Research Article

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# Novel varieties of broccoli for optimal bioactive components under saline stress

Raul Dominguez-Perles,[a] Maria Carmen Martinez-Ballesta,[b] Fernando Riquelme,[b] Micaela Carvajal,[b] Cristina Garcia-Viguera[a] and Diego A Moreno[a*]

## Abstract

**BACKGROUND: Consumption of broccoli is increasing steadily worldwide because of the interest in its bioactive composition and nutritive value for health promotion. Novel broccoli cultivars to be established under current adverse conditions in production areas (aggressive environmental conditions and saline irrigation waters) need to maintain physical and nutritional quality for consumption and year-round supply to the markets. The newly introduced cultivars 'Naxos' and 'Parthenon' have been selected as potential candidates to replace the currently underperforming 'Nubia' variety. We aimed to compare the physical and phytochemical quality (glucosinolates, hydroxycinnamic acids, flavonoids, vitamin C and minerals), as well as the *in vitro* antioxidant capacity of these three cultivars under conditions of environmental stress.**

**RESULTS: 'Parthenon' showed equal productivity and nutritional composition to 'Nubia', whereas 'Naxos' presented in general the best results when compared to 'Nubia' and 'Parthenon'. For phenolic compounds 'Nubia' presented the highest contents, although 'Naxos' seemed better adapted to saline stress conditions, as suggested by the lowest degree of variation in the contents of healthy phytochemicals, including phenolic compounds, when grown under such conditions.**

**CONCLUSION: 'Naxos' broccoli performed best and is a suitable candidate to replace 'Nubia' for marketable, nutritive and phytochemical quality, especially in areas of production under adverse conditions as found in Mediterranean southeast Spain (semiarid climate with saline irrigation water).**


**Keywords:** *Brassica oleracea*; quality; composition; glucosinolate; phenolic compounds; antioxidant; minerals

## INTRODUCTION

The critical relevance of the dietary intake of plant-origin nutrients and bioactive phytochemicals beneficial for health and well-being has become a paradigm in the design of modern diets and plans. Among the most widely consumed vegetables, broccoli (*Brassica oleracea* var. *Italica*) appears to be one of the best inducers of mammalian detoxification enzymes (also known as phase II enzymes) associated with a reduced risk of developing various chronic conditions and diseases, including carcinogenic processes and cardiovascular pathologies. Additionally, it has been suggested as a health-promoting food for the digestive tract.[1] The health-related broccoli attributes are based on its content of nitrogen–sulfur compounds (glucosinolates), phenolic compounds (hydroxycinnamic acid derivatives and flavonoids), vitamins and minerals (including K, P, Fe, Zn, Cu and Se).[2,3] The phytochemical composition of the inflorescences of broccoli has been described as dependent on genetic (cultivar), physiological (organ and age) and abiotic factors (saline stress, temperature, photoperiod, season and fertilization).[4–8]

Broccoli production is one of the major agro-economical activities in the Murcia region (southeast Spain), with exports surpassing 64 000 t in 2008. The principal cultivars exploited in the last 10 years have been 'Marathon', 'Nubia', and 'Monaco'. However, today the total absence of 'Marathon' and the search for novel cultivars with additional advantages to adapt to local growth conditions have prompted the sector to innovate with new varieties. 'Parthenon', now cultivated worldwide, and 'Naxos', ideal for the temperatures of late winter/early spring in southeast Spain, constitute new varieties introduced into the Murcia region. This region currently underwent a water shortage that has forced the use of saline irrigation water. This situation constitutes an important stress factor, negatively affecting agricultural productivity (in terms of yield and nutritional composition) and it must be taken into account when selecting novel cultivars to be grown in production areas. The pernicious effect of salinity on the morphological, physiological and biochemical characteristics

* Correspondence to: Diego A Moreno, Phytochemistry Laboratory, Department of Food Science and Technology, Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Post Office Box 164 Espinardo, Murcia 30100, Spain. E-mail: dmoreno@cebas.csic.es

a Department of Food Science and Technology, Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Espinardo, Murcia 30100, Spain

b Department of Plant Nutrition, Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Espinardo, Murcia 30100, Spain

of broccoli has been widely reported elsewhere and also from our group.[6,8–12] Under such conditions plants undergo an osmotic adjustment, and the compatible solutes accumulated include various secondary metabolites.[12] Therefore, we aimed to study and characterize the response of the new cultivars to the saline stress prevailing in the production areas for selecting the best cultivar for this growing environment that would guarantee physical quality and composition for consumption.

Previous studies performed on 'Marathon', 'Nubia' and 'Monaco',[9–11] widely distributed varieties in recent years (up to 60% of the broccoli produced in the area of study), demonstrated the effects of saline stress on the nutritional value of broccoli, mainly in terms of glucosinolate content.[6,8,12] These data represent a useful background to compare and progress with newly introduced cultivars 'Naxos' and 'Parthenon': potential candidates for the current environmental conditions of the Mediterranean semiarid zones of production (southeast Spain, Murcia, with warm autumn and winter conditions). There is a need for year-round production of high-quality broccoli, demanded not only for high quality standards of physical appearance but also because of its nutritive and health-promoting phytochemical profile.

## EXPERIMENTAL

### Plant material and growing conditions

Broccoli cultivars 'Nubia', 'Naxos' and 'Parthenon' were cultivated in the autumn–winter season (November 2008 to February 2009) in a greenhouse of CEBAS-CSIC ('La Matanza' Experimental Farm, Santomera, Murcia, southeast Spain), under a semiarid Mediterranean climate.

Broccoli seeds of 'Nubia' were obtained from Ramiro Arnedo SA (Murcia, Spain). 'Parthenon' and 'Naxos' seeds were obtained from SAKATA® (Sakata Seed Ibérica SLU, Valencia, Spain). Seeds were pre-hydrated with aerated, deionized water for 12 h and then germinated, and spread on to vermiculite trays for 2 days in the dark at 28 °C in an incubator. They were then transferred to a growth chamber with a 16 h light and 8 h dark cycle, at air temperatures of 25 and 20 °C, respectively. The relative humidity was 60% (day) and 80% (night) and photosynthetically active radiation was 400 $\mu$mol $m^{-2}$ $s^{-1}$, provided by a combination of fluorescent tubes (Philips TLD 36 W/83, New York, USA, and Sylvania F36W/GRO, Munich, Germany) and metal halide lamps (Osram HGI.T400W, Munich, Germany). After 4 days, the seedlings were placed in 15 L containers filled with perlite and irrigated every 2 days with half-strength Hoagland nutrient solution, completely replaced every week.

