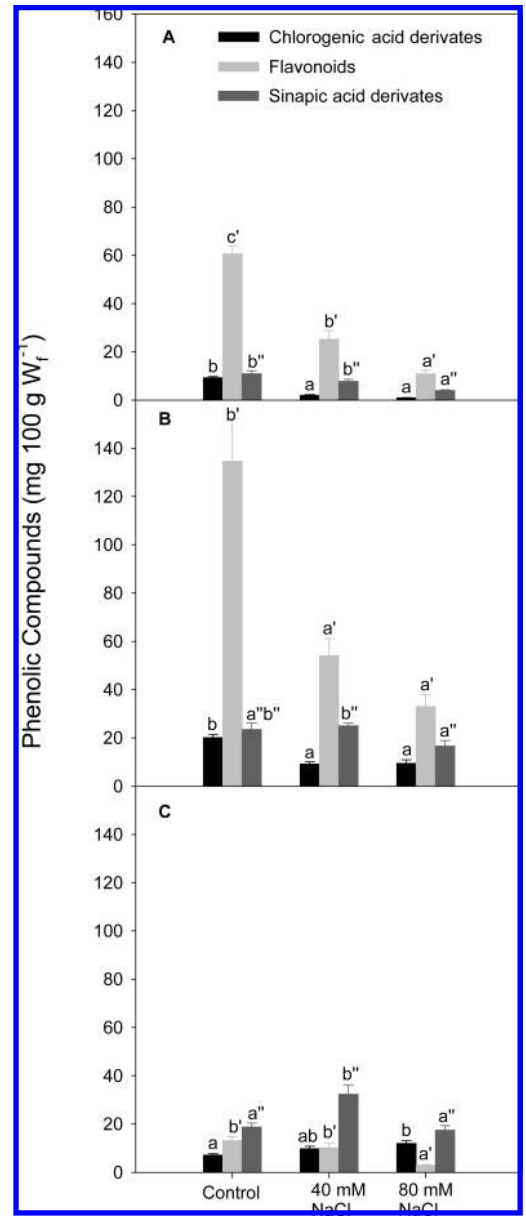

Figure 2. Phenolic compounds (flavonoids, chlorogenic and sinapic acid derivatives) (mg 100 g^{-1} of fresh weight) in the old leaves (**A**), young leaves (**B**), and florets (**C**) of broccoli plants grown under different saline treatments (control, 40 mM NaCl, 80 mM NaCl). Means ($n = 5$; $P < 0.001$) with the same letter are not significantly different at $P < 0.05$ according to Tukey's test.

Analysis of Mineral Anions and Cations. For the mineral ion analysis, the edible floret tissues and leaf xylem sap were collected and diluted (*17*). For this, florets and leaves were placed in Eppendorf tubes with holes at the bottom and frozen rapidly in liquid nitrogen. These tubes were then centrifuged twice within assay tubes, at 4000*g* for 4 min (4 °C), using a Hettich-Universal 132R centrifuge, in such a way that all of the sap was extracted from the samples (*18*). The anions were determined by injection into a Dionex-D-100 ion chromatograph. An Ionpac AS12A (4 × 250 mm) (10-32) column and guard column were used. The flow rate was 1.5 mL min^{-1} with an eluent of 2.7 mmol L^{-1} Na_2CO_3/0.3 mmol L^{-1} $NaHCO_3$. The cation analysis was performed by optical inductively coupled plasma (ICP) spectrometry (ICP-OES spectrometer, IRIS Intrepid II XDL, Thermo Electron Corp., Franklin, MA), equipped with a 2000 W RF generator and full-wavelength coverage and quantified by comparison of peak areas with those of known standards (*17*).The mineral content was expressed as milligrams per 100 g of fresh weight.

