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(RESEARCH ARTICLE)

Effects of salinity on the nutritional quality of amaranth (*Amaranthus cruentus*) leaves: case of local and resistant varieties

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## Abstract

Climate change is a real obstacle to agriculture because of the difficulty of water supply. Farmers are trying to mitigate this problem by developing several strategies including those based on irrigation. Unfortunately, these irrigation techniques favour the entry of sodium in food crops. In response to this situation, research has been conducted in Benin to develop a salinity resistant variety of local amaranth (called line 23).

The objective of this study is to determine the nutritional values of this resistant amaranth variety grown under salt stress and to compare them with the nutritional values of local amaranth grown under the same conditions.

Local and resistant amaranth plants were grown in real environment and then stressed with salt concentrations of 0; 7.1 and 19.6 mmol NaCl. After harvesting, the leaves were sent to the laboratories for the determination of nutritional elements. Three replicates were done. Analysis of variance was used to compare the means of the nutritional elements assayed using JMP software (SAS Institute MC 2007).

The results show a significant improvement in calcium, phosphorus, potassium and vitamin A levels in line 23 in contrast to the reference cultivar. However, the sodium content, although decreasing as the salt concentration increases, remains above acceptable limits.

As for the reference cultivar, the results show an increase in the concentrations of several elements: iron, calcium, magnesium, including that of sodium, which is harmful to cardiovascular health.

Keywords: Salinity; Nutritional values; Amaranth; Resistant line

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## 1. Introduction

Agriculture is one of the main sources of food supply for countries today. For agriculture to flourish, water is an indispensable element [1], even if its distribution is unequal according to regions. Global warming also induces the drainage of groundwater for cultivation [1].

In order to alleviate the difficulties related to water supply, countries are implementing several approaches: strategies based on wastewater and subterranean water irrigation, water from dams [2]. However, these techniques cause an acceleration of soil salinity [3, 4], which creates an accumulation of soluble salts. According to the United Nations Agricultural Organization, soil degradation due to salinity causes a very heavy economic loss to countries [2]. Indeed, when agricultural products are cultivated on soils with a high rate of salinization, there is an increase in oncotic pressure that makes water difficult to mobilize by plants, a toxicity in Cl- and Na+ ions and an alteration of certain nutritional elements such as vitamins and proteins [5].

In West Africa, vegetable cultivation is an integral part of food agriculture. Unfortunately, the urbanization phenomenon in cities is leading to a decrease in cultivable land [6], which is pushing farmers in these cities to develop other strategies, including farming in sandy and coastal areas.

In Africa and Asia, vegetable species number about 884, which are cultivated and consumed by the populations [7]. Among the vegetable species cultivated and consumed, leafy vegetables are in high demand because of their high vitamin and mineral content [8]. In addition, these vegetables can, among other things, compensate for major deficiencies in micronutrients such as iron, vitamin A and iodine [7, 9].

In Benin, several studies have shown that amaranth is the vegetable most appreciated by consumers and farmers [10]. It is the most popular vegetable because of its vitamin A and iron content. To overcome the alteration induced by salinization in amaranth plants, a variety of amaranth resistant to salinity was developed by the Laboratory of Plant Physiology and Study of Environmental Stresses of the Faculty of Science and Technology of the University of Abomey Calavi in Benin. Although this line has been developed as resistant, it cannot be marketed unless thorough studies are carried out on its nutrient content, especially mineral salts.

Indeed, several studies carried out on the cultivation of vegetables under salt stress show that in the presence of high salt concentrations, a decrease in plant length, root weight, water content [11], sugar content, number of fruits per plant and fresh mass of fruits [12] is observed.

Significant increases were observed in iron, vitamins [10], potassium [11], but also in sodium [11], which can be harmful to health. It was therefore necessary to create a variety of vegetables that would resist salt stress but above all whose nutritional profile would not be altered.

The general objective of the present study is thus to compare the nutritional values of the local amaranth considered in this study as being the reference cultivar cultivated in the presence of salt stress with those of the resistant line called line 23 and cultivated under the same conditions.

Specifically, the aim is to describe the cultivation process of the vegetables (mutants and reference cultivars), to present the nutritional compositions and finally to judge the impact of salinity on the amaranth plants.

# 2. Material and methods

### 2.1. Presentation of the different categories of vegetables

Two categories of vegetables were used as plant materials in this research: the local amaranth cultivar called "reference cultivar" and the second category is a salinization-resistant line obtained and called "line 23" [13]. This line is expected to be resistant to soil salt stress.

