Research Article

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Salinity differently affects antioxidant content and amino acid profile in two cultivars of *Amaranthus cruentus* differing in salinity tolerance

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Abstract

BACKGROUND: *Amaranthus cruentus* is a promising leafy vegetable with high nutritional value and is able to cope with salt stress but the impact of sodium chloride (NaCl) on its main properties have not been studied in detail. Plants from two contrasting cultivars (Rouge: salt-tolerant and Locale: salt-sensitive) were exposed to NaCl (0, 30, 60 and 90 mmol L^{-1}) in nutrient solution for 2 weeks. Plant growth, mineral content, oxidative status and antioxidant concentration, salicylic acid concentration, protein content and amino acid profile were analyzed in the harvested leaves.

RESULTS: Low dose (30 mmol L^{-1} NaCl) increased plant growth while Na^+ accumulated to higher extent in salt-sensitive Locale than in salt-tolerant Rouge. A total of 30 mmol L^{-1} NaCl increased magnesium (Mg), phosphorus (P) and iron (Fe) content, as well as total antioxidant activity, ascorbate, phenolics, α-tocopherol and carotenoids content to higher extent in cultivar (cv.) Rouge than in cv. Locale. Low (30 mmol L^{-1}) and moderate salinities (60 mmol L^{-1}) increased γ-tocopherol and total protein in cv. Locale. They also increased lysine, valine, methionine and proline concentration as well as chemical score of protein in this cultivar. The highest NaCl (90 mmol L^{-1}) dose had a detrimental impact on both cultivars.

CONCLUSIONS: It is concluded that *A. cruentus* is a promising plant species for saline agriculture since moderate doses of salt improve both quantitative and qualitative parameters in cultivar dependent manner.

Keywords: *Amaranthus*; antioxidant; NaCl; salinity; saline agriculture

INTRODUCTION

Soil salinity is a major environmental constraint compromising agricultural production in the world. It already affects 830 million hectares and is expected to impact 50% of arable land by 2050.[1] An excess of soluble salts [mainly sodium chloride (NaCl)] compromises water uptake by plants and lead to numerous disturbances in relation to Na^+ and Cl^- accumulation and salt-induced deficiencies of essential elements.[2,3] The ultimate consequence is a global decrease in crop yield which constitutes a threat to global food security in numerous areas of the world.[1,4] Most of the cultivated plant species are rather salt-sensitive (glycophyte species) since they were selected over thousands of years for yield potential in non-saline environments.[5] An alternative is to focus on marginal plants already domesticated but which still exhibit rusticity and capacity to cope with environmental abiotic constraints to meet future demands of foods from a growing world population.

For this purpose, *Amaranthus cruentus* is an ideal candidate.[6] Both grain and leaves of *A. cruentus* are consumed as foods due to its high nutritive values.[6,7] The leaves of *A. cruentus* are extremely rich in vitamins including β-carotene, vitamin B6, vitamin C, riboflavine and folate, as well as dietary minerals with extremely high iron (Fe) and zinc (Zn) content and *A. cruentus* also displays a suitable amino acid profile for human nutrition.[6,8–10] Leaf extracts also exhibit a very high antioxidant capacity and high concentrations of phenolic acids and flavonoids.[11–13]

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Field observation suggests that amaranth can cope with abiotic stresses, including drought and salinity.[13–17] Numerous bioactive compounds, including vitamins, polyphenols and flavonoids are issued from the secondary metabolism which is frequently activated in response to salt stress[13,16,17] but data on *A. cruentus* are crucially missing.

There is no evidence that cultivars differing in their mean level of salinity tolerance respond in the same way to NaCl. To the best of our knowledge, no study until now compared the influence of NaCl on nutritive status of leaves from contrasting *A. cruentus* cultivars. The present study therefore reports the effects of NaCl on amino acid profile, protein, minerals, sugar, salicylic acid and antioxidant (including ascorbate and α-tocopherol) content in two cultivars of *A. cruentus* differing in their mean level of salinity tolerance.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of two *A. cruentus* cultivars (Locale and Rouge) were obtained from a Market Gardening Crops Program from the Benin National Institute for Agricultural Research (INRAB); cultivar (cv.) Locale is considered as salt-sensitive while cv. Rouge is regarded as salt-tolerant.[18] Seventeen days after germination, the obtained seedlings were transferred to plastic tanks containing 10 L of permanently aerated Hoagland nutrient solution. Salt stress was applied 18 days after plant transfer in order to reach a final concentration of 0 (control), 30, 60 or 90 mmol L^{-1} NaCl. Plants were exposed to salinity in a phytotron under fully controlled environmental conditions [photoperiod 12 h d^{-1}, mean light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR), relative humidity of 70% and temperature of 25 ± 1 °C during the day and 20 ± 2 °C during the night].

