

Full Length Research Paper

Salinity increased vitamins concentration in *Amaranthus cruentus* leaves

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Salt stress is one of the major environmental constraints limiting agricultural productivity and influencing the concentration of bioactive compounds of vegetables. In this study, the effect of NaCl salt stress on nutrient contents of leaves in a cultivar of amaranth, an important leafy vegetable cultivated in some tropical regions worldwide, was evaluated. The experiment was carried out in a screen house at Center for Agricultural Research of Agonkanmey, Benin Republic as a randomized complete block design (RCBD) with three replications. Three weeks old plants were subjected in pots containing a mixture of potting soil and sand, to three concentrations (0, 30 and 90 mM) of NaCl by irrigation every two days. Nutrient contents in leaves were determined at maturity, after four weeks of stress, using standards methods. Proteins, total sugars, reducing sugars, lipids, potassium, calcium, vitamins C and B3 contents were not significantly affected by NaCl. Iron content increased significantly only at 30 mM NaCl but vitamins A, B1 and B2 contents increased significantly with increase in NaCl concentration. Thus, salt stress did not reduce nutritional values of this amaranth cultivar but improved its leaves nutritional quality by increasing vitamins A, B1 and B2 content.

Key words: Amaranth, NaCl, proteins, lipids, sugars, mineral, vitamins.

INTRODUCTION

Amaranthus, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial

plants. Some amaranth species are cultivated as leafy vegetables which are essentially required to safeguard health, particularly by precluding human diseases as they are good source of vitamins, mineral nutrients and antioxidants (Prasad et al., 2014). They exhibit a high nutritive value but also a fascinating ability to adapt to diverse harsh environments (Omami et al., 2006). As tropical leafy vegetables, they are acquiring increasing importance as potential subsidiary food crop considering their excellent quality in protein and endogenous micronutrients content (Devadas and Saroja, 1980; Prakash and Zaidi, 2000). Presently, some amaranth species are cultivated in semi-arid regions, where salinity problem is acute (Bhattacharjee, 2008). Vegetable crops are predominantly cultivated in the southern part of Benin, in urban and suburban areas and in the valley of *Ouémé* (Adorgloh-Hessou, 2006). In Benin, amaranth species are mainly cultivated as leaf vegetable in the cultivable lands of the coastal areas where soil and irrigation water's salinity constitute real problems hampering crop production. Salt stress is one of the major environmental constraints limiting agricultural productivity (Boyer, 1982; Wei et al., 2003). As environmental stress, it may have a strong influence on the concentration of bioactive compounds of vegetables (Prasad et al., 2014). However, despite a substantial amount of literature on responses of plants to salinity stress, data on the effect of salt stress on nutrient contents in leaves of amaranth are lacking. Moreover, only little research work has focused on the response of amaranth cultivars produced in Benin to salt stress. Since amaranths are mainly used in Benin as leafy vegetable, it is important to show if NaCl stress induced modification in leaves nutrient contents. The present study aims at evaluating NaCl stress effects on protein, sugar, lipid, mineral and vitamin concentrations of the main amaranth cultivar grown in Benin.

MATERIALS AND METHODS

The main *Amaranthus cruentus* cultivar produced in Benin named 'Locale' was used. Seeds were obtained from the Market Gardening Crops Program of the Benin National Institute for Agricultural Research (INRAB).

Experimental conditions

The experiment was carried out in a screen house at Center for Agricultural Research of Agonkanmey (Abomey-Calavi, Benin Republic) from March to May 2016. Plants were cultivated at a temperature of 26/22°C day/night with natural light and a relative humidity of 55%.

Seeds were incubated for germination in tanks filled with potting moistened soil for two weeks. Young seedlings were then

transferred to earthen small pots of 5.8 cm diameter and 6 cm height containing a mixture of potting soil and sandy loam soil 50:50 (one plant/pot) and cultivated one week before stress application. Plants of cultivar *Locale* were subjected to salt stress in earthen big pots of 11.3 cm diameter and 14 cm height filled with 3 kg of the same mixture. Treatments consisted of plant irrigation every two days with 100 ml/pot of 0, 30 or 90 mM NaCl solution corresponding respectively to an electric conductivity of 0, 1.91 and 8.39 dS.m⁻¹ determined by a conductimeter (VWR; CO310). The experiment was laid out as a randomized complete block design (RCBD) with one factor (NaCl concentrations) and three replications.

Leaf nutrient determination

Nutrient contents were determined at plant maturity after four weeks exposure to stress. Plants were then fifty days old. The mature leaves of these plants were used for estimating proteins, total sugars, reducing sugars, lipids, potassium, calcium, iron, vitamin A, thiamine (vitamin B₁), riboflavin (vitamin B₂), niacin (vitamin B₃) and ascorbic acid (vitamin C) contents. Total and reducing sugars were determined using the method of Dubois et al. (1965) and proteins were assayed with method of Gornall et al. (1949). Total lipids were extracted using Soxhlet modified methods with acetone as solvent (Randall, 1974). Each probe was immersed in the boiling solvent and then rinsed in the cold one. After extraction, the solvent was evaporated and recovered by condensation. The residue of total lipids was determined gravimetrically after drying. For mineral ion determination, leaves were annealed in a muffle furnace at 550°C for 24 h; the ashes thus obtained were dissolved in 5 ml hydrochloric acid 6N which was evaporated on a hot plate at 125°C. The viscous residue obtained is again dissolved and recovered using HNO₃; mineral ions were then determined by atomic absorption spectrophotometer. Vitamin A was determined with the method of Jedlicka and Klimes (2005) and Kini et al. (2008); vitamins B₁, B₂ and B₃ with the method of Benmoussa et al. (2003) and Gregory (1954), and vitamin C with the method of Karboue and Nesrallah (2014).

Statistical analysis

For all parameters, each value was presented in the form of mean ± standard error from three independent samples values per treatment. Analysis of the main effects of stress intensity was based on a one-way analysis of variance (ANOVA). Differences among means were compared through Student, Newman and Keuls (SNK) test. All statistical analyses were performed by SPSS 16.0. (SPSS Inc. Released, 2007).

RESULTS

NaCl effect on sugars, proteins and lipids concentration

NaCl effects on leaf total sugars, reducing sugars, proteins and lipids concentrations are shown in Figure 1. A non-significant increase was observed at 30 mM NaCl

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