### 2.2. Methodology for growing vegetables

Seeds of the reference cultivar were provided by the Agonkamey Research Center located at the National Institute of Agricultural Research of Benin (INRAB) and those of line 23 by the Laboratory of Plant Physiology and Environmental Stress Studies (LAPVESE) of the Faculty of Science and Technology (FAST) of the University of Abomey-Calavi in Benin.

Three saline concentrations were used, namely: the control T0, the treatments T1 and T2. The treatment T0 corresponds to the drilling water used on the culture site; the treatments T1 and T2 correspond to saline concentrations of 7.1 mmol and 19.6 mmol of NaCl respectively and come from a dilution of sea water with the drilling water used on the site.

## 2.3. Conduct of the experiment

The trial was carried out in a real environment on the INRAB site at Sèmè Kraké 6°22'28"N and 2°41'12"S. The plants were grown at an average temperature of 33° during the day and 24° at night with natural light and a relative humidity of 74%. The procedure used for the experiment was identical to that used by the market gardeners at the site. The seeds were placed in the ground for germination to form nurseries in the soil. They were covered with straw for 21 days. The young seedlings were transported and transplanted to previously designed beds in sea sand according to categories. The dimensions of the beds were 3 meters long and 1 meter wide and the spacing between plants was 25 centimeters. The number of seedlings transplanted on each bed was 48. The seedlings were amended with poultry droppings on the same day and continued to grow. During this phase, all plants were watered with borehole water (which is the control) twice a day with quantities of 12 liters per bed: in the morning at 7 a.m. and in the evening at 6 p.m. Urea was applied ten days after the first amendment. A second amendment was made two weeks after the first and a third ten days after the second. Just after the third amendment, i.e. the next day, the salt stress started and lasted three weeks, i.e. 21 days.

Once the plants reached maturity, leaf harvesting was done. Approximately 500g of fresh material from each cultivar per replicate per treatment was collected. The collected leaves were then rinsed once with borehole water to remove dirt and then twice in a row with distilled water. Each sample was then labeled and kept cool in a cooler containing ice beforehand. All samples were sent for extraction and assay.

### 2.4. Obtaining dry matter for the determination of protein, calcium, iron, magnesium, phosphorus

The amaranth leaves are dried in a ventilated oven at 105°C for 72 hours (to constant weight).

The dried samples are then weighed to identify water and dry matter contents.

### 2.5. Determination of nitrogen content

Nitrogen was determined by the KJELDAHL method from micro-distillation of the digested sample mineralization with sulfuric acid in the presence of a selenium catalyst. This microdistillation was done by steam stripping in the presence of a normal soda solution. The distillate collected in boric acid was titrated with sulfuric acid in the presence of a methyl red indicator.

The nitrogen content thus obtained was multiplied by the factor 6.25 to obtain the protein content of the sample [14].

### 2.6. Determination of calcium, iron, sodium, potassium and magnesium content

The samples submitted for analysis were reduced to powder. The resulting product was incinerated in a muffle furnace at 550°C for 24H, with a programmed temperature increase not to exceed 0.6°C per second. The ash was dissolved in 2cc of hydrochloric acid, 6N which is evaporated on hot plate at 125°C. The more or less viscous residue obtained was again dissolved and recovered with HNO3, 0.1M in a flask of known volume. The solution thus obtained is used to determine the nutritional elements by Atomic Absorption Spectrophotometry (AAS).

At a specific wavelength, part of the light energy emitted by the hollow cathode lamp (a lamp emitting light that is also specific to the element) is absorbed by the sample solution. This amount of energy is used by the element to go from its "ground state" to a "metastable excited state": this is the excitation energy. It is proportional to the concentration of elements in the solution. Thus knowing the amount of energy absorbed by a sample solution, the concentrations have been deduced.

For this purpose, standard solutions (prepared or commercially acquired solutions) of precisely known concentration was used. For a range of standards, the optical device of the apparatus calculates a parameter (absorbance) for each standard. It then performs a parametric regression that allows it to determine the concentration of each sample from the same parameter determined and a regression curve for the sample.

To allow the atoms of the element to be measured to be uniformly exposed to the light source, the measuring solution is drawn through a very fine capillary and then sprayed into the flame fed by a mixture of air and acetylene. The atoms released in this way can then absorb some of the energy emitted by the hollow cathode lamp and thus enable the absorption to be determined.

The AAS optical system is equipped with precise and powerful detectors and amplifiers that facilitate the manipulation of the emitted signals. To minimize the effect of ionic interference during the absorption process, Lanthanum at 10g/L has been added to the extracts of specific reagents for the determination of calcium and/or magnesium. For the determination of sodium and / or potassium, the extracts are combined with Cesium Chloride at 2g/L [13].