Statistical Analysis. Data were analyzed statistically, using the SPSS 13.0 software package, by analysis of variance (ANOVA) and by Tukey's test. Significant differences were determined at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Salinity on Glucosinolates, Phenolic Compounds, and Vitamin C. The fresh weight (W_f) of the aerial part decreased with salt stress, and the changes were more pronounced as salinity levels increased. Thus, the reductions in W_f of the aerial parts were 60% (from 836.00 to 329.94 g) with regard to the control for 40 mM NaCl treated plants and 70% (from 836.00 to 243.35 g) for the 80 mM NaCl treated plants. The florets reduced their W_f by 80% (from 217.28 to 52.15 and 49.26 g) in both saline treatments compared to the control. It is known that salt stress affects both leaf growth and water status. and similar reductions in the leaf weight were previously

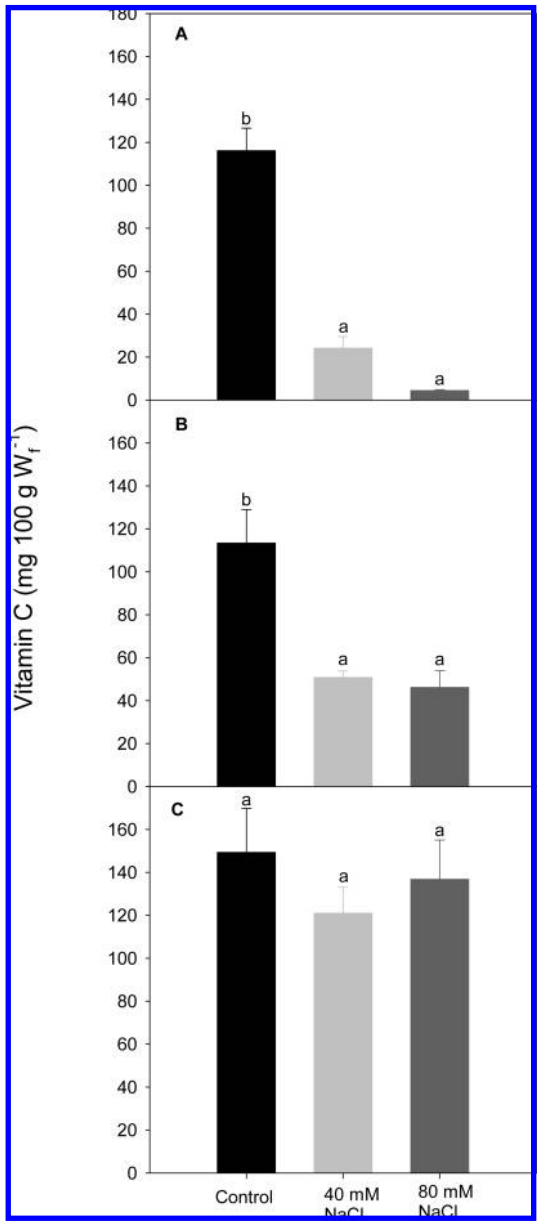

Figure 3. Vitamin C (mg 100 g^{-1} of fresh weight) in the old leaves (**A**), young leaves (**B**), and florets (**C**) of broccoli plants grown under different saline treatments (control, 40 mM NaCl, 80 mM NaCl). Means ($n = 5$; $P < 0.001$) with the same letter are not significantly different at $P < 0.05$ according to Tukey's test.

observed in broccoli and cauliflower plants under salinity (*18*). These results indicated that salt stress becomes one component of a more complex scenario, which includes also events such as pH modifications in substrate that may seriously compromise plant growth and yield (*19*).