Broccoli plants (30 days old) were placed in a non-controlled-environment aluminium-framed greenhouse with polyethylene covers and mechanical ceiling windows for passive venting. Daily mean relative humidity and temperature were calculated from measurements taken every 10 min using dataloggers (AFORA SA, Barloworld Scientific, Murcia, Spain). Relative humidity averaged 40/70% (day/night) and air temperature 20/9 °C (day/night). The greenhouse was vented when daytime temperatures exceeded 28 °C. A total of 90 broccoli plants were placed in a randomized design, using 10 plants per treatment and cultivar, with each plant being grown in a perlite-filled 20 L container. All plants were grown under the same conditions and irrigated with half-strength Hoagland nutrient solution (about 2 dS $m^{-1}$ electrical conductance (EC)) twice a week under natural light conditions, until 20 days after transplantation (DAT). At that time, the application of 40 and 80 mmol $L^{-1}$ NaCl in the nutrient solution was begun (about 4 and 7 dS $m^{-1}$ EC, respectively). The plants did not show any symptoms of deficiency or toxicity. At 90 DAT all the plants were harvested.

### Plant harvest and head quality parameters

At harvest (90 DAT), plants had reached at least 100–130 mm of head diameter, which corresponded to the typical size at the mature stage (Fig. 1). Harvesting was performed when more than 90% of the plants presented mature commercial flowering heads and their quality parameters (compactness, homogeneity of grain, absence of secondary heads, absence of minor leaves in the head, as well as total absence of hollow stems) corresponded to 'marketable' class. All parameters were visually evaluated to establish that heads presenting more than two unacceptable physical parameters would be considered as 'non-marketable'.

Plants were processed as previously described.[6–8] Broccoli plants were cut off and weighed fresh (total above-ground biomass; Table 1).[13] Heads were separated from the rest of the plant once in the laboratory and the physical characters were recorded quickly and gently: head diameters (major and minor), circularity, length of heads for market size and diameter of the stalk (Fig. 1). The sampled heads were then cut into pieces (edible florets) and mixed thoroughly, again to be bulked into three well-mixed replicates per treatment and cultivar ($n = 3$). The samples were then flash-frozen using liquid nitrogen and kept at −80 °C until freeze-dried (model Christ Alpha 1-4D; Christ, Osterode am Harz, Germany). The complete processing operations were carried out in less than 4 h, in order to reduce the deleterious effect of handling on the composition of the broccoli florets.[1,2,6,8] After freeze-drying, the samples were ground to a fine powder and stored at −20 °C for further analysis.

### Extraction and determination of intact glucosinolates and phenolic compounds

Freeze-dried powder (50 mg) was extracted in 1.5 mL of 70% methanol for 30 min at 70 °C, vortexed every 5 min to improve extraction and then centrifuged (20 min, 10 000 × $g$, 4 °C) (model Sigma 1–13, B Braun Biotech International, Osterode, Germany). Supernatants were collected and methanol was removed using a rotary evaporator. The dried residue was reconstituted in ultrapure water up to 1 mL and filtered through a 0.22 $\mu$m polypropylene membrane filter (ANOTOP 10 plus; Whatman, Maidstone, UK). Each sample (20 $\mu$L) was analyzed in a Waters high-performance liquid chromatography (HPLC) system (Waters Cromatografía SA, Barcelona, Spain) consisting of a W600E multi-solvent delivery system, inline degasser, W717plus autosampler and W2996 PAD. The compounds were separated in a Luna C18 column (25 cm × 0.46 cm, 5 $\mu$m particle size; Phenomenex, Macclesfield, UK) with a security guard C18-ODS (4 × 30 mm) cartridge system (Phenomenex). The mobile phase was a mixture of water–trifluoroacetic acid (99.9 : 0.1, v/v) (A) and a mixture of acetonitrile–trifluoroacetic acid (99.9 : 0.1, v/v) (B). The flow rate was 1 mL $min^{-1}$ in a linear gradient, starting with 1% B for 5 min to reach 17% B at 15 min, which was maintained for 2 min, then 25% B at 22 min, 35% B at 30 min, 50% B at 35 min and 99% B at 40 min. The monitored compounds, glucosinolates (227 nm) and phenolic compounds (330 nm), were eluted from the column in 35 min. Glucosinolates present in the samples were then identified using a previously described liquid chromatographic–mass spectrometric method[14] and quantified using sinigrin as standard (sinigrin monohydrate from *Sinapis nigra*, Phytoplan Diehm & Neuberger

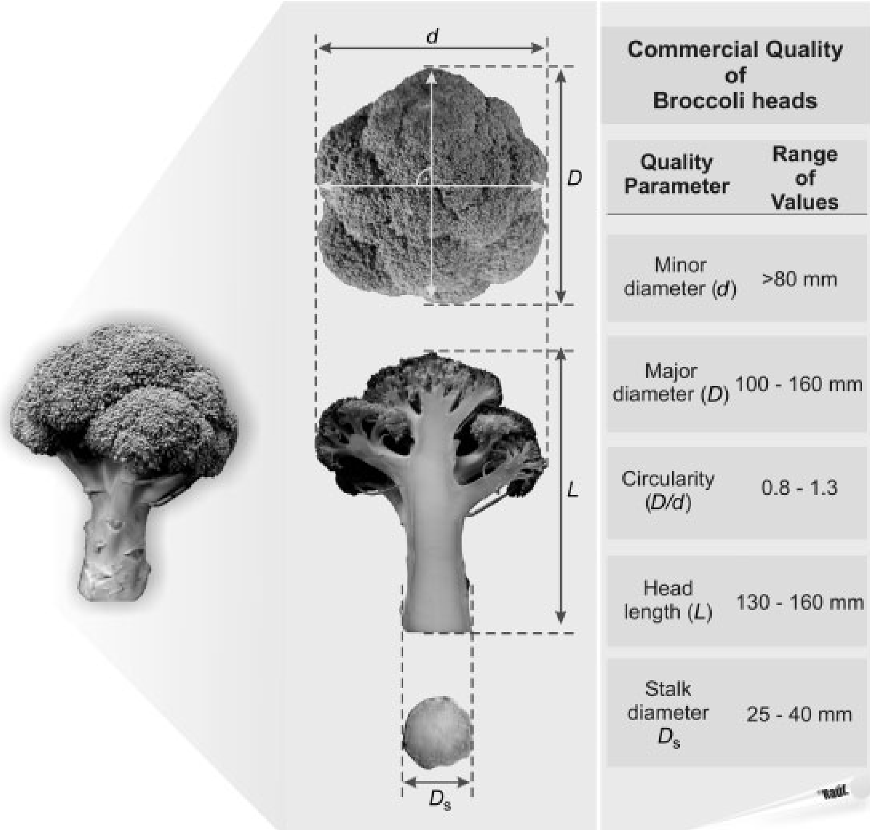


**Figure 1.** Schematic representation of physical quality parameters measured in marketable broccoli heads. Head diameter (minor (*d*) and major (*D*)), ratio of diameters, named circularity (*C*), length of heads for market size (*L*) and the stalk diameter ($D_s$) were all measured in the lab immediately after sampling as part of the operations included in the sample preparation.