## 2.7. Determination of phosphorus content

From the mineralization obtained, phosphorus is determined by colorimetry after development of a colored complex (blue) by using a sulfo-molybdic reagent (dissolution of ammonium hepta-molybdate and sulfuric acid) and ascorbic acid at 660nm [14].

### 2.8. Dosing of carbohydrates

The term carbohydrate in this article reflects the content of reducing sugars. Carbohydrates were determined by the 3,5-dinitrosalicylate method of Dubois and al (1965) [15].

### 2.9. Determination of Vitamin A, B9 and C

The amaranth leaves were dried in the shade and then powdered. A dissolution was then made in pure ethanol without water for vitamin A and in water for vitamins B9 and C. The samples were subjected to absorbance at 325 nanometers, then the reading was made on the spectrum with the appropriate wavelengths. A comparison is then made at an absorbance of 1 g / 100 ml [16].

### 2.10. Statistical Analysis

For all parameters, each value is presented as mean +/- standard error with three replicates per treatment. Means were compared using the one or two factor ANOVA test as appropriate.

Analyses were performed using JMP software (SAS Institute MC 2007).

## 3. Results

Table I shows the mean values of nutrient concentrations of the reference cultivar by treatment and Table II shows the concentrations relative to line 23.

Elements	T0 Mean ± SD	T1 Mean ± SD	T2 Mean ± SD	P-value
Carbohydrates (g)	4.805±0.29ª	4.910±0.01 <sup>a</sup>	5.220±0.00ª	0.2816
Total nitrogen (%)	2.610±0.00 <sup>a</sup>	2.295±0.00 <sup>b</sup>	2.135±0.02°	0.0001
Crude protein (%)	16.185±0.11 <sup>a</sup>	$14.405 \pm 0.00^{b}$	13.190±0.01 <sup>c</sup>	0.0001
Calcium (mg)	238.110±1.20 <sup>c</sup>	319.090±0.90 <sup>b</sup>	399.240±0.50ª	0.0001
Iron (mg)	22.970±0.21 <sup>c</sup>	26.340±0.49 <sup>b</sup>	30.330±0.56ª	0.0001
Fat (g)	$0.300 \pm 0.01^{b}$	$0.340 \pm 0.00^{a}$	$0.350 \pm 0.00^{a}$	0.011
Magnesium (mg)	60.685±0.37°	76.595±0.39⁵	82.495±0.29ª	0.0001
Phosphorus (mg)	101.345±1.05°	112.665±0.22 <sup>b</sup>	122.425±0.33 <sup>a</sup>	0.0001
Potassium (mg)	4033.71±1.20 <sup>a</sup>	3753.89±30.21 <sup>b</sup>	3121.26±11.76°	0.0001
Sodium (mg)	510.955±0.78°	570.595±0.25 <sup>b</sup>	591.415±0.38 <sup>a</sup>	0.0001
Vitamin A (µg)	10.605±0.30°	24.530±0.90 <sup>a</sup>	16.905±0.06 <sup>b</sup>	0.0001
Vitamin B9 (mg)	117.715±0.23 <sup>c</sup>	151.680±0.18 <sup>b</sup>	178.520±0.40 <sup>a</sup>	0.0001
Vitamin C (mg)	29.975±0.11ª	22,490±0,28 <sup>b</sup>	19.725±0.17°	0.0001

**Table 1** Average nutrient concentration values of the reference cultivar according to each treatment.

Means within columns with different letters are statistically different T0: 0 mmol NaCl; T1: 7.1 mmol NaCl; T2: 19.6 mmol NaCl

Elements	T0 Mean ± SD	T1 Mean ± SD	T2 Mean ± SD	P-value
Carbohydrates (g)	6.630±0.13 <sup>b</sup>	7.465±0.12 <sup>a</sup>	7.730±0.04 <sup>a</sup>	0.001
Total nitrogen (%)	2.340±0.00 <sup>a</sup>	2.295±0.00 <sup>b</sup>	2.205±0.00 <sup>c</sup>	0.0001
Crude protein (%)	14.675±0.01ª	14.010±0.00 <sup>b</sup>	13.695±0.01°	0.0001
Calcium (mg)	357.165±0.48°	398.125±1.69 <sup>b</sup>	502.400±0.34 <sup>a</sup>	0.0001
Iron (mg)	25.700±0.16ª	23.785±0.12 <sup>b</sup>	21.010±0.02°	0.0001
Fat (g)	$0.305 \pm 0.00^{b}$	$0.335 \pm 0.00^{a}$	0.340±0.00 <sup>a</sup>	0.0001
Magnesium (mg)	101.945±0.35ª	98.360±0.16 <sup>b</sup>	83.955±0.22°	0.0001
Phosphorus (mg)	103.705±0.62°	135.710±0.30 <sup>b</sup>	165.645±0.91ª	0.0001
Potassium (mg)	3186.45±2.04 <sup>c</sup>	3199.85±1.01 <sup>b</sup>	3232.26±3.81ª	0.0001
Sodium (mg)	994.750±0.50ª	752.050±0.55 <sup>⊾</sup>	691.000±0.58°	0.0001
Vitamin A (µg)	69.565±0.26 <sup>c</sup>	71.295±0.26 <sup>b</sup>	76.73±0.22ª	0.0001
Vitamin B9 (mg)	167.270±0.35 <sup>a</sup>	158.955±0.35 <sup>b</sup>	150.890±0.07°	0.0001
Vitamin C (mg)	21.955±0.21ª	20.225±0.20 <sup>b</sup>	16.595±0.23°	0.0001