Total glucosinolates [expressed by the addition of glucoiberin, glucoraphanin, glucoerucin, glucobrassicin, methoxy-, neo- and hydroxy-glucobrassicin, all determined by HPLC-DAD as in López-Berenguer et al. (*20*)] showed no significant differences ($P < 0.05$) in the old leaves of salt-treated plants with respect to the control plants (**Figure 1**). However, in young leaves a marked increase with salinity was observed, although the rise was higher for the 40 mM NaCl treatment than for the 80 mM NaCl addition. In the florets, the glucosinolate levels were enhanced in both saline treatments compared to the control, but independently of the concentrations of NaCl applied. Similar results were also found previously after the application of 40 mM NaCl and elicitors during head formation in broccoli plants (*21*). It has been reported previously that, in *Brassica napus* L., glucosinolates were accumulated under low water potential when the leaves lost turgor (*22*), suggesting that, during drought, the primary metabolism and growth were restricted, but not the secondary metabolism and the production of glucosinolate precursors, which were utilized later (*23*). In addition, it has been observed in *B. napus* that during drought, the gene expression of *btg*-26 increased 6-fold (*24*); this gene probably is related with the synthesis of osmoprotective compounds and may be responsible for the increase in the production of glucosinolates. Variations in glucosinolate concentration under different mineral stress situations (*25*) have also been detected, supporting the hypothesis of a cross signaling between ionic stress and biotic stress defense through the enhancement of organic metabolites (*26*).

In addition, variation in the amount and pattern of glucosinolates has been attributed to genetic and environmental factors, including different soil metals, plant age, temperature, water stress, and soil type (*26*−*28*). In this study, the increase of total glucosinolates with salinity was more pronounced in the florets than in the young fully expanded leaves (**Figure 1**), probably due to a higher de novo synthesis or the increased transport to this physiological sink via the phloem, although the mechanism for glucosinolate transport in the phloem is still unknown (*29*). The lack of an active metabolism for glucosinolate synthesis in the old leaves or the above-mentioned transport to a sink organ may also explain the glucosinolate levels after the salt treatments. Total and individual glucosinolate contents per head unit may be an essential criterion in considering enhancement of health-promoting phytochemicals in broccoli (*21, 23*), and salinity increased this glucosinolate production.

In this study, natural antioxidants in broccoli are expressed by the content of phenolic compounds (chlorogenic and sinapic acid derivatives and flavonoids) and vitamin C. In the leaves, flavonoids were significantly higher ($P < 0.001$) than the chlorogenic and sinapic acid derivatives, whereas in the edible inflorescences sinapic acid derivates were the most abundant phenolic compound (**Figure 2**). In general, there was a decrease of phenolic compounds with salinity in the old leaves compared to the control, and the loss was higher for flavonoids (60.2 and 82.0% for 40 and 80 mM NaCl treated plants, respectively) than for sinapic acid derivates (reductions of 29.1 and 63.8% for 40 and 80 mM NaCl treated plants, respectively). Similar results were observed in the young leaves, although in this case there were no significant differences ($P < 0.05$) in the sinapic acid derivatives between control and salt-treated plants. It has been shown that salt stress induced disturbances in the secondary metabolic pathways, leading to an increase in phenolic compounds (*28*). However, our results showed a decrease in the phenolic compounds under salinity in leaves at a higher rate when salinity levels increased. On the other hand, in inflorescences, the sinapic acid was increased at 40 mM NaCl treatment (**Figure 2**), pointing out that polyphenols could improve the nutritive value of broccoli (*31*) and enhance the defense system against stresses, especially oxidative stress. The phenolic content of romaine lettuce, a low salt tolerant plant, declined with short-term salt treatment (*32*). However, previous results showed that phenolic compounds in *Bruguiera parviflora* and in the shoots of two clones of *Saccharum* sugar canes decreased when the plants were subjected to long-term NaCl treatments (7−45 and 10−30 days, respectively) (*33, 34*). Our results, and the evidence provided by other studies, suggest that phenolic compound contents in plants could be altered by salinity stress, but this is

Table 1. Anion Concentration of Old and Young Leaves and Florets of Broccoli Plants Grown under Different Saline Treatments (Control, 40 mM NaCl, 80 mM NaCl)[a]