Plant harvest, water content and mineral concentration

Plants were harvested after 2 weeks of treatment: five plants per treatment (cultivar × NaCl dose) were separated in roots, stems and leaves and weighed. Roots were quickly rinsed in sterile deionized water; organs were separately weighed (fresh weight estimation) and then incubated in an oven at 70 °C during 48 h for dry weight (DW) estimation. The leaves of the remaining plants were harvested, quickly frozen in liquid nitrogen and then stored at −80 °C until final analysis. For mineral content, 50 mg DW of leaf and root tissues were exposed to 4 mL nitric acid (HNO_3) 68% at 80 °C. After complete evaporation, residues were dissolved with HNO_3 68% + hydrochloric acid (HCl) (1:3, *v/v*) and filtered on Whatman Grade A filter. Cation concentrations were estimated by inductively coupled plasma (ICP) analysis (MPX type; Varian, Palo Alto, CA, USA) in triplicate for each sample.

Plant oxidative status and antioxidant content

Malondialdehyde (MDA) as an indicator of oxidative stress and lipid peroxidation was quantified in the leaves by the thiobarbituric acid method according to Heath and Paker.[19] Hydrogen peroxide (H_2O_2) was estimated spectrophotometrically at 508 nm using the titanium reagent in phosphate buffer K_2HPO_4/KH_2PO_4 at pH 7.8 according to Arasimowicz *et al.*[20] The total antioxidant activity was estimated through the free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH) method according to Brand-Williams *et al.*[21]: the data are expressed as µmol L^{-1} Trolox equivalent g^{-1} fresh weight (FW) and are provided separately for the hydrophilic (AOAM) and hydrophobic (AOAD) fraction.

Reduced glutathione (GSH) and total glutathione (GSH + GSSG) were quantified with a fluorescence Shimadzu RF-0A detector at 420 nm with excitation at 340 nm after high-performance liquid chromatography (HPLC) separation and *ortho*-phthalaldehyde (OPA) pre-column derivatization.[22] Ascorbate concentration was estimated as recommended by Kampfenkel *et al.*[23] The concentration of α-tocopherol and its oxidation product α-tocopherol quinone were estimated after extraction in ice-cold *n*-hexane containing 1 ppm butylated hydroxytoluene and separation on a Partisil 10 ODS-3 column at a flow rate of 1 mL·min^{-1} with a methanol/water (95:5, v/v) solvent. The α-tocopherol and α-tocopherol-quinone were detected at 283 and 265 nm, respectively.[24] Total carotenoids were extracted from fresh leaves using 80% acetone and determined spectrophotometrically as previously detailed.[13]

Total phenolics, flavonoids, red pigments and salicylic acid concentration

Phenols were extracted with 2 mL of 80% methanol. The mixture was centrifuged at 10 000 × *g* for 10 min at 4 °C. Total phenolic content was determined using the Folin–Ciocalteu reagent.[25] Total flavonoids were estimated using the colorimetric assay recommended by Dewanto *et al.*[26] using catechin as standard. The red pigments concentration (anthocyanins + betalains) concentration was estimated using the method of Mancinelli[27] and calculated as cyanidin-3-glucoside using 29 600 as extinction coefficient and 444.5 as molecular weight.

Salicylic acid (SA) was extracted in methanol 90% in two steps preventing sublimation of SA by addition of 0.2 mol L^{-1} sodium hydroxide[28]: dried residues were resuspended in 250 µL trichloroacetic acid (TCA) 5% + 800 µL acetate/cyclohexane. A subsequent acid hydrolysis of the aqueous phase allows the liberation of SA and its glycosyl derivative. Samples were then injected on a HPLC system equipped with an Inertsil ODS-3; 250 mm × 3 mm with 3 µm particles. Mobile phase consists in a gradient of water/acetonitrile from 10% to 100% of acetonitrile and elution was performed at 30 °C with a flow rate of 1.0 mL min^{-1}.

Sugar and protein content and amino acid profile

Total soluble sugars were extracted and quantified with an anthrone reagent according to Yemm and Willis.[29] Total soluble protein was estimated according to Bradford.[30] Amino acid profile and concentrations were determined according to Meussen *et al.*[31] after acid hydrolysis and derivatization using OPA reagent in combination with 9-fluorenylmethyl chloroformate (FMOC). Samples (300 mg FW) were exposed to 500 µL of HCl (6 mol L^{-1}) containing 1% (*w/v*) of phenol and incubated during 18 h at 110 °C. Solution was then dried under vacuum and samples were resuspended in 400 µL methanol +500 µL MilliQ water. A double derivatization process was performed in pre-columns using (i) 2-mercaptoethanol 4% + 25 mg OPA dissolved in 0.5 mL methanol in a total volume of 5 mL borate buffer pH 10.4 and (ii) FMOC 0.25% in acetonitrile. Samples were injected on a Zorbax Eclipse Plus column (Agilent Technologies, Santa Clara, CA, USA) maintained at 40 °C (3.5 µm particle size; 150 mm × 21 mm) using two mobile phases (A: phosphate buffer 40 mmol L^{-1} pH 8.4; B: acetonitrile/methanol/water (45:45:10, *v/v/v*) at a flow rate of 0.42 mL min^{-1} (100% A–0% B 0.5 min; progressive increase from 0% to 57% B 0.5–25 min). OPA-derivatized amino acids were detected at 350 nm excitation and 450 nm detection wavelengths and FMOC-derivatized ones were analyzed at 260 nm

(excitation) and 325 nm (emission). Bovine serum albumin (BSA; 1 mg mL^{-1}) was used as standard.