**Table 1.** Total aboveground biomass and flowering head fresh weight of broccoli at harvest (kg per plant)

| | | Saline treatment (mmol L$^{-1}$ NaCl) | | |
|---|---|---|---|---|
| Organ | Cultivar | 0 | 40 | 80 |
| Whole plant | 'Nubia' | 0.831b | 1.003b | 0.808b |
| | 'Naxos' | 1.150a | 1.129a | 1.146a |
| | 'Parthenon' | 0.811b | 0.991b | 0.679c |
| | ANOVA *P*-value | ** | ** | *** |
| | LSD ($P < 0.05$) | 0.084 | 0.049 | 0.087 |
| Flowering heads | 'Nubia' | 0.218b | 0.299a | 0.193ab |
| | 'Naxos' | 0.323a | 0.343a | 0.314a |
| | 'Parthenon' | 0.209b | 0.323a | 0.151b |
| | ANOVA *P*-value | ** | n.s. | ** |
| | LSD ($P < 0.05$) | 0.056 | 0.062 | 0.139 |

Means ($n = 3$) within a column followed by the same lower-case letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
n.s., non-significant ($P > 0.05$); significant at: ** $P < 0.01$; *** $P < 0.001$.

GmbH, Heidelberg, Germany). Caffeoylquinic acid derivatives were quantified using chlorogenic acid (Sigma, St Louis, MO, USA), flavonoids with quercetin-3-rutinoside (Sigma), and sinapic acid derivatives using sinapinic acid (Sigma).

### Extraction and determination of vitamin C

Total vitamin C, as the sum of ascorbic acid (AA) and dehydroascorbic acid (DHAA), was determined according to Moreno *et al.*, with further modifications.[15] The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-*b*]-quinoxaline-1-one (DFQ) with 1,2-phenylenediamine dihydrochloride (OPDA). Samples (20 µL) were analyzed with a Merck Hitachi HPLC system (Tokyo, Japan), equipped with an L-4000 UV detector and an L-6000 pump. Separation of DFQ and AA was achieved on a Lichrospher RP18 100 µm particle size 250 × 4 mm column (Scharlab, Barcelona, Spain). The mobile phase was methanol–water (5 : 95, v/v) containing 5 mmol L$^{-1}$ cetrimide and 50 mmol L$^{-1}$ potassium dihydrogen phosphate at pH 4.5. The flow rate was 0.9 mL min$^{-1}$. The detector wavelength was initially set at 348 nm and, after the elution of DFQ, was shifted manually to 261 nm for L-AA detection.

### Antioxidant capacity

To analyze the antioxidant capacity of broccoli extracts, the 2,2-diphenyl-picryl-hydrazyl (DPPH$^\bullet$) (Sigma) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$) (Sigma) assays were used as well as the ferric reducing ability (FRAP assay), following the method described by Llorach *et al.*[16] Freeze-dried material (50 mg) was extracted in 70% methanol using an ultrasound bath for 5 min, with centrifuging at 10 000 × *g* for 10 min at 4 °C; it was then used for all antioxidant determinations.

  

*DPPH• assay*
Free radical scavenging activity was evaluated by adding 25 µL of the corresponding diluted sample to a cuvette containing 975 µL of 0.094 mmol $L^{-1}$ free radical DPPH• solution (up to absorbance 1). The final volume of the assay was 1 mL and the lack of DPPH• was measured by decrease in absorbance at 515 nm for 50 min at 25 °C in a spectrophotometer (model V-630, JASCO, Tokyo, Japan). Results were expressed as Trolox equivalents.

*ABTS•+ assay*
Free radical scavenging capacity was determined by adding 5 µL of each sample to the cuvette containing a 32 µmol $L^{-1}$ water solution (995 µL) of the free radical (ABTS•+). The final volume of the assay was 1 mL and the disappearance of ABTS•+ was determined by measuring the decrease in absorbance at 414 nm for 60 min at 25 °C in a V-630 JASCO spectrophotometer.

*FRAP assay*
The freshly prepared FRAP was 10 mL of a 0.3 mmol $L^{-1}$ dissolution in an acetate buffer (pH 3.6), including 2.5 mL of 20 mmol $L^{-1}$ 2,4,6-Tripyridyl-s-triazine (TPTZ) solution in 40 mmol $L^{-1}$ HCl and 2.5 mL of 20 mmol $L^{-1}$ ferric chloride ($FeCl_3.6H_2O$). Ferric reducing ability was determined by adding 5 µL of each sample to 950 µL FRAP solution warmed at 37 °C. The increase in absorbance was measured at 593 nm for 45 min in a V-630 JASCO spectrophotometer. Calibration curves were made for each assay using Trolox as the standard.

**Analysis of mineral elements**
The analysis of P, S, Na, K, Ca, Mg, B, Cu, Fe, Mn and Zn was carried out after $HNO_3$–$HClO_4$ (2 : 1) acid digestion of the dried plant material. Each was determined by inductively coupled plasma spectrometry (OES Thermo ICAP 6000 Series®, Thermo Electron Corp., Franklin, MA, USA) in a dilution with $LaCl_3$ + CsCl of the extract aliquot, as reported elsewhere.[13] The total C and N in the samples were determined in an αThermo FlashEA 1112 autoanalyzer (Thermo Fisher Scientific SA, Madrid, Spain).

**Statistical analysis**
Data were processed using the SPSS 17.0 software package (LEAD Technologies, Inc., Chicago, IL, USA), and a multifactorial analysis of variance (ANOVA) and multiple range test (Duncan's test) were carried out. In addition, a multifactor correlation analysis was performed to corroborate relationships between selected parameters (at $P < 0.05$).

## RESULTS AND DISCUSSION

**Yield and quality parameters**
The total biomass yield at harvest of 'Naxos' was the highest, surpassing 'Nubia' (by 28%) and 'Parthenon' (by 30%), in non-stressed plants. In the 40 mmol $L^{-1}$ NaCl treatment, 'Naxos' also performed best (surpassing 'Nubia' by 9% and 'Parthenon' by 10%), and also at 80 mmol $L^{-1}$ NaCl (surpassing 'Nubia' by 30% and 'Parthenon' by 41%). In addition, at 80 mmol $L^{-1}$ NaCl 'Nubia' recorded a 15% higher fresh weight than 'Parthenon' (Table 1). With regard to the hydric contents, no differences were observed between the cultivars, with humidity values of 89%, 87% and 85% on average at 0, 40 and 80 mmol $L^{-1}$ NaCl, respectively.