	Cl^-	SE	NO_3^-	SE	PO_4^{3-}	SE	SO_4^{2-}	SE
				Old Leaves				
control	0.007	±0.001a	0.005	±0.000a	0.000	±0.000a	0.069	±0.003b
40 mM NaCl	0.308	±0.026b	0.037	±0.007b	0.002	±0.000b	0.037	±0.007a
80 mM NaCl	0.387	±0.055b	0.024	±0.002b	0.001	±0.000a	0.031	±0.005a
				Young Leaves				
control	0.013	±0.002a	0.039	±0.005b	0.003	±0.001a	0.057	±0.003b
40 mM NaCl	0.222	±0.020b	0.025	±0.005ab	0.010	±0.001b	0.039	±0.005a
80 mM NaCl	0.364	±0.030c	0.017	±0.002a	0.004	±0.001a	0.027	±0.004a
				Florets				
control	0.013	±0.002a	0.009	±0.001a	0.004	±0.001a	0.019	±0.001a
40 mM NaCl	0.050	±0.005b	0.005	±0.001a	0.020	±0.002c	0.033	±0.003b
80 mM NaCl	0.080	±0.007c	0.021	±0.001b	0.013	±0.002b	0.027	±0.001b

[a] Concentrations are expressed as mmol per g of fresh weight. Data are mean ± SE ($n = 5$). Columns with the same letters are not significantly different ($P < 0.05$, Tukey's test).

Table 2. Cations Concentration of the Old and Young Leaves and Florets of Broccoli Plants Grown under Different Saline Treatments (Control, 40 mM NaCl, 80 mM NaCl)[a]

	Na^+	SE	Ca^{2+}	SE	K^+	SE	Mg^{2+}	SE
				Old Leaves				
control	0.032	±0.002a	0.301	±0.023b	0.746	±0.069b	5.87	±0.449b
40 mM NaCl	0.668	±0.041b	0.154	±0.021a	0.377	±0.034a	2.05	±0.211a
80 mM NaCl	0.703	±0.034b	0.164	±0.011a	0.269	±0.014a	1.82	±0.171a
				Young Leaves				
control	0.021	±0.001a	0.162	±0.010b	0.609	±0.019b	3.87	±0.218b
40 mM NaCl	0.486	±0.024b	0.099	±0.011a	0.359	±0.027a	1.76	±0.235a
80 mM NaCl	0.565	±0.009c	0.110	±0.010a	0.283	±0.014a	1.48	±0.157a
				Florets				
control	0.013	±0.0004a	0.024	±0.001a	0.450	±0.017ab	2.16	±0.149a
40 mM NaCl	0.173	±0.017b	0.039	±0.006a	0.480	±0.029b	2.03	±0.156a
80 mM NaCl	0.235	±0.027b	0.064	±0.008b	0.377	±0.016a	2.29	±0.110a

[a] Concentrations are expressed as mmol per g of fresh weight. Data are mean ± SE ($n = 5$). Columns with the same letters are not significantly different ($P < 0.05$, Tukey's test).

critically dependent on the salt sensitivity of the given species. Therefore, the agronomical factors and the level of saline stress should be taken into consideration if the intent is to obtain broccoli for functional ingredients or phytochemically enriched vegetables.

The content of vitamin C suffered a very high significant reduction ($P < 0.001$) in the leaves, more pronounced in the old than in the young ones (**Figure 3**). In many studies, it has been observed that, in salt-sensitive species, the content of ascorbate decreases with salinity (*35*). In addition, studies with the *Arabidopsis thaliana* ascorbate-deficient vtc-1 mutant showed that ascorbate content quickly decreased under salt stress (*36*). These results are in agreement with the observed decrease in the leaves of salinized plants and could be due to the ascorbate participation in the reduction of H_2O_2 through the increased ascorbate peroxidase (AP) activity (*36*). Nonetheless, the ROS-detoxification mechanisms must be studied because, in addition to its role as an antioxidant, ascorbate is involved in hormone synthesis, gene expression, cell division, growth, and apoptosis (*37*). However, in the edible florets the levels of vitamin C were found to be unchanged ($P < 0.05$) after the salt treatments compared to the control. According to previous results (*21*), the absence of effects on vitamin C is also a positive outcome for broccoli. Moreno et al. established that the changes in vitamin C (AA and DHAA) could be more related to the environmental conditions than to the stress treatments, and not always the concentration of the bioactive compound may be negatively affected as for the ascorbic acid.