Table 2 Average values of nutrient concentrations of line 23 according to each treatment.

Means within columns with different letters are statistically different; T<sub>0</sub>: 0 mmol NaCl; T<sub>1</sub>: 7.1 mmol NaCl; T<sub>2</sub>: 19.6 mmol NaCl

Carbohydrate content increased with salt stress for the reference cultivar from 4.805 g at 0 mmol NaCl to 5.220 g at 19.6 mmol NaCl but this increase was not significant (p = 0.2816). For line 23, there was an increase in carbohydrate concentrations compared to the reference cultivar from 6.630 g at 0 mmol NaCl to 7.730 g at 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that the carbohydrate content increases with the degree of salinity with a greater increase in line 23.

Lipid content increased with salt stress for the reference cultivar from 0.300 g at 0 mmol NaCl to 0.350 g at 19.6 mmol NaCl (p = 0.011). For line 23, a slight increase in lipid content was also noted, from 0.305 g for a concentration of 0 mmol NaCl to 0.340 g for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that lipid content increases slightly with the degree of salinity with lower contents in line 23.

Total nitrogen content decreased with salt stress for the reference cultivar from 2.61% at 0 mmol NaCl to 2.14% at 19.6 mmol NaCl (p < 0.0001). For line 23, a decrease in total nitrogen content compared to the reference cultivar was noted, from 2.34% at 0 mmol NaCl to 2.21% at 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that the total nitrogen content decreases with the degree of salinity. The same trend was observed for crude protein content, which decreased from 16.19% at 0 mmol NaCl to 13.19% at 19.6 mmol NaCl (p < 0.0001) for the reference cultivar. For line 23, crude protein content decreased slightly from 14.68% at 0 mmol NaCl to 13.70% at 19.6 mmol NaCl (p < 0.0001).

Calcium content increased with salt stress for the reference cultivar from 238.110 mg at 0 mmol NaCl to 399.240 mg at 19.6 mmol NaCl (p < 0.0001). For line 23, there was an increase in calcium concentrations compared to the reference cultivar from 357.165 mg for a concentration of 0 mmol NaCl to 502.400 mg for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that calcium content increases with the degree of salinity with a greater increase at line 23.

Iron content increased with salt stress for the reference cultivar, from 22.970 mg at 0 mmol NaCl to 30.330 mg at 19.6 mmol NaCl (p < 0.0001). For line 23, a decrease in iron concentrations compared to the reference cultivar was noted from 25.700 mg for a concentration of 0 mmol NaCl to 21.010 mg for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that iron content increases with salinity level for the reference cultivar while it decreases at line 23.

Magnesium content increased with salt stress for the reference cultivar, from 60.685 mg at 0 mmol NaCl to 82.495 mg at 19.6 mmol NaCl (p < 0.0001). For line 23, there was a decrease in magnesium concentrations from 101.945 mg at 0 mmol NaCl to 83.955 mg at 19.6 mmol NaCl (p < 0.0001). However, magnesium levels remained higher at line 23.

Phosphorus content increased with salt stress for the reference cultivar from 101.345 mg at 0 mmol NaCl to 122.425 mg at 19.6 mmol NaCl (p < 0.0001). For line 23, there was a clear increase in phosphorus concentrations compared to the reference cultivar, from 103.705 mg for a concentration of 0 mmol NaCl to 165.645 mg for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that phosphorus content increases with the degree of salinity with a greater increase at line 23.

Potassium content decreased with salt stress for the reference cultivar from 4033.71 mg at 0 mmol NaCl to 3121.26 mg at 19.6 mmol NaCl (p < 0.0001). For line 23, there was an increase in potassium concentrations compared to the reference cultivar from 3186.45 mg for a concentration of 0 mmol NaCl to 3232.26 mg for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that potassium content increases with salt stress for line 23 while it decreases with salinity for the reference cultivar.