The marketable yield (flowering heads) of 'Nubia' and 'Parthenon' in the control plants was lower than in 'Naxos', which surpassed 'Nubia' by 33% and 'Parthenon' by 35%. At 40 mmol $L^{-1}$ NaCl, non-significant differences between cultivars were found, whereas at 80 mmol $L^{-1}$ NaCl 'Naxos' also showed much higher fresh weight of inflorescence compared to 'Nubia' (by 39%) and 'Parthenon' (by 52%). Therefore, a significant effect on the broccoli biomass under saline stress was observed, especially at 80 mmol $L^{-1}$ NaCl. We studied the effects on florets (adult plants), but our results are also in agreement with the findings of Yuan *et al*. on radish sprouts, which reported that whereas saline treatment of up to 50 mmol $L^{-1}$ entails a positive effect on the fresh weight of radish sprouts, higher salinity (100 mmol $L^{-1}$ NaCl) produces a deleterious effect on plant yield.[17] The higher above-ground biomass of 'Naxos' indicated better adaptation to the environmental stress factors,[13,18] confirmed also by the best results of marketable yield and the absence of statistical differences between saline treatments.

Taking into account the relative contribution of the inflorescences to the above-ground biomass in each cultivar, the percentage was higher in 'Naxos' (32–45%) than in 'Nubia' (24–27%) and 'Parthenon' (22–29%), reinforcing the suitability of 'Naxos' under Mediterranean semiarid conditions.

The characterization of commercial physical quality of the broccoli inflorescences showed that minor and major head diameters were variable between cultivars (Fig. 2(A, B)), showing significantly higher values in 'Naxos' than in 'Nubia' or 'Parthenon'. The circularity, major : minor diameters ($D : d$) and the commercial head length were very homogeneous groups (Fig. 2(C, D)). Stalk diameters were significantly higher in 'Nubia' than in 'Naxos' or 'Parthenon' (Fig. 2(E)). Therefore, the parameters of head quality showed that practically all the plants and studied samples presented commercially acceptable heads.[13]

**Intact glucosinolates**
The content of intact aliphatic and indole glucosinolates in the inflorescences of broccoli has been reported to be affected by the cultivar and growth conditions.[10] A detailed analysis of the differences between cultivars showed that the total glucosinolates in 'Naxos' heads (at 0, 40, and 80 mmol $L^{-1}$ NaCl) significantly surpassed 'Nubia' (by 30–48%) and 'Parthenon' (by 8–70%) (Fig. 1(A)). The total glucosinolates in inflorescences of 'Nubia', 'Naxos', and 'Parthenon' were in the ranges previously described for commercial broccolis like 'Emperor', 'Shogun' or 'Marathon' (0.302–0.576 g $kg^{-1}$ fresh weight (fw)).[19] These data also highlighted the importance of the genetic background to drive the phytochemical profile of edible broccoli heads in cultivars better adapted to the saline conditions in the local area.

Analysis of the content of single aliphatic glucosinolates in the heads of 'Naxos' control plants (0 mmol $L^{-1}$ NaCl) showed glucoraphanin as the main aliphatic glucosinolate (about 10-fold higher than glucoerucin). The level of glucoraphanin was higher in 'Naxos' (∼0.200 g $kg^{-1}$ fw) than in 'Nubia' and 'Parthenon' (Fig. 3(B)). The glucoerucin in flowering heads of 'Nubia' and 'Naxos' had similar concentrations (∼0.005 g $kg^{-1}$ fw), well below the level of 'Parthenon' (∼0.02 g $kg^{-1}$ fw) (Fig. 3(C)). The glucoraphanin content was higher in 'Naxos', also less affected by the saline treatments, in comparison to 'Nubia' and 'Parthenon'. 'Nubia' broccoli showed higher content of glucoerucin at 40 and 80 mmol $L^{-1}$ NaCl, surpassing 'Naxos' and 'Parthenon', whereas in 'Parthenon' glucoerucin was higher under the control condition, suggesting the role of the genetic factor in the adaptation of



  

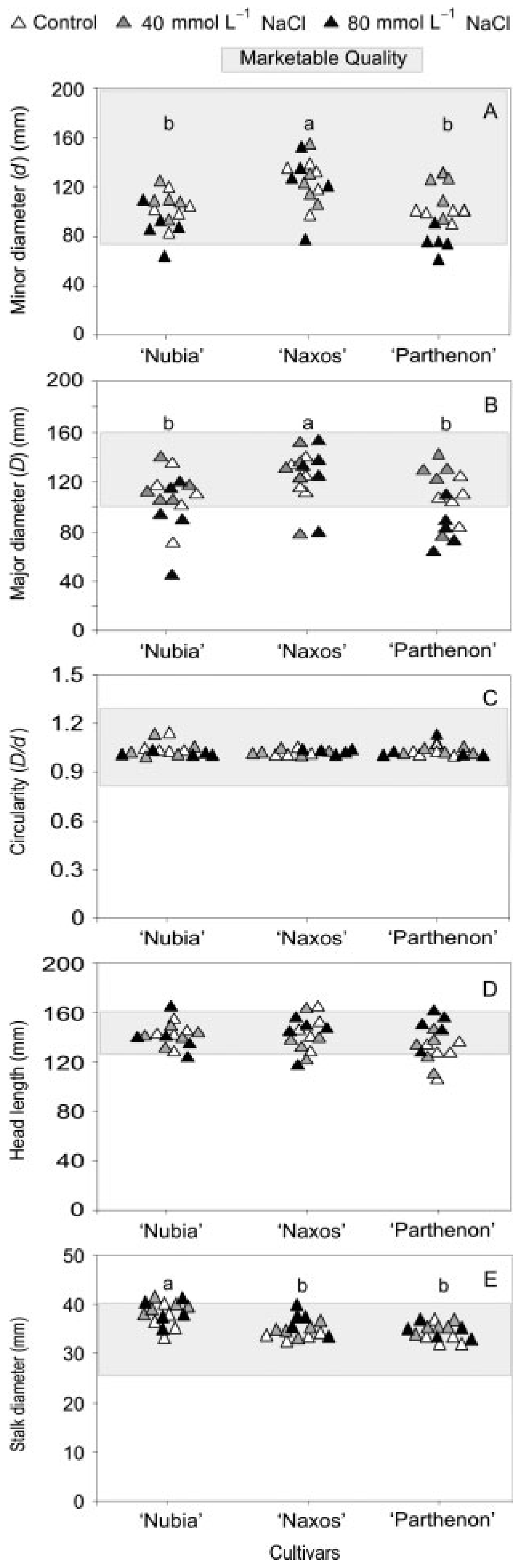


**Figure 2.** Representation of the physical quality parameters of 'Nubia', 'Naxos' and 'Parthenon' heads. Each data cloud informs about each floret physical parameter of non-treated plants (△), and the 40 mmol $L^{-1}$ NaCl (▲) and 80 mmol $L^{-1}$ NaCl (▲) treatments. Statistical analysis was done for flowering head samples of every cultivar and treatment. Different lower-case letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

broccoli to salinity[10] as well as the relevance of abiotic factors for the selection of newly introduced cultivars to growth under adverse conditions.