Effect of Salinity on Mineral Content. Mineral nutrient analysis was carried out in the leaves and edible parts of broccoli. Different anion levels were observed depending on the part of the plant and the salt treatment (**Tables 1** and **2**). Thus, concentrations of Na^+ and Cl^- were higher in the leaves than in the florets, in agreement with the fact that under salt stress the broccoli plants minimized the concentration of toxic ions in their reproductive organs (*19*). In addition, the more pronounced accumulation of Na^+ and Cl^- in the old leaves than in the young fully expanded leaves revealed the compartmentization of excess ions in the vacuoles of old leaves as a possible mechanism to avoid damage in the photosynthetically active leaves (*38*).

Chloride (Cl^-) and nitrate (NO_3^-) ions play an interchangeable role in osmoregulation; the former are able to prevent excessive nitrate concentration by replacing the latter and may have a positive effect on nitrogen content in plant organs (*38*). Chapagain et al. (*39*) have shown that fruit nitrate decreased and chloride increased upon increasing the chloride concentration in the nutrient solution; this agrees with the earlier findings on antagonism between the chloride and nitrate uptake in plants. This well-known process could explain the reduced NO_3^- levels in young leaves; however, the relationhip NO_3^-—salinity is very complex, and papers describe conflicting results, with decrease (*40*), increase, or no effect (*41*). The NO_3^- levels in the florets of 80 mM NaCl treated plants were in consonance with those found in different cuciferous species, such as radish (203.0 mg 100 g_{fw}^{-1}) or lettuce (148.9.0 mg 100 g_{fw}^{-1}).

Evidence regarding the effect of nitrate on human health is conflicting. However, in view of the large number of its harmful effects on human health [for a review see Anjana and Muhammad (*40*)], it seems reasonable to take preventive measures to decrease accumulation of nitrate in plants and its subsequent consumption by human beings. Therefore, under saline stress, the reduction in nitrate content could add value to broccoli edible florets, already very popular for their nutritional and therapeutic properties.

The PO_4^{3-} content in leaves was increased after the 40 mM NaCl application and with both saline additions in the broccoli florets. The nutritive value of the inflorescences found at higher phosphorus content is similar to other vegetables such as peas (113 mg 100 g_{fw}^{-1}) or Brussels sprout (84 mg 100 g_{fw}^{-1}) (*42*). Therefore, there is a need to undertake relative studies on the patterns of P remobilization and redistribution within various plant parts of *Brassica* cultivars under saline stress, because a better nutritional and growth status may be the result of a more efficient nutrient absorption and/or use by the cultivar.

The SO_4^{2-} content was reduced in the old and young leaves after salt addition compared to control, without marked differences ($P < 0.05$) between the saline treatments. These reductions are in agreement with the previous results observed for broccoli plants treated with 80 mM NaCl (*43*). However, the decrease of SO_4^{2-} content was accompanied by a marked increase of the total glucosinolates content, which could be related to the role of SO_4^{2-} in the regulation of glucosinolate biosynthesis, although the mechanism for this regulation is still unknown. Thus, total glucosinolate levels were high at sufficient sulfur supply or high nitrogen levels in broccoli plants (*44*).

With regard to cation determinations, Ca^{2+} content was lower than control in the old and young leaves after salt addition, but increased in the edible part of the inflorescences (**Table 2**). This could be regarded as a good source of available calcium, and consumption in the diet may contribute to adequate calcium nutrition. Concerning K^+ and Mg^{2+} levels, a decrease was observed with both saline treatments in the old and young leaves. In general, under saline conditions, Na^+ in the growth medium might interact with other cations such as K^+, Ca^{2+}, and Mg^{2+}, resulting in the low absorption of the latter ions by the root (*45*). However, our results in the inflorescence showed that K^+ and Mg^{2+} concentrations were not affected by salinity ($P < 0.05$) and could be the result of osmotic regulation of these cations in this particular sink organ. Moreover, this might have important implications in terms of the nutritional value of *Brassica* foods, because it has been reported that the mineral content is correlated with the ability of Cruciferae sprout extracts to in vitro scavenge the superoxide anion radicals (*46*).