As for sodium, its content increased with salt stress for the reference cultivar, from 510.955 mg for a concentration of 0 mmol NaCl to 591.415 mg for a concentration of 19.6 mmol NaCl (p < 0.0001). For line 23, there was a decrease in sodium concentrations from 994.750 mg for 0 mmol NaCl to 691.000 mg for 19.6 mmol NaCl (p < 0.0001). Although a decrease in sodium was observed with line 23, its levels remained higher than those obtained with the reference cultivar.

Vitamin A content varied randomly for the reference cultivar from 10.605  $\mu$ g at T0 to 19.905  $\mu$ g at T2 with a peak of 24.530 at T1 (p < 0.0001). For line 23, there was an increase in vitamin A content compared to the reference cultivar, from 69.565  $\mu$ g for a concentration of 0 mmol NaCl to 76.73  $\mu$ g for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that vitamin A content varies with the degree of salinity with a greater increase at line 23.

Vitamin B9 content increased with salt stress for the reference cultivar from 117.715 mg at 0 mmol NaCl to 178.520 mg at 19.6 mmol NaCl (p < 0.0001). For line 23, there was a decrease in vitamin B9 concentrations compared to the reference cultivar, from 167.270 mg at 0 mmol NaCl to 150.890 mg at 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that the vitamin B9 content decreases with the degree of salinity for line 23 compared to the reference cultivar.

Vitamin C content decreased with salt stress for the reference cultivar, from 29.975 mg at 0 mmol NaCl to 19.725 mg at 19.6 mmol NaCl (p < 0.0001). This same decrease was observed for line 23, from 21.955 mg for a concentration of 0 mmol NaCl to 16.595 mg for a concentration of 19.6 mmol NaCl (p < 0.0001). Thus, it is inferred that the vitamin C content decreases with the degree of salinity.

## 4. Discussion

The effects of salt stress on nutritional quality were studied in the present research. In general, the different levels obtained in the present research for the studied parameters are not in agreement with the values available in the literature. Several researches testify to the disparate results of studies on amaranth, especially the variety *A. cruentus* [-17-20]. Although these studies focused on mineral and/or vitamin contents, the differences could be explained by differences in the cultivation process. In the study by Fasuyi and al [18], the seeds were obtained from the farmers and the leaves were sun-dried for three days. In this case, the lack of scientific certainty in the quality of the seeds and the probable alteration of the quality of the leaves by the sun's rays explain the decrease in the values obtained in comparison with those of the present study. As for the study by Musa and Oladiran [19], there are several reasons that could justify the variability between the contents obtained and those of the present research. Firstly, in the Musa and Oladiran study, phosphorus and potassium were added, whereas in the present study, the addition was organic, i.e. poultry droppings, and then urea. Secondly, the culture was done in real environment on beds in the present research while in the study of Musa and Oladiran, the plants were cultivated in pots. Finally, harvesting was done at the vegetative phase in the Musa and Oladiran study while it was done at the mature phase in the present research. The study of Wouyou and al [20] was carried out in a greenhouse, which implies absolute control over parameters such as humidity, luminosity, interactions related to sea spray non-existent.

The carbohydrate content obtained in the present study increased with increasing salinity. This increase was not significant for the local cultivar but was significant for line 23. The studies of Kinsou and al. and Wouyou and al. [10,21] on tomato and amaranth show a decrease in sugar content with increasing salt concentration. This difference in behavior under salt stress could be explained by the resistant characteristic of line 23, which shows the beneficial effect of this vegetable variety under salt stress. Similarly, some research [22,23] shows that the increase in sugar content in the presence of salt stress in salinity-tolerant plants could be explained by the fact that salt stress induces a stimulation of the production and retention of sugars and starch within the cells of these plants.

In the present research, he noted a decrease in protein content as NaCl concentration increased. In the literature, there are no data on the effect of salt stress on protein content in *A. cruentus* pigweed. A study by Umankata and al. on *A tricolor* pigweed shows an increase in protein content as NaCl content increased [24]. This difference can be explained by the difference in amaranth varieties. Indeed, the response mechanisms of these two varieties could be different. The decrease in nitrogen and crude protein levels can be explained by the relationship between salinity and nitrogen fixation. Indeed, in the presence of salt stress, severe disturbances occur in protein metabolism, especially in nitrogen metabolism and a decrease in protein synthesis [5]. Furthermore, salt stress induces a significant reduction or even total suppression of nodulation and biological nitrogen fixation at the cellular level. All these mechanisms could explain the reduction of nitrogen and protein contents at the plant level [25]. However, the decrease in protein content is really less at the level of line 23.