Statistical treatments

For each parameter, analyses were performed on at least five biological replicates. For each sample (replicate) quantifications were made on technical triplicates. Normality of the data were estimated using Shapiro–Wilk tests and homoscedasticity was verified using the Leven test. Variance analyses were performed with two crossed fixed factors (cultivar × NaCl dose) using the generalized linear model function with SAS Entreprise Gide 4.2. software. Two independent experiments were performed (2018 and 2019) and provided similar data.

RESULTS AND DISCUSSION

Plant growth and mineral concentration

All plants remained alive until the end of the treatment, confirming the high level of salt-tolerance of *A. cruentus* and its putative interest for culture in salt-affected soils. The leaf dry weight of cv. Rouge significantly increased in response to 30 mmol L^{-1} NaCl (Fig. 1(A)) and remained similar to control in response to 60 mmol L^{-1} NaCl, while the leaf DW of cv. Locale slightly decreased at 60 and 90 mmol L^{-1} NaCl. The root DW of both cultivars increased in response to 30 mmol L^{-1} NaCl.

The leaf sodium ion (Na^+) concentration increased in response to NaCl, being always significantly higher in cv. Locale than in cv. Rouge ($P = 0.0031$) (Fig. 1(C)). The leaf potassium ion (K^+) concentration significantly increased in response to 30 mmol L^{-1} NaCl in both cultivars ($P = 0.0090$) but to a higher extent in cv. Rouge than in cv. Locale. The leaf K^+ content decreased in Locale at 60 and 90 mmol L^{-1} NaCl. The Na^+ and K^+ ions share numerous chemical properties, especially similar ionic radii: Na^+ is consequently commonly absorbed by poorly selective K^+ transporters. In *A. cruentus* exposed to a low NaCl dose, however, K^+ accumulation occurred concomitantly with Na^+ absorption, which suggests that *A. cruentus* may efficiently regulate ion transporters in order to perform osmotic adjustment. A high endogenous Na^+ concentration constitutes a drawback for the consumption of leaves produced in salt-affected soils. At 30 mmol L^{-1} NaCl, leaves from cv. Rouge however contained higher amounts of K^+ and lower amounts of Na^+ than leaves of cv. Locale. Some authors reported