Changes in essential mineral nutrients and their metabolism in broccoli seemed to be the mechanisms involved to compensate for the injurious effect of NaCl in the broccoli, when the threshold of resistance may be exceeded. Accumulation of excess ions (Na^+ and Cl^-) into the leaves may be a functional tool to preserve the integrity of reproductive organs such as the inflorescence. In general, the mineral composition of the edible parts of broccoli is in the range of the recommended amounts for human consumption.

In conclusion, significant effects of saline treatments on bioactive compounds in broccoli indicate that the use of salt stress at head induction and development may serve the purpose of enhancing the nutritional quality of broccoli cultivars to deliver a health-promoting food. In addition, under salt stress an increase in glucosinolate contents appears to be involved in the response of broccoli to salinity, but more biosynthetic and metabolic information has to be assessed. Differences between the bioactive compounds may result from the salt stress intensity, and it has been observed to be highly variable depending on the organ of the plant. Thus, although the salinity induces physiological effects such as a growth reduction, an improvement in the nutritional quality of the edible inflorescences was observed. Nonetheless, research is also necessary to understand the environmental factors that contribute to the phytochemical and mineral content of crops to deliver highly nutritious foods to the public. Critical goals in this area could be to provide valid information of the effect of salinity on the mineral bioavailability from plants and their absorption by plant roots.

LITERATURE CITED

(1) Mansour, M. M. F.; van Hasselt, P. R.; Kuiper, P. J. C. NaCl effects on root plasma membrane ATPase of salt tolerant wheat. *Biol. Plant.* **2000**, *43*, 61–66.

(2) Munns, R. Comparative physiology of salt and water stress. *Plant, Cell Environ.* **2002**, *25*, 239–250.

(3) Tester, M.; Davenport, R. Na^+ tolerance and Na^+ transport in higher plants. *Ann. Bot.* **2003**, *91*, 503–527.

(4) Greenway, H.; Munns, R. Mechanism of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* **1980**, *31*, 149–190.

(5) Blum, A.; Munns, R.; Passioura, J. B.; Turner, N. C. Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations. *Plant Physiol.* **1996**, *110*, 1051–1051.

(6) Qasim, M.; Ashraf, M.; Ashraf, M. Y.; Rehman, S. U.; Rha, E. S. Salt-induced changes in two canola cultivars differing in salt tolerance. *Biol. Plant.* **2003**, *46*, 629–632.

(7) Cohen, J. H.; Kristal, A. R.; Stanford, J. L. *J. Natl. Cancer Inst.* **2000**, *92*, 61–68.

(8) Moreno, D. A.; Carvajal, M.; López-Berenguer, C.; García-Viguera, C. Chemical and biological characterisation of nutraceutical compounds of broccoli. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1508–1522.

(9) López-Berenguer, C.; García-Viguera, C.; Carvajal, M. Are root hydraulic conductivity responses to salinity controlled by aquaporins in broccoli plants? *Plant Soil.* **2006**, *279*, 13–23.

(10) Finley, J. W. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds. *Ann. Bot.* **2005**, *95*, 1075–1096.

(11) Charron, C. S.; Sams, C. E. Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *J. Am. Soc. Hortic. Sci.* **2004**, *129*, 321–330.

(12) Rosa, E. A. S.; Rodrigues, A. S. Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late seasons. *HortScience* **2001**, *36*, 56–59.

(13) Cartea, M. E.; Velasco, P. Glucosinolates in *Brassica* foods: bioavilability in food and significance for human health. *Phytochem Rev.* **2008**, *7*, 213–229.