Calcium content increased with the degree of salinity without distinction of the vegetable variety. However, the highest levels were found in the resistant variety. Increases in calcium content were also observed in the studies of Umankata and al [24] and Mahjuba and al [26] on tricolor amaranth and rice plants. This increase could be explained by the responses of cell membranes to salinity. Indeed, under salt stress, the calcium channels present at the cell membranes capture a wide range of  $Ca^{2+}$  ion in order to better cope with the dehydration caused by the stress [27]. This accumulation could therefore justify the intrusion of calcium inside cells in the presence of salinity.

In the present study, iron content in vegetables decreased under salt stress in the reference cultivar while it increased in line 23. A decrease in iron content was also observed in plants grown under salt stress in several studies [28, 29]. This decrease is explained by the fact that sodium accumulation in the root part under salt stress induces a reduction in plant growth [29]. Sodium accumulation interferes with iron uptake by plants [29, 30], which reduces iron mobilization by the plant. The opposite results obtained in line 23 are evidence of just such resistance of the line to salt intrusion. On the other hand, the study of Wouyou and al. showed an increase in iron content with salinity between 0 and 30 mmol NaCl [10], but this difference in results is explained by the difference in the experimental culture methodology, as the vegetables in the present research were grown under field conditions and those in the study of Wouyou and al. were grown in a controlled environment.

In the present study, sodium content increased with salinity for the local cultivar while it decreased for line 23. The increase in sodium in the reference cultivar is consistent with results found in several studies [28, 31] and is merely the result of a natural mechanism in which excess sodium in the soil leads to significant sodium intrusion into the plants. The decrease in sodium in the face of increasing salinity is evidence of the resistance of line 23, although the levels remain very high and toxic to the organism.

In the present study, there was a decrease in potassium content with increasing salinity in the reference cultivar, while an increase was observed in line 23. These two results, which are already in agreement with the evolution of sodium levels in each line, could be explained by the mechanism of the Na+/K+ pump, which functions in such a way that the intrusion of sodium through the membranes leads to an expulsion of potassium. Indeed, potassium and sodium ions are two positively charged ions that use the same channels [32]. Similarly, the saline environment accelerates sodium intrusion at the root level [28], which inhibits potassium ion transporters [32-33]. Finally, one reason that could also explain the decrease in potassium levels in the reference cultivar is that the presence of NaCl in the growing medium limits the binding of major cations such as potassium at the plant level [5].

Magnesium content increased with salinity in the reference cultivar while an opposite reaction was noted with line 23. This phenomenon of decreasing magnesium content in the presence of salt stress was also reported by Mahjuba and al [26] and Kaussar and al [34]. This could be explained by the competition induced by the excessive accumulation of sodium. However, further studies deserve to be conducted to explain the relationships between magnesium depletion in the presence of salt stress.

Phosphorus levels in both lines increased as the salt concentration increased. To date, we have not been able to find any study that measured phosphorus levels in vegetables under salt stress. However, the increase in phosphorus levels could be explained by the interaction between phosphorus and calcium. Indeed, these two minerals are involved in the constitution of cells.

Thus, the increase in calcium levels could also lead to an increase in phosphorus levels.

Vitamin A content varied randomly in the presence of salt stress for the reference cultivar. Indeed, an increase was first observed between 0 and 7.1 mmol of NaCl and then a drop-in content at 19.6 mmol of NaCl. The study by Wouyou and al [10] also shows a disparity in vitamin A content.

The vitamin C content decreased in the present study in both lines. These results are consistent with those of Ratnakar and Rai, Seth and al. and Mandhania and al. [35-37] for other amaranth varieties. However, these results are contrary to those of Wouyou and al. [10]. The contradiction in these results could be explained by the differences in culture media on the one hand, but also and especially by the harvesting stages on the other hand. Indeed, the study of Wouyou and al. took place in a controlled environment while this one took place at the sea shore. The sea spray could therefore have been a source of additional stress for the plants, which could have caused a strong mobilization of vitamin C to protect the plants, thus inducing a decrease in reserves. Similarly, the present research was conducted until the amaranth plants were consumable, whereas most studies stop at the physiological maturity phase for nutrient determination.

Vitamin B9 content increased under salt stress in the reference cultivar and decreased in line 23. Most studies have not looked at the determination of vitamin B9 in particular, but the study by Wouyou and al [10] shows an improvement in the content of some B-complex vitamins in the presence of salt stress, especially vitamins B1 and B2.