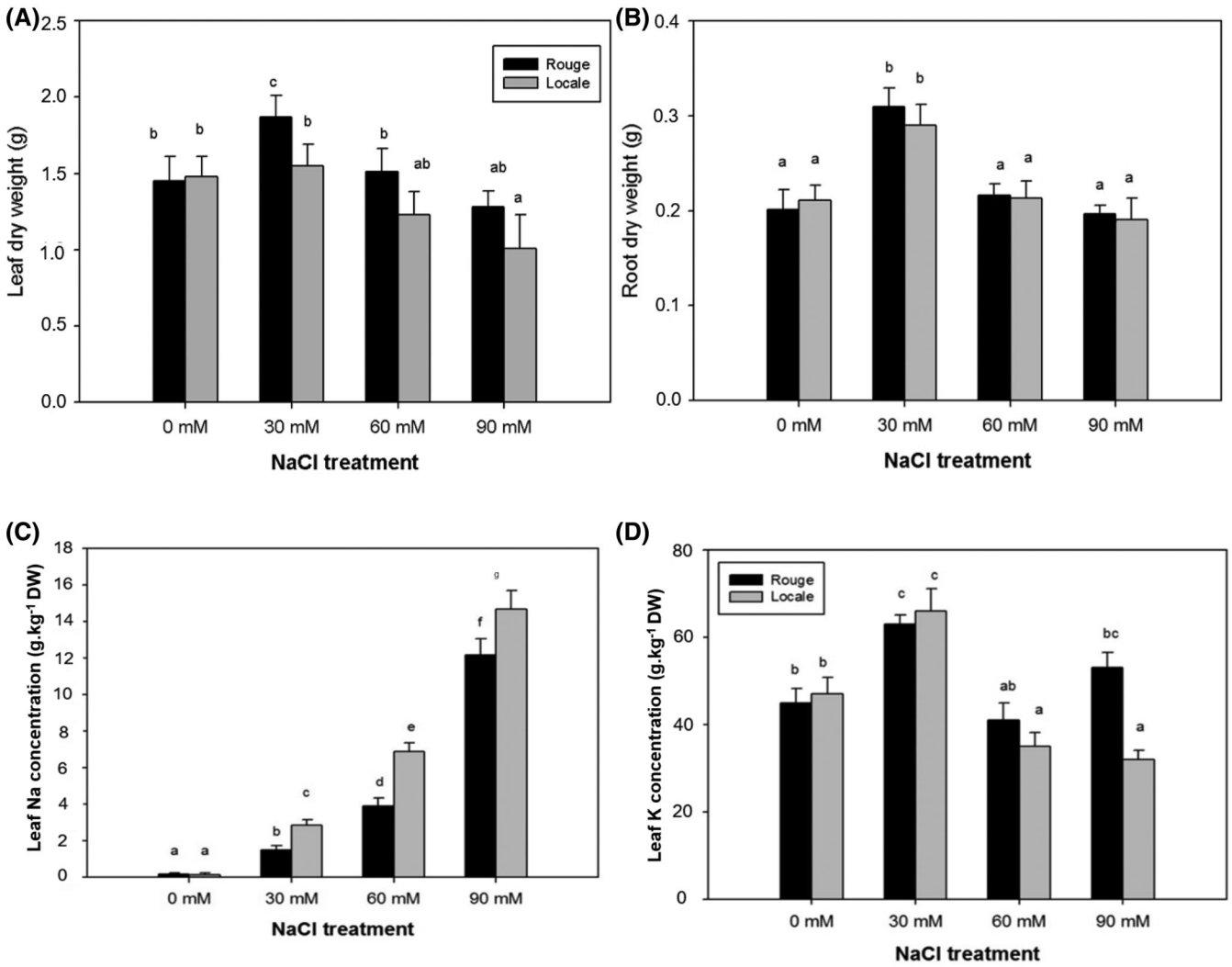

Figure 1. Impact of NaCl (0, 30, 60, 90 mmol L^{-1}) on leaf (A) and root (B) dry weight and on leaf sodium (Na) concentration (C) and leaf potassium (K) concentration (D) in two cultivars of *Amaranthus cruentus* (Locale: salt-sensitive; Rouge: salt-tolerant). Plants were exposed for 16 days to salinity. Each value is the mean of five replicates and vertical bars are standard error. Values sharing similar letter are not significantly different at $P < 0.05$ according to Tukey's multiple range test.

Table 1. Total element concentrations in the leaves of two cultivars of *Amaranthus cruentus* (cv. Locale and cv. Rouge) cultivated during 2 weeks in the presence of sodium chloride (NaCl) (0, 30, 60 and 90 mmol L^{-1})

Element	Cultivar	0 mmol L^{-1}	30 mmol L^{-1}	60 mmol L^{-1}	90 mmol L^{-1}
Calcium (Ca)	Locale	8.12 ± 0.54 b	8.37 ± 0.7 b	7.93 ± 0.63 ab	7.90 ± 0.52 ab
	Rouge	7.31 ± 0.11 a	7.47 ± 0.25 a	7.84 ± 0.33 ab	7.32 ± 0.10 a
Magnesium (Mg)	Locale	12.3 ± 1.0 a	14.1 ± 1.4 b	13.9 ± 1.1 b	12.1 ± 0.8 a
	Rouge	12.2 ± 1.5 a	15.2 ± 0.9 c	14.8 ± 0.5 b	14.3 ± 1.3 b
Phosphorus (P)	Locale	7.32 ± 0.61 a	8.26 ± 0.55 b	7.41 ± 0.50 a	7.07 ± 0.62 a
	Rouge	8.13 ± 0.24 b	9.47 ± 0.47 c	9.21 ± 0.74 c	8.94 ± 0.34 bc
Iron (Fe)	Locale	121 ± 12 a	142 ± 9 b	111 ± 14 a	117 ± 12 a
	Rouge	145 ± 7 b	191 ± 17 d	170 ± 11 c	163 ± 13 bc
Zinc (Zn)	Locale	30.1 ± 2.3 b	26.7 ± 1.8 b	29.7 ± 2.1 b	20.1 ± 2.4 a
	Rouge	28.7 ± 2.1 b	27.1 ± 1.1 b	38.2 ± 3.6 c	30.3 ± 3.2 b
Manganese (Mn)	Locale	248 ± 22 c	239 ± 18 bc	221 ± 23 b	199 ± 10 a
	Rouge	222 ± 24 b	234 ± 25 bc	217 ± 31 b	192 ± 13 a
Copper (Cu)	Locale	10.3 ± 2.1 a	9.8 ± 1.9 a	11.2 ± 2.4 a	9.7 ± 0.9 a
	Rouge	11.4 ± 2.7 a	10.4 ± 1.5 a	9.5 ± 1.8 a	10.9 ± 1.2 a

Concentrations of Ca, Mg and P are in g kg^{-1} dry weight (DW) and concentrations of Fe, Zn, Mn and Cu are in mg kg^{-1} DW.
Each value is the mean of five replicates ± standard error. For a given element, values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's multiple range test.

a low level of genetic variability in *A. cruentus*[32,33] but these studies were conducted in the absence of salt stress and we demonstrate here that the tested salt-tolerant and salt-sensitive genotypes of *A. cruentus* clearly differ for monovalent cation accumulation that is corroborative to the results of salt-stressed *A. tricolor*.[13]