When analyzing the indole glucosinolates hydroxyglucobrassicin, glucobrassicin, methoxyglucobrasscin and neoglucobrassicin (Fig. 3(D, E, F, G) respectively), it was observed that under control conditions hydroxyglucobrassicin was the major indole glucosinolate in 'Naxos' (∼0.600 g $kg^{-1}$ fw), followed by 'Parthenon', with 'Nubia' being the lowest (Fig. 3(D)). Glucobrassicin, which constitutes one of the main indole glucosinolates present in broccoli,[19] showed similar levels in florets of 'Nubia' and 'Naxos' (∼0.250 g $kg^{-1}$ fw), whereas 'Parthenon' had a much lower concentration in comparison (Fig. 3(E)). Methoxyglucobrassicin was always higher in 'Naxos' (∼0.030 g $kg^{-1}$ fw), and the lowest value was recorded in 'Nubia' at 0 mmol $L^{-1}$ NaCl (Fig. 3(F)), whereas the other reported major indole glucosinolate of broccoli, neoglucobrassicin,[19] presented its highest levels in 'Nubia' (∼0.100 g $kg^{-1}$ fw) (Fig. 3(G)). From these results we can outline that the saline stress (40 and 80 mmol $L^{-1}$ NaCl) was better supported by 'Naxos' broccoli, conferring an additional agricultural and food value of adaptation to the adverse conditions of the southeast Spain production area, since the highest total and single glucosinolate contents recorded in 'Naxos' at 0 mmol $L^{-1}$ NaCl are maintained upon increased NaCl applications through the irrigation water.

The three cultivars 'Nubia', 'Naxos' and 'Parthenon' showed a content of total glucosinolates in the range accepted for health-promoting foods under all the tested conditions. Nonetheless, 'Naxos' showed the highest contents and the smallest variations regarding the glucosinolate contents. The response of 'Parthenon' and 'Nubia' of moderate sensitivity to the NaCl applied emphasized in our results what was also observed in previous findings on the co-influence of inner and external factors with regard to the metabolism of glucosinolates.[6,20] Despite the fact that the resistance of 'Naxos' to saline stress was not a character for its selection (intended to resist higher temperatures of late winter/early spring in Mediterranean areas), it appeared as a 'collateral advantage' for growing broccoli in Mediterranean semiarid regions (as in Murcia, southeast Spain), where local stress conditions also included low quality of the irrigation waters together with the semiarid climate.

### Phenolic compounds

The total phenolic compounds by HPLC–diode array detection (DAD) as the addition of the hydroxycinnamic acid derivatives and flavonoids analyzed in the flowering heads of plants grown at 0 and 40 mmol $L^{-1}$ NaCl showed higher contents in 'Nubia', surpassing 'Naxos' (by 58% and 8%, respectively) and 'Parthenon' (by 75% and 56%, respectively). The highest load of total phenolics, at 80 mmol $L^{-1}$ NaCl, was recorded in 'Naxos' – higher than 'Nubia' (by 8%) and 'Parthenon' (by 50%) (Fig. 4(A)). 'Naxos' underwent the lowest reductions and under NaCl stress regarding the phenolic compounds. Further analysis on the separate classes of phenolic compounds present in broccoli (i.e. chlorogenic and sinapic acid derivatives and flavonoids) showed that the flavonoids were the major group of phenolics in the three cultivars, and therefore the major group responsible for the total phenolics response to salinity treatments. When examining the content of chlorogenic acid derivatives in flowering heads (plants grown at 0 mmol $L^{-1}$ NaCl), 'Nubia' and 'Naxos' revealed similar amounts, approximately twofold higher than in 'Parthenon' (Fig. 4(B)). The flavonoids were higher in the flowering heads of 'Nubia',



  