## 5. Conclusion

Nutritional analyses show a resistance of line 23 to salt stress, which is reflected in a decrease in sodium content with increasing salt stress. However, the lowest sodium content obtained in line 23 is significantly higher than the sodium content obtained in the local cultivar grown at  $T_2$ .

Analyses also show an improvement in the content of some nutrients, notably carbohydrates, calcium, phosphorus, potassium and vitamin A in line 23. Although protein, magnesium and vitamin B9 concentrations decreased under salt stress, they were still higher than the maximum values obtained for the reference cultivar. As for iron and vitamin C, the levels remain within acceptable limits. However, the high sodium content that persists in line 23 does not allow its use for men because of the repercussions that this could have on the arterial health of individuals.

Pending results from blood pressure studies, additional studies are warranted to re-investigate the line's resistance to salinity to further reduce sodium mobilization in plants.

### **Compliance with ethical standards**

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### Disclosure of conflict of interest

The authors declare that there is no conflict o interest regarding the publication of this paper.

#### References

- [1] Ashour EK, Al-Najar H. The Impact of climate change and soil salinity in irrigation water demand in the gaza strip. J Earth Sci Climat Change. 2012; 3 (2): 120.
- [2] Food and Agriculture Organization of the United Nations. State of the world's soil resources, Technical summary. Rome; 2016 p 1-92.
- [3] Sifola I, Postiglione L. The effect of increasing NaCl in irrigation water on growth, gas exchange and yield of tobacco burley type. Fied Crops Research. 2002; 74: 81-91.
- [4] De Pascale S, Maggio A, Ruggiero C, Barbieri G. Growth, water relations, and ion content of field-grown celery under saline irrigation. J. Am. Soc. Hortic. Sci. 2003; 128: 128-35.
- [5] Khaled LB, Ouarraqi EM, Zid E. Impact of NaCl on growth and nutrition of Massa durum wheat variety grown in hydroponic environment. Acta Bot Gallica. 2007; 154(1): 101-16.
- [6] Satterthwaite D, McGranahan G, Tacoli C. Urbanization and its implications for food and farming. Philis Trans R Soc Lond B Biol Sci. 2010; 365 (1554): 2809-20.
- [7] Kahane R, Temple L, Brat P, De Bon H. Leafy vegetables in tropical countries: diversity, economic wealth and health value in a very fragile context. Col Ang. 2005: 3-14.