The leaf concentrations in calcium (Ca), magnesium (Mg), Fe, phosphorus (P), manganese (Mn) and copper (Cu) are provided in Table 1. Calcium was hardly modified by NaCl treatment but was significantly higher in Locale than in Rouge ($P = 0.033$). In contrast, Mg, P and Fe content all increased at 30 mmol L^{-1} NaCl for the two cultivars and still remained higher in stressed plants than in controls for cv. Rouge but not for cv. Locale. The NaCl dose of 60 mmol L^{-1} also significantly increased Zn concentration in cv. Rouge ($P = 0.016$) but not in cv Locale. Salinity decreased the leaf Mn content at the highest dose (90 mmol L^{-1} NaCl). Numerous studies confirmed that *A. cruentus* is a valuable source of elements, especially considering Zn and Fe content.[8–10] In *A. tricolor*, Sarker and Oba[34,35] reported that drought stress reduces Fe content in the leaves. Although salt stress induces both an osmotic and an ionic constraint, it obviously increased Fe content in the leaves of *A. cruentus*. Because of this high Fe content, *A. cruentus* consumption may be recommended as a cheap strategy to reduce anemia in the population of rural areas[6,10,36,37] and our work suggests that moderate doses of NaCl even increase both leaf biomass and Fe content. Increase in the total Fe content is not a guarantee of food improvement since phytate may reduce Fe bioavailability for the human body.[36] We did not quantify phytate in the present work, but accumulation of phytic acid was reported to mainly occur in the grain while Fe is sequestered in ferritin within the leaves.[10] The present mineral profile was established on pooled leaves while for adult mature plants, the mineral status may vary depending on the leaf position.[9]

Oxidative status and antioxidant concentration

As a marker of lipid peroxidation, MDA content significantly increased at all NaCl doses in cv. Locale ($P = 0.017$) but at 90 mmol L^{-1} only in cv. Rouge (Fig. 2(A)). This suggests that salt-induced oxidative stress was correctly managed in the salt-tolerant cv. Rouge as confirmed by the fact that H_2O_2 concentration remained constant in this cultivar while it increased at 60 and 90 mmol L^{-1} in cv. Locale (Fig. 2(B)). The present findings are corroborative to the results of *A. tricolor*.[38,39] This hypothesis is also supported by the results of the total antioxidant power. For both AOAM (hydrophilic; Fig. 2(C)) and AOAD (hydrophobic; Fig. 2(D)) fractions, cv. Rouge constitutively exhibited significantly higher total antioxidant activity than cv. Locale ($P = 0.0012$ for AOAM and $P = 0.041$ for AOAD). The AOAM and AOAD values increased in response to 30 mmol L^{-1} in both cultivars, but still remained lower in cv. Locale than in cv. Rouge. Total antioxidant activity of AOAM significantly decreased at 60 mmol L^{-1} in cv. Locale but not in cv. Rouge. At 90 mmol L^{-1} NaCl both AOAM and AOAD fractions significantly decreased. Free radical scavenging activity was found to be constitutively high in *A. cruentus* extracts.[11] The total antioxidant capacity increased in *A. tricolor* in response to moderate levels of salinity[13] and drought.[34] It could be considered as an attempt of the plants to cope with salt-induced synthesis of reactive oxygen species and a similar increase was reported in other pseudocereal species such as buckwheat sprouts.[40] The present work demonstrates that an improvement of antioxidant capacity occurred in *A. cruentus* but that the pattern may differ depending on cultivars, values being higher in salt-tolerant than in salt-sensitive ones, even in the absence of salt.

Total antioxidant activity correlates with concentration of endogenous antioxidant such as glutathione, α-tocopherol, ascorbic acid and phenolic compounds like hydroxybenzoic and hydroxycinnamic acids,[41] flavanols, flavonols, flavanones and flavones.[12,42,43] Glutathione (Fig. 2(E)) and ascorbate (vitamin C; Fig. 2(F)) significantly increased in cv. Rouge in response to 30 and 60 mmol L^{-1} NaCl while they increased only in response to 30 mmol L^{-1} in cv. Locale. Both compounds are water-soluble and thus recovered in the AOAM fraction. Cultivar Rouge