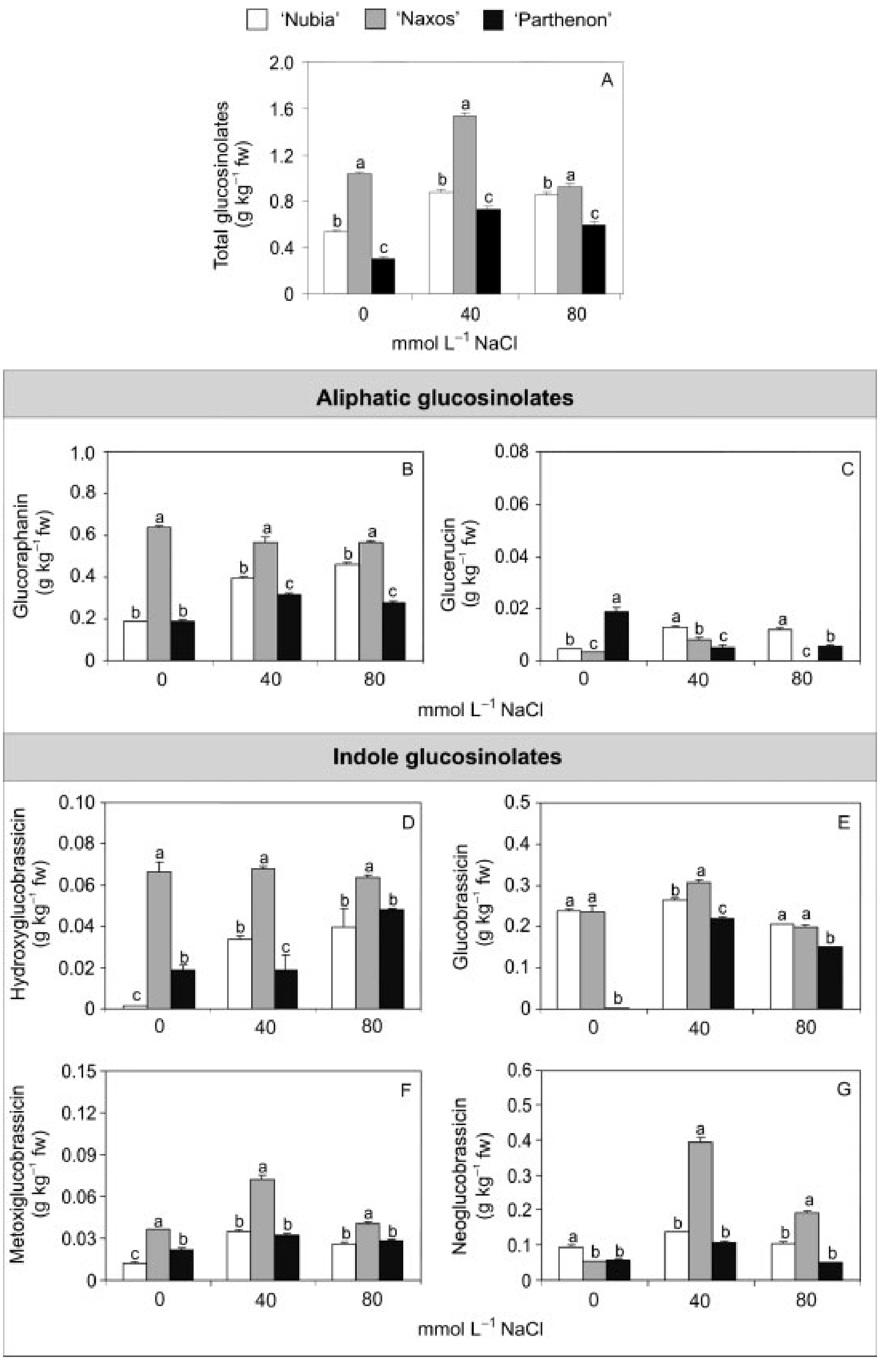


**Figure 3.** Total and single intact glucosinolates (g kg$^{-1}$ fw) in flowering heads of novel broccoli cultivars. Total glucosinolates (A), glucoraphanin (B), glucoerucin (C), hydroxyglucobrassicin (D), glucobrassicin (E), methoxyglucobrassicin (F) and neoglucobrassicin (G) content in the flowering heads of widely grown cultivar 'Nubia' (white bar) compared with the novel cultivars 'Naxos' (grey bar) and 'Parthenon' (black bar) grown hydroponically and under distinct saline conditions. Data presented are mean ± SD ($n = 3$). Different lower-case letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

surpassing 'Naxos' by three times and 'Parthenon' by seven times (Fig. 4(C)). Finally, the sinapic acid derivatives also appeared in the highest concentration in 'Nubia': twofold higher than in 'Naxos' or 'Parthenon' (Fig. 4(D)).

Under saline conditions the sense of the differences between cultivars, regarding the content in total phenolics and the separate groups considered (hydroxycinnamic acids and flavonoids), was reduced, given the stronger decrease in their levels found in 'Nubia' as compared to 'Naxos' or 'Parthenon' (Fig. 4(B–D)), indicating that 'Nubia' was a valuable cultivar and also confirming the necessity of establishing novel substitutes to guarantee the market demand of high-quality broccoli.

It has previously been shown that salt stress induces an increase in phenolic compounds as a result of the disturbance caused in secondary metabolic pathways.[19,21,22] Other authors have also described a decrease in phenolics, and mainly in flavonoids, secondary to extended NaCl treatment.[8,23] The data obtained in the present work support a decrease in phenolic compounds



  

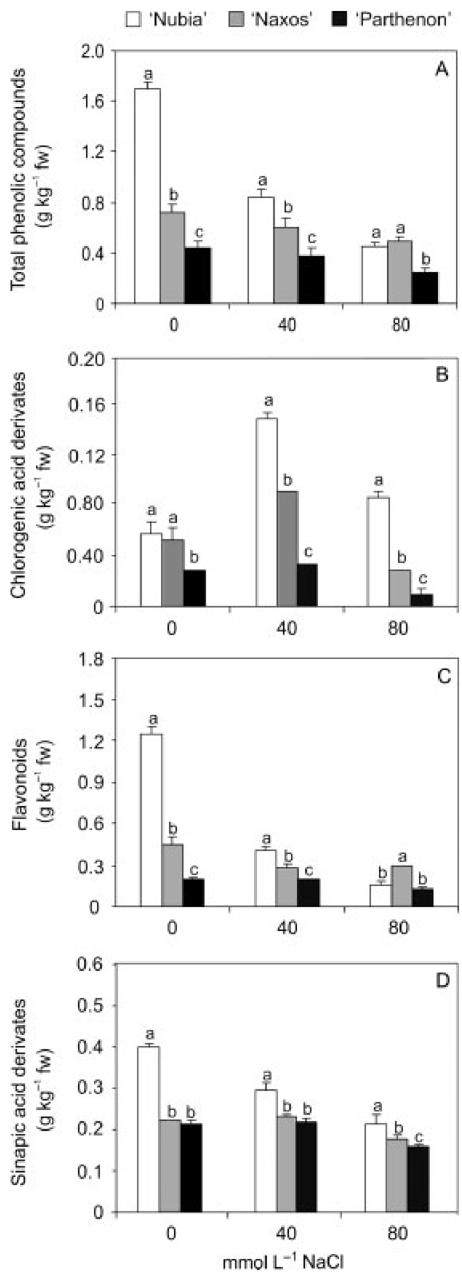


**Figure 4. T**otal phenolic compounds (A), chlorogenic and sinapic acid derivates (B and D, respectively), and flavonoid (C) content (g $kg^{-1}$ fw) in the flowering heads of 'Nubia' (white bar), 'Naxos' (grey bar) and 'Parthenon' (black bar) grown hydroponically and under distinct saline conditions. Data presented are mean ± SD ($n = 3$). Different lower-case letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

as an invariable consequence of saline treatment and pointed out that the influence of cultivar to define the magnitude of the response to the deleterious effect of salinity on the phenolic metabolism in the context of a multifactorial process.[23,24]

### Vitamin C

The content of total vitamin C, as the sum of AA and DHAA showed non-significant differences when comparing 'Nubia', 'Naxos' and 'Parthenon', in plants grown at 0 mmol $L^{-1}$ NaCl (Fig. 5). When studying AA and DHAA separately, it was observed that the AA content in flowering heads of plants grown at 0 mmol $L^{-1}$ NaCl was similar for 'Nubia' and 'Naxos', whereas 'Parthenon' showed a lower content. In 'Naxos', higher content of AA was recorded at 40 mmol $L^{-1}$ NaCl, whereas non-significant differences were recorded at 0 or 80 mmol $L^{-1}$ NaCl (Fig. 5).

It has been previously described how the level of vitamin C is closely related to the cultivar analyzed.[24] Our data also support the relationship between the concentration of this natural antioxidant and biological and agronomic factors.[25] Considering all this, the relative influence of salinity on vitamin C concentration is conditioned by the cultivar.