(14) Krauss, R. M.; Eckel, R. H.; Howard, B.; Appel, L. J.; Daniels, S. R.; Deckelbaum, R. J. AHA dietary guidlines: a statement for healthcare professionals from the nutrition committee of the American Heart Association. *Circulation* **2000**, *10*, 2284–2299.

(15) Martinez-Sanchez, A.; Allende, A.; Bennett, R. N.; Ferreres, F.; Gil, M. I. Microbial. nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biol. Technol.* **2006**, *42* (1), 86–97.

(16) Vallejo, F.; Tomas-Barberan, F. A.; Garcia-Viguera, C. Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. *Eur. Food Res. Technol.* **2003b**, *216*, 395–401.

(17) Martínez-Ballesta, M. C.; Bastías, E.; Zhu, C.; Shäffner, A. R.; González-Moro, B.; González-Murua, C.; Carvajal, M. Boric acid and salinity effects on maize roots. Response of aquaporins ZmPIP1 and ZmPIP2, and plasma membrane H^+-ATPase, in relation to water and nutrient uptake. *Physiol. Plantarum* **2008**, *132*, 479–490.

(18) De Pascale, S.; Maggio, A.; Barbieri, G. Soil salinization affects growth, yield and mineral composition of cauliflower and broccoli. *Eur. J. Agron*. **2005**, *23*, 254–264.
(19) Hachicha, M.; Cheverry, C.; Mhiri, A. The impact of long-term irrigation on changes of ground water level and soil salinity in northern Tunisia. *Arid. Soil Res. Rehab*. **2000**, *14*, 175–182.
(20) Lopez-Berenguer, C.; Carvajal, M.; Moreno, D. A.; García-Viguera, C. Effects of microwave cooking conditions on bioactive compounds present in broccoli inflorescences. *J. Agric. Food Chem*. **2007**, *55*, 10001–10007.
(21) Moreno, D.; López-Berenguer, C.; Martínez-Ballesta, M. C.; Carvajal, M.; García-Viguera, C. Basis for the new challenges of growing broccoli for health in hydroponics. *J. Sci. Food Agric*. **2008**, *88*, 1472–1481.
(22) Jensen, C. R.; Mogensen, V. O.; Mortensen, G.; Fieldsend, J. K.; Milford, G. F. J.; Andersen, M. N.; Thage, J. H. Seed glucosinolate, oil and protein contents of field-grown rape (*Brassica napus* L.) affected by soil drying and evaporative demand. *Field Crops Res*. **1996**, *47*, 93–105.
(23) Gershenzon, J. Changes in the levels of plant secondary metabolites under water and nutrient stress In *Phytochemical Adaptation to Stress*; Recent Advances in Phytochemistry; Timmerman B. N., Steelink, C., Loewus, F. A., Eds.; Plenum: New York, 1984; Vol. 18, pp 273−320.
(24) Stroeher, V. L.; Boothe, J. G.; Good, A. G. Molecular cloning and expression of a turgor-responsive gene in *Brassica napus*. *Plant Mol. Biol*. **1995**, *27*, 541–551.
(25) Tolrá, R.; Pongrac, P.; Poschenrieder, C.; Vogel-Mikus, K.; Regvar, M.; Barceló, J. Distinctive effects of cadmium on glucosinolate profiles in Cd hyperaccumulator *Thlaspi praecox* and non-hyperaccumulator *Thlaspi arvense*. *Plant Soil* **2006**, *288*, 333–341.
(26) Poschenrieder, C.; Tolra, R.; Barceló, J. Can metals defend plants against biotic stress? *Trends Plant Sci*. **2006**, *11*, 288–295.
(27) Brown, P. D.; Tokuhisa, J. G.; Reichelt, M.; Gershenzon, J. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* **2003**, *62*, 471–481.
(28) Farnham, M. W.; Wilson, P. E.; Stephenson, K. K.; Fahey, J. W. Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. *Plant Breed*. **2004**, *123*, 60–65.