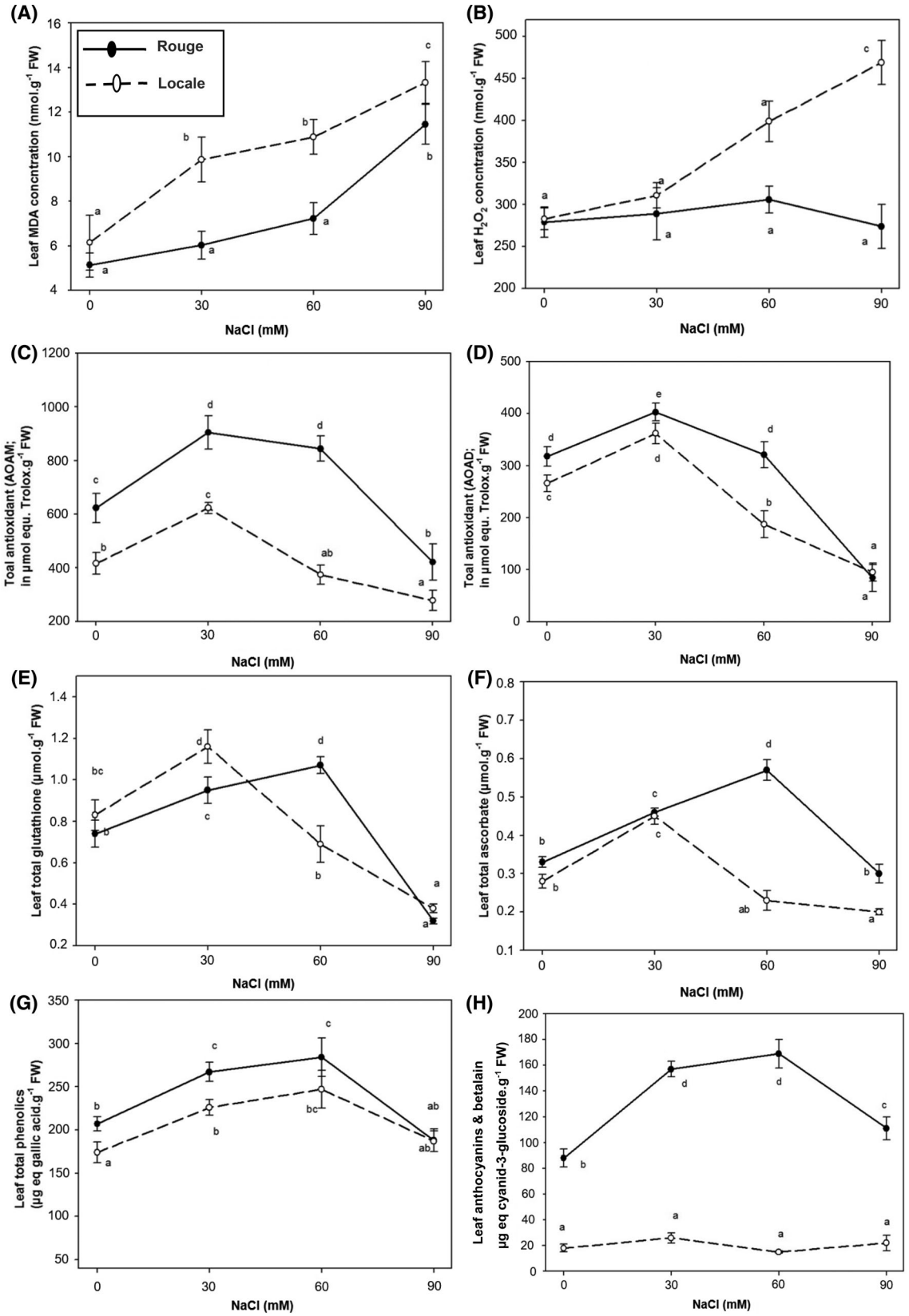

Figure 2. Impact of NaCl (0, 30, 60, 90 mmol L^{-1}) on leaf malondialdehyde (MDA) (A), hydrogen peroxide (H_2O_2) (B), total antioxidant activity in hydrophilic (AOAM) fraction (C) and lipophilic (AOAD) fraction (D), total glutathione (E), total ascorbate (F), total phenolic compounds (G) and anthocyanins concentration (H) in two cultivars of *Amaranthus cruentus* (Locale: salt-sensitive; Rouge: salt-tolerant). Plants were exposed for 16 days to salinity. Each value is the mean of five replicates and vertical bars are standard error. Values sharing similar letter are not significantly different at $P < 0.05$ according to Tukey's multiple range test.

Table 2. Concentrations in α-, β- and γ-tocopherol (in μg g^{-1} dry weight) and total carotenoid (in mg g^{-1} fresh weight) in the leaves of two cultivars of *Amaranthus cruentus* (cv. Locale and cv. Rouge) cultivated during 2 weeks in the presence of sodium chloride (NaCl) (0, 30, 60 and 90 mmol L^{-1})

Cultivars	NaCl (mmol L^{-1})	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Carotenoids
Locale	0	14.2 ± 1.3 a	0.12 ± 0.04 a	0.19 ± 0.03 a	0.23 ± 0.04 a
	30	18.2 ± 1.5 b	0.07 ± 0.02 a	0.65 ± 0.06 b	0.27 ± 0.05 a
	60	12.3 ± 2.9 a	0.25 ± 0.04 b	0.91 ± 0.10 c	0.43 ± 0.02 b
	90	15.6 ± 1.6 ab	ND	1.36 ± 0.07 d	0.44 ± 0.08 b
Rouge	0	19.3 ± 0.2 b	0.13 ± 0.05 a	0.24 ± 0.07 a	0.25 ± 0.04 a
	30	28.1 ± 1.4 c	ND	ND	0.82 ± 0.08 c
	60	33.1 ± 2.9 c	ND	0.17 ± 0.03 a	0.84 ± 0.07 c
	90	22.2 ± 0.4 c	ND	ND	0.97 ± 0.02 d

Each value is the mean of five replicates ± standard error. For a given element, values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's multiple range test.
ND, not detected.

contained significantly higher amounts of ascorbate than cv. Locale ($P = 0.030$) and this could partly explain the higher antioxidant capacity recorded in cv. Rouge, even in the absence of salt.