### *In vitro* antioxidant activity (DPPH$^\bullet$, ABTS$^{\bullet+}$ and FRAP assays)

Comparing cultivars (at 0 mmol $L^{-1}$ NaCl), the flowering heads of 'Nubia' had the highest DPPH$^\bullet$ and ABTS$^+$ radical scavenging capacity and ferric reducing ability, surpassing 'Naxos' (between 30% and 45%) and 'Parthenon' (by 45–65%). In addition, the antioxidant capacity exhibited by extracts of flowering heads of broccoli grown under saline stress (40 and 80 mmol $L^{-1}$ NaCl) was also higher in 'Nubia' than in 'Naxos' and 'Parthenon' (Table 2).

Results of antioxidant tests showed a reduction at 40 and 80 mmol $L^{-1}$ NaCl in general, although 'Naxos' and 'Parthenon' underwent a less intense decrease of antioxidant power than 'Nubia', even though the three cultivars showed a range in the levels of health-promoting activity estimated over a wide variety of vegetables, including broccoli.[26]

Antioxidant capacity constitutes a practical expression of the functionality of phenolic compounds and AA present in broccoli.[24,27] Phenolic compounds and AA showed a separate trend in plants grown under saline conditions. Whereas phenolic compounds showed an invariable decrease in plants grown under NaCl treatments in the three cultivars, AA appeared to be practically unaffected (especially in 'Naxos' and 'Parthenon'). Taking together the concentration of these compounds in plants grown at 0, 40 and 80 mmol $L^{-1}$ NaCl, these data suggest that the major constituents responsible for antioxidant capacity are phenolic compounds (the concentration of flavonoids and sinapic acid derivatives showed a significant positive correlation to the DPPH$^\bullet$ ($r^2$ 0.722 ($p < 0.001$) and $r^2$ 0.518 ($p < 0.01$), respectively) and ABTS$^+$ ($r^2$ 0.764 ($p < 0.001$) and $r^2$ 0.817 ($p < 0.001$), respectively) radical scavenging capacities and ferric reducing ability ($r^2$ 0.666 ($p < 0.001$) and $r^2$ 0.605 ($p < 0.01$), respectively) and their reduction entails a minor antioxidant power of inflorescences of the plants grown under saline stressing conditions. Vitamin C, which was less affected by saline growing conditions, is able to guarantee healthy antioxidant power in broccoli plants grown under the saline conditions present in the local area (southeast Spain).

Therefore, although as a general rule the antioxidant power attributed to *Brassica oleracea* has been related to their natural antioxidants, phenolics and vitamin C,[16,28–30] the relative participation of phenolic compounds and vitamin C is not yet fully understood, and probably the inherent genetics of each cultivar govern the relative phytochemical profile, since the distinct compounds taking part in the whole bioactive machinery of broccoli respond to the physiological disturbance caused by saline stress in independent pathways that are modified more or less severely depending on the genetic information of each cultivar, constituing a compensatory mechanism to resist the adverse conditions to which the plants or broccoli are exposed.

  

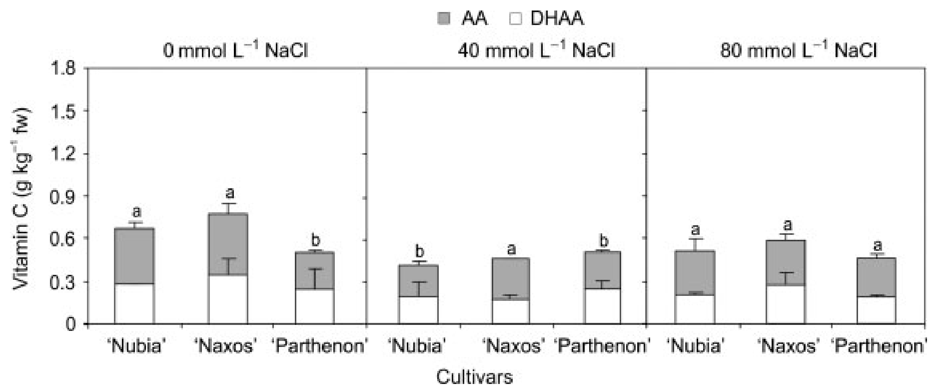


**Figure 5.** Vitamin C content (g $kg^{-1}$ fw) in the flowering heads of 'Nubia', 'Naxos' and 'Parthenon' grown hydroponically under control conditions, and 40 and 80 mmol $L^{-1}$ NaCl saline treatments. Data are showed as mean ± SD ($n = 3$) of ascorbic acid (grey bar) and dehydroascorbic acid (white bar). Total vitamin C is represented as addition of ascorbic and dehydroascorbic acids. Different lower-case letters indicate significant differences of the total vitamin C content between cultivars at $P < 0.05$ according to Duncan's multiple range test.

**Table 2.** Analysis of *in vitro* antioxidant activity of 'Nubia', 'Naxos' and 'Parthenon' broccolis grown hydroponically

| Treatment (mmol $L^{-1}$ NaCl) | Cultivar | Antioxidant activity (mmol $L^{-1}$ Trolox $kg^{-1}$ fw) DPPH | ABTS | FRAP |
|---|---|---|---|---|
| 0 | 'Nubia' | 245.015a | 276.075a | 157.057a |
| | 'Naxos' | 170.462b | 145.740b | 106.493b |
| | 'Parthenon' | 83.268c | 134.850b | 82.532b |
| | ANOVA *P*-value | *** | ** | * |
| | LSD ($P < 0.05$) | 32.108 | 65.31 | 3.9196 |
| 40 | 'Nubia' | 195.137a | 174.701a | 145.583a |
| | 'Naxos' | 163.347a | 127.766b | 84.394b |
| | 'Parthenon' | 99.400b | 115.483b | 86.717b |
| | ANOVA *P*-value | ** | * | * |
| | LSD ($P < 0.05$) | 48.437 | 36.485 | 49.547 |
| 80 | 'Nubia' | 181.973a | 141.770a | 91.024b |
| | 'Naxos' | 155.125b | 122.151a | 121.135a |
| | 'Parthenon' | 97.286c | 93.222a | 63.488c |
| | ANOVA *P*-value | *** | n.s. | ** |
| | LSD ($P < 0.05$) | 11.817 | 57.012 | 11.159 |

Means ($n = 3$) within a column followed by a different lower-case letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.
Non-significant ($P > 0.05$); significant at: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

### Mineral nutrients and trace elements

Broccoli constitutes an essential dietary source of minerals.[31] Analysis of the plants grown under control conditions indicated that the three cultivars presented a much high nutritional value regarding mineral content. In the flowering heads of 'Nubia', 'Naxos' and 'Parthenon', carbon and nitrogen remained practically invariable. It was observed that 'Naxos' presented a higher content of S than 'Nubia' and 'Parthenon', which may correlate with content in glucosinolates. Regarding P, S, Na, K, Ca and Mg, their contents in flowering heads remained within the range of previously reported data for broccoli.[32] Under saline treatments, higher contents of P and K were recorded in 'Nubia' as compared to 'Naxos' or 'Parthenon', at 40 mmol $L^{-1}$ NaCl (Table 3).