- [8] Otles S, Ozgoz S. Health effects of dietary fiber. Sci Pol Tech Alim. 2014; 13 (2): 191202.
- [9] Akindahunsi AA, Salawu SO. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. Afr Jour of Biotech. 2005; 4 (6): 497-501.
- [10] Wouyou AD, Ahissou EA, Gandonou CB, Assougba KF, Houngbèmè A, Gbaguidi FA. Salinity increased vitamins concentration in *amaranthus cruentus* leaves. Affr. J. Biotechnol. 2017; 16 (4): 2106-11.
- [11] Karoune S, Kechebar MSA, Halis Y, Djellouli A, Rahmoune C. Effect o salt stress on morphology, physiology and biochemistry of Acacia albida. JARA. 2017; 14: 60-73.
- [12] Kinsou E, Amoussa AM, Mensah ACG, Kpinkoun JK, Assogba KF, Ahissou H and al. Effect of salinity on flowering, fruiting and nutritional quality of fruits of the local tomato cultivar Akikon (Lycopersicon esculentum Mill.) from Benin. 2021; 15 (2): 737-49.
- [13] Atou R, Tonouewa G, Wouyou A, Kpochemè E, Missihoun AA, Montcho D and al. Screening of Amaranth (*Amaranthus cruentus* L.) Mutant Lines for Salinity Tolerance. IJPSS. 2022; 34 (22): 785-97.
- [14] Kanninkpo C. Synthesis of some organic and soil analysis methods at the Laboratory of Soil, Water and Environmental Sciences based on working papers. 2013: 1-4.
- [15] Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1965; 28: 350-6.
- [16] Clarkes EGC, Moffat AC. Clarke's isolation and identification of drugs in pharmaceuticals, body fluids, and postmortem material. 2<sup>nd</sup> edition. London: A. C. Moffat; 1986.
- [17] Achigan-Dako EG, Sogbohossou OED, Maundu P. Current knowledge on Amaranthus spp.: research avenues for improved nutritional value and yield in leafy amaranth in subSaharan Africa. Euphytica. 2014; 197 : 303-17.
- [18] Fasuyi AO, Akindahunsi AO. Nutritional evaluation of Amarathus cruentus leaf meal based broiler diets supplemented with cellulase/glucanase/xylanase enzymes. Am J of Food Tech. 2009; 103 : 757-65.
- [19] Musa A, Oladiran J. Effect of heading on some micronutrients, anti-nutrients and toxic substances in *Amaranthus cruentus* grown in Minna, Niger State, Nigeria. Am J of Food and Nut. 2011; 1: 147-54.
- [20] Wouyou A., Gandonou C. B., Assogba Komlan F., Montcho D., Zanklan S. A., Lutts S., Gnancadja S. L. (2017a). Salinity Resistance of Five Amaranth (*Amaranthus cruentus*) Cultivars at Young Plants Stage, International Journal of Plant and Soil Science, 14(3): 1-13.
- [21] Kinsou E, Amoussa AM, Mensah ACG, Kpinkoun JK, Assogba KF, Ahissou H and al. Effect of salinity on flowering, fruiting and nutritional quality of fruits of the local tomato cultivar Akikon (Lycopersicon esculentum Mill.) from Benin. 2021; 15 (2): 737-49.
- [22] Yong-Gen Y, Yoshie K, Atsuko S, Satoru K, Naova F, Hiroshi E, Sumiko S, and al. Salinity induces carbohydrate accumulation and sugar-regulated starch biosynthetic genes in tomato (Somanum lycopersicum L. cv. Micro-Tom) fruits in ABA- and osmotic stress-independent manna. J Exp Bot. 2010 61(2): 563-74.
- [23] Ahmad A, Ismun A, Taib M. Effects of salinity stress on carbohydrate metabolism in Cryptocoryne elliptica cultures. J Trop Plant Physiol. 2017; 9: 1-13.
- [24] Umakanta S, Tofazzal I, Shinya O. Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in Amaranthus tricolor leaves. PloS ONE. 2018; 13(11): 1-18.
- [25] Swaraj K, Bishnoi NR. Effect of salt stress on nodulation and nitrogen fixation in legumes. Indian J Exp Biol. 1999; 37(9): 843-8.
- [26] Mahjuba A, Hiroki O. Effect of saline irrigation on accumulation of Na+, K+, Ca2+ an Mg2+ ions in rice plants. Agriculture. 2018; 8: 1-16.
- [27] Seifikalhor M, Aliniaeifard S, Shomali A, Azad N, Hassani B, Lastochkina O and al. Calcium signalling and salt tolerance are diversely entwined in plants. Plant Signaling and Behaviour. 2019; 14(11): 1-15.
- [28] Abbas G, Saqib M, Akhtar J, Haq MA. Interactive effects of salinity and iron deficiency on different rice genotypes. JPLN. 2015; 178(2): 306-11.
- [29] Zhao D, Gao S, Zhang X, Zhang Z, Zheng H, Rong K and al. Impact of saline stress on the uptake of varous macro and micronutrients and their associations with plant biomass and root traits in wheat. Plant Soil Environ. 2021; 67(2): 61-70.

- [30] Rabhi M, Barhoumi Z, Ksouri R, Abdelly C, Gharsalli M. Interactive effects of salinity and iron deficiency in Medicago ciliaris. C R Biol. 2007; 330(11): 779-88.
- [31] Bouassaba K, Chougui S. Effect of salt stress on biochemical and anatomical behavior in two varieties of chili pepper (Capsicum Annuum L.) in Mila/Algeria. Eur Sci J. 2018; 14(15): 159-74.
- [32] Adams E, Shin R. Transport, signalling and homeostasis of potassium and sodium in plants. J Integr Plant Biol. 2014; 56: 236-49.
- [33] Sun J, Dai S, Wang R, Chen S, Li N, Zhou S and al. Calcium mediates roots K+ / Na+ homeostasis in poplar uptake of tomato plants grown under salt stress. Environ Exp Bot. 2007; 59: 173-8.
- [34] Gull M, Kausar A. Influence of salinity stress on the uptake of magnesium, phosphorus, and yield of salt susceptible and tolerant sorghum cultivars (Sorghum bicolor L). JABB. 2019; 7(3): 53-8.
- [35] Ratnakar A, Rai A. Effect of NaCl Salinity on β-carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of Amaranthus polygamous L var. Pusakirti. Octa J Environ res. 2013 ; 1(3) : 211-6.
- [36] Seth SP, Sharma V, Khandelwal SK. Effect of salinity on antioxidant enzymes in wheat. Ind J Plant Physiol. 2007; 12(2)): 186-8.
- [37] Mandhania S, Madan S, Sheokand S. Differential response in salt tolerant and sensitive genotypes of wheat in terms of ascorbate, carotenoids, proline and plant water relations. Asian J Exp Biol Sci. 2010; 1(4): 792-7