Plant phenolics receive considerable attention due to their positive impact on human health in relation to prevention of numerous diseases. Total phenolics concentrations were significantly higher in cv. Rouge than in cv. Locale ($P = 0.037$) (Fig. 2(G)) and increased in both cultivars in response to 30 ($P = 0.018$) and 60 ($P = 0.022$) mmol L^{-1} NaCl. Total flavonoids followed the same trend but differences between cultivars were more prominent than for polyphenols (detailed data not shown). This was even more obvious for red pigments (Fig. 2(H)) which mean value in control plants was five times higher in cv. Rouge than in cv. Locale, such a high difference explaining the typical purple color exhibited by the foliage of cv. Rouge. Betalains and anthocyanin are water-soluble vacuolar red pigments acting as antioxidant but also as anti-cancer and anti-aging effectors.[44] Although the spectrophotometric quantification did not discriminate between the two classes of compounds, it is commonly considered that their occurrences in the plant kingdom are mutually exclusive and that *A. cruentus* accumulate betalain such as amaranthine which has numerous chemopreventive actions in human health.[45]

Beside the AOAM fraction, the antioxidant capacity of the AOAD lipophilic fraction also increased in NaCl-treated plants. Tocopherols are important lipophilic bioactive molecules protecting lipids from oxidation. As far as amaranth is concerned, tocopherol profile is known to be drastically different in leaves and seeds.[46] As detailed in Table 2, α-tocopherol constitutes the major contributor in total tocopherol in both cultivars, confirming previous data[12] and recorded values were higher in Rouge than in Locale. The γ-tocopherol concentrations exhibited a progressive increase in response to NaCl in cv. Locale ($P = 0.0098$), while it was almost undetectable in cv. Rouge: γ-tocopherol exhibits the greatest antioxidant capacity and is thus also a valuable component of vitamin E activity.[47]

Carotenoids also generate interest for human health, particularly as a source of provitamin A (α- and β-carotene). β-Carotene is an important lipid-soluble antioxidant protecting cell membranes; xanthophylls such as lutein, zeaxanthine and neoxanthine also protect against light-induced eye and skin damage.[48] We quantified total carotenoid concentration which interestingly significantly increased in response to moderate salt levels (30 and 60 mmol L^{-1}) reaching higher concentration in Rouge than in Locale (Table 2).

Salicylic acid, sugar, and protein content and amino acid profile

SA is an important hormonal compound in plants and has demonstrated an improvement in plant salinity tolerance.[49] It also counteracts development of numerous human diseases and almost 100 human proteins were recently demonstrated to interact with SA acting as a therapeutic agent explaining why salicylate-rich medicinal plants continue to be used extensively worldwide.[50] SA significantly increased in response to 30 and 60 mmol L^{-1} NaCl in both cultivars and then remained stable in cv. Rouge while it decreased in cv. Locale at 90 mmol L^{-1} NaCl (Fig. 3(A)). Total soluble sugars remained unchanged in cv. Rouge and only slightly increased in cv. Locale at 30 mmol L^{-1} NaCl ($P = 0.009$) (Fig. 3(B)).

Total soluble proteins slightly declined in cv. Rouge but increased in cv. Locale (Fig. 3(C)); soluble proteins were always higher in Locale than in Rouge, whatever the NaCl dose ($P = 0.001$). The amino acid profile is presented in Table 3. Threonine and arginine co-eluted and the used methodology was unable to provide an estimation for tryptophane. Most amino acids were present in lower concentration in Rouge than in Locale. Interestingly, we found a significant increase in lysine concentration in cv. Locale exposed to 30 mmol L^{-1} NaCl. Lysin is an essential amino acid which is deficient in numerous cereal grains.[51] Lysine content reported in the salt-stressed amaranth leaves were higher than those previously reported for plants growing in the absence of NaCl.[52] We also observed a salt-induced significant increase in methionine and proline: proline is a well-known osmocompatible solute accumulating in stress conditions while methionine is the precursor of several compounds such as ethylene or polyamines overproduced in salt stress conditions.[2–4] The overproduction of these free amino acids may to some extent interfere with our estimation of proteic amino acid. The amino acid chemical score was calculated considering the mean essential amino acid content of the sample divided by essential amino acid content of the FAO/WHO reference pattern.[53] In control plants of Locale, high values were recorded for

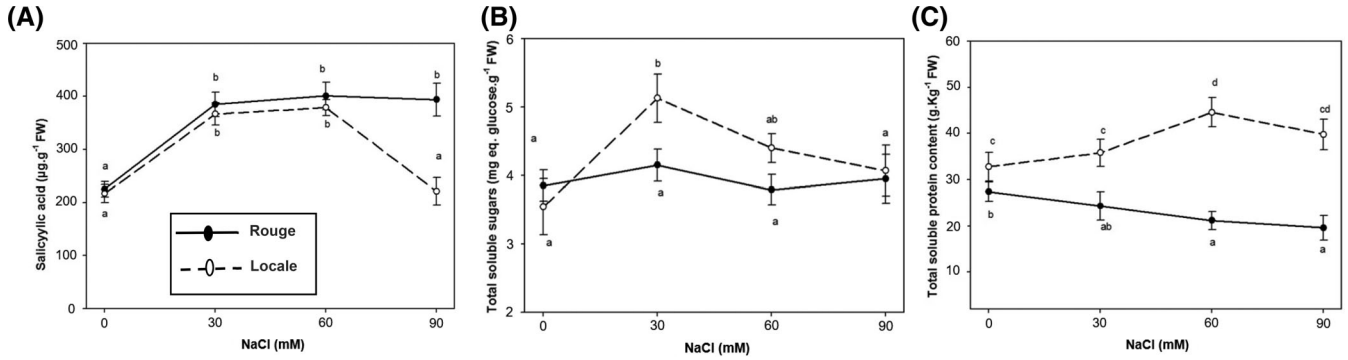

Figure 3. Impact of NaCl (0, 30, 60, 90 mmol L^{-1}) on leaf salicylic acid (A), leaf soluble sugars (B) and leaf total protein (C) concentration in two cultivars of *Amaranthus cruentus* (Locale: salt-sensitive; Rouge: salt-tolerant). Plants were exposed for 16 days to salinity. Each value is the mean of five replicates and vertical bars are standard error. Values sharing similar letter are not significantly different at $P < 0.05$ according to Tukey's multiple range test.

valine (149), isoleucine (144) methionine + cysteine (285) with leucine as limiting factor. The chemical score of protein (omitting tryptophane) for plants in the absence of NaCl was higher than 100 for both cultivars (cv. Locale: 127; cv. Rouge: 118) and the value even culminated at 141 for plants of cv. Locale exposed to 30 mmol L^{-1} NaCl, which is a high value for plant protein.

Table 3. Amino acid concentrations in the leaves (in mg g^{-1} fresh weight) of two cultivars of *Amaranthus cruentus* (cv. Locale and cv. Rouge) cultivated during 2 weeks in the presence of sodium chloride (NaCl) (0, 30, 60 and 90 mmol L^{-1})

Amino acid	Cultivar	0 mmol L^{-1}	30 mmol L^{-1}	60 mmol L^{-1}	90 mmol L^{-1}
Cys	Locale	1.32 c	1.40 d	1.13 bc	1.20 c
	Rouge	1.07 b	0.89 a	0.84 a	0.79 a
Met	Locale	0.99 b	1.39 c	1.58 cd	1.63 d
	Rouge	0.73 a	0.77 a	0.69 a	0.74 a
Val	Locale	1.87 ab	2.69 d	2.62 d	2.31 c
	Rouge	1.65 a	1.74 a	1.86 ab	1.92 b
Ile	Locale	2.01 cd	1.95 c	1.92 c	2.23 d
	Rouge	1.68 b	1.75 b	1.45 b	1.23 a
Leu	Locale	2.85 c	2.63 b	2.51 b	2.61 b
	Rouge	2.20 a	2.32 ab	2.05 a	2.13 a
Thr + Arg	Locale	3.69 b	3.81 c	3.59 b	3.89 c
	Rouge	3.21 a	3.08 a	3.17 a	3.34 ab
Phe	Locale	1.72 bc	1.63 bc	1.80 c	1.75 bc
	Rouge	1.54 b	1.78 c	1.23 a	1.35 a
Tyr	Locale	1.37 ab	1.64 b	1.55 b	1.59 b
	Rouge	1.23 a	1.18 a	1.21 a	1.25 a
His	Locale	0.85 a	0.95 a	0.98 a	1.07 b
	Rouge	0.96 a	1.01 a	0.88 a	0.93 a
Lys	Locale	2.61 b	3.09 c	3.29 d	3.18 c
	Rouge	2.51 ab	2.57 b	2.44 a	2.61 b
Asx	Locale	2.61 b	2.89 b	2.94 b	2.86 b
	Rouge	1.99 a	2.07 a	1.88 a	1.94 a
Glx	Locale	4.83 d	4.68 c	4.22 ab	4.39 b
	Rouge	4.61 c	4.31 b	4.33 b	4.07 a
Gly	Locale	2.31 b	2.52 c	2.28 ab	2.63 c
	Rouge	2.11 a	2.29 ab	2.19 a	2.17 a
Ser	Locale	1.65 b	1.83 c	1.89 c	1.75 bc
	Rouge	1.32 a	1.43 a	1.36 a	1.41 a
Ala	Locale	2.26 b	1.95 a	1.96 a	2.30 b
	Rouge	2.17 ab	2.03 a	1.89 a	2.0 a
Pro	Locale	1.59 a	2.91 c	2.95 cd	3.02 d
	Rouge	1.40 a	2.63 b	2.55 b	2.89 c
Hyp	Locale	0.31 a	0.41 b	0.38 ab	0.48 b
	Rouge	0.27 a	0.20 a	0.29 a	0.31 a

Each value is the mean of five replicates and standard errors are not provided for the sake of clarity. For a given amino acid, values followed by the same letter are not significantly different at $P = 0.05$ according to Tukey's multiple range test.

CONCLUSIONS

Amaranthus cruentus is a promising leafy vegetable containing high amounts of antioxidant and interesting proteins for human diet. Moderate salinities increased plant growth but also antioxidant and Fe content in a salt-tolerant cultivar and concentration of essential amino acid in a salt-sensitive one. *Amaranthus cruentus* is thus an ideal candidate for agronomic production on salt-affected areas. The impact of salinity on antinutrient contents, such as oxalate or phytic acid however still remains to be determined.

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