Essential trace elements (B, Cu, Fe, Mn, and Zn) in florets of 'Nubia', 'Naxos' and 'Parthenon' ranged within health-promoting values of the recommended daily allowance.[31] Not many significant differences were found between cultivars, except for B in 'Naxos', which showed almost double the content of 'Nubia' and 'Parthenon'. At 40 mmol $L^{-1}$ NaCl, 'Nubia' showed the highest content of B and Cu, and 'Parthenon' showed the lowest content of Fe, Mn and Zn (Table 4).

The Fe, Mn, Zn, and Cu status in the broccoli plants was not negatively affected by NaCl stress, as seen in others experiments.[33,34] Consequently, current conditions of high-saline irrigation waters for crops in southeast Spain are not limiting the mineral quality of broccoli[7] for nutrition.

## CONCLUSION

The cultivars 'Nubia', 'Naxos' and 'Parthenon' showed distinct responses to NaCl stress in the irrigation water and the climate conditions prevailing in the production area (southeast Spain). Even though the three cultivars presented a nutritional composition in the range of health-promoting foods, 'Parthenon' and 'Nubia' were less rich in bioactive compounds and nutrients. The 'Naxos' cultivar appeared to be the most suitable alternative to 'Nubia' in terms of 'marketable' yield as well as profile and content of bioactive components, showing moderate variations under saline stress. The new cultivars guarantee availability of high-quality broccoli under saline stress conditions (Mediterranean semiarid area and low quality of water for irrigation) – an adverse situation to overcome for a quality produce.

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**Table 3.** Variation in mineral nutrient contents of 'Nubia', Naxos' and 'Parthenon' broccoli grown hydroponically

| Saline treatment (mmol $L^{-1}$ NaCl) | Cultivar | Mineral nutrient (mmol $kg^{-1}$ fw) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | N | P | S | Na | K | Ca | Mg |
| 0 | 'Nubia' | 3.69a | 3.73a | 31.23a | 42.71b | 4.75a | 117.23a | 11.98a | 12.64a |
| | 'Naxos' | 3.79a | 3.41a | 30.86a | 50.39a | 5.71a | 120.38a | 20.92a | 15.78a |
| | 'Parthenon' | 3.67a | 3.74a | 27.61a | 39.40b | 4.11a | 99.28a | 11.48a | 11.43a |
| | ANOVA *P*-value | n.s. | n.s. | n.s. | * | n.s. | n.s. | n.s. | n.s. |
| | LSD ($P < 0.05$) | 0.28 | 0.34 | 11.75 | 2.63 | 3.24 | 23.01 | 9.19 | 3.56 |
| 40 | 'Nubia' | 3.64a | 3.24a | 34.49a | 45.61b | 10.72ab | 118.43a | 9.89a | 12.49a |
| | 'Naxos' | 3.97a | 3.36a | 29.88b | 52.08a | 14.46a | 103.74b | 9.46a | 12.03a |
| | 'Parthenon' | 3.70a | 3.37a | 22.63c | 31.91c | 7.29b | 81.82c | 8.24a | 8.74b |
| | ANOVA *P*-value | n.s. | n.s. | *** | * | ** | ** | n.s. | ** |
| | LSD ($P < 0.05$) | 0.56 | 0.29 | 4.27 | 2.03 | 4.49 | 12.85 | 7.89 | 3.18 |
| 80 | 'Nubia' | 3.93a | 3.67b | 40.84a | 49.10c | 16.17a | 118.70a | 10.59a | 12.83a |
| | 'Naxos' | 3.70a | 3.37b | 28.03b | 59.47a | 17.56a | 96.20b | 13.46a | 12.26a |
| | 'Parthenon' | 3.77a | 4.18a | 40.20a | 53.73b | 14.64a | 72.38c | 11.15a | 12.86a |
| | ANOVA *P*-value | n.s. | * | * | * | n.s. | ** | n.s. | n.s. |
| | LSD ($P < 0.05$) | 0.35 | 0.31 | 11.82 | 3.83 | 8.49 | 21.24 | 4.11 | 2.27 |

Means ($n = 3$) within a column followed by a different lower-case letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.
Non significant ($P > 0.05$); significant at: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

**Table 4.** Variation of trace element contents of 'Nubia', Naxos' and 'Parthenon' broccoli grown hydroponically

| Saline treatment (mmol $L^{-1}$ NaCl) | Cultivar | Trace element (µmol $kg^{-1}$ fw) | | | | |
|---|---|---|---|---|---|---|
| | | B | Cu | Fe | Mn | Zn |
| 0 | 'Nubia' | 277.7b | 7.3a | 84.9a | 19.1a | 71.4a |
| | 'Naxos' | 404.7a | 8.8a | 100.9a | 23.0a | 73.3a |
| | 'Parthenon' | 264.7b | 7.1a | 73.1a | 17.6a | 70.0b |
| | ANOVA *P*-value | * | n.s. | n.s. | n.s. | n.s. |
| | LSD ($P < 0.05$) | 54.0 | 4.8 | 58.2 | 15.8 | 34.8 |
| 40 | 'Nubia' | 317.1a | 9.3a | 63.9a | 22.8ab | 67.4a |
| | 'Naxos' | 295.1b | 7.2ab | 77.6a | 25.1a | 64.8a |
| | 'Parthenon' | 226.3c | 5.6b | 42.6b | 15.0b | 45.3b |
| | ANOVA *P*-value | ** | * | * | * | ** |
| | LSD ($P < 0.05$) | 15.0 | 2.1 | 27.5 | 10.0 | 18.8 |
| 80 | 'Nubia' | 342.1a | 12.4a | 75.5a | 18.8a | 86.9a |
| | 'Naxos' | 311.6a | 5.6b | 66.0a | 19.2a | 63.0a |
| | 'Parthenon' | 318.3a | 10.3a | 1045a | 24.9a | 104.3a |
| | ANOVA *P*-value | n.s. | * | n.s. | n.s. | n.s. |
| | LSD ($P < 0.05$) | 28.4 | 2.2 | 39.8 | 17.4 | 44.0 |

Means ($n = 3$) within a column followed by a different lower-case letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.
Non-significant ($P > 0.05$); significant at: * $P < 0.05$; ** $P < 0.01$.

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