(29) Petersen, B. L.; Chen, S. X.; Hansen, C. H.; Olsen, C. E.; Halkier, B. A. Composition and content of glucosinolates in developing *Arabidopsis thaliana*. *Planta* **2002**, *214*, 562–571.
(30) Ayaz, F. A.; Kadioglu, A.; Turgut, R. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rosc.) Eichler. *Can. J. Plant Sci* **2000**, *80*, 373–378.
(31) Higdon, J. V.; Delage, B.; Williams, D. W.; Dashwood, R. H. Cruciferous vegetables and human cancer risk: epidemiological evidence and mechanistics basis. *Pharmacol. Res*. **2007**, *55*, 224–236.
(32) Kim, H. J.; Fonseca, J. M.; Choi, J. H.; Kubota, C.; Young- Kwon, D. Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). *J. Agric. Food Chem* **2008**, *56*, 3772–3776.
(33) Wahid, A.; Ghazanfar, A. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *J. Plant Physiol*. **2006**, *163*, 723–730.
(34) Parida, A. K.; Das, A. B.; Das, P. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J. Plant Biol*. **2002**, *45*, 28–36.
(35) Ben Amor, N.; Jimenez, A.; Megdiche, W.; Lundqvist, M.; Sevilla, F.; Abdelly, C. Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. *Physiol. Plant*. **2006**, *126*, 446–457.
(36) Huang, C. H.; He, W. L.; Guo, J. K.; Chang, X. X.; Su, P. X.; Zhang, L. X. Increased sensitivity to salt stress in an ascorbate-deficient *Arabidopsis* mutant. *J. Exp. Bot*. **2005**, *56*, 3041–3049.
(37) Arrigoni, O.; De Tullio, M. C. Ascorbic acid: much more than just an antioxidant. *Biochim. Biophys. Acta* **2002**, *1569*, 1–9.
(38) Dorais, M.; Papadoulos, A. P.; Gosselin, A. Greenhouse tomato fruit quality. *Hortic. Rev.* **2001**, *26*, 262–319.
(39) Chapagain, B. P.; Wiesman, Z.; Zaccai, M.; Imas, P.; Magen, H. Potassium chloride enhances fruit appearance and improves quality of fertigated greenhouse tomato as compared to potassium nitrate. *J. Plant Nutr*. **2003**, *26*, 643–658.
(40) Anjana, S. U.; Muhammad, I. Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. *Agron. Sustain. Dev*. **2007**, *27*, 45–57.
(41) Grattan, S. R.; Grieve, C. M. Salinity−mineral nutrient relations in horticultural crops. *Sci. Hortic*. **1998**, *78*, 127–157.
(42) Belizt, H. D.; Grosch, W.; Schieberle, P. Minerals. In *Food Chem.*; Belizt, H. D., Grosch W., Schieberle, P., Eds.; Springer: Berlin, Germany, 2004; pp 427−433.
(43) López-Berenguer, C.; Carvajal, M.; García-Viguera, C.; Alcaraz, C. F. Nitrogen, phosphorous and sulphur nutrition in broccoli plants grown under salinity. *J. Plant Nutr*. **2007**, *30*, 1855–1870.
(44) De Pascale, S.; Maggio, A.; Pernice, R.; Fogliano, V.; Barbieri, G. Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. *Eur. J. Agron*. **2007**, *26*, 418–424.
(45) Martínez-Ballesta, M. C.; Silva, C.; López-Berenguer, C.; Cabañero, F. J.; Carvajal, M. Plant aquaporins: new perspectives on water and nutrient uptake in saline environment. *Plant Biol*. **2006**, *8*, 535–546.
(46) Zielinski, H.; Frias, J.; Piskula, M. K.; Kozowska, H.; Vidal-Valverde, C. Vitamin B_1 and B_2, dietary fiber and minerals content of Cruciferae sprouts. *Eur. Food Res. Technol*. **2005**, *221*, 78–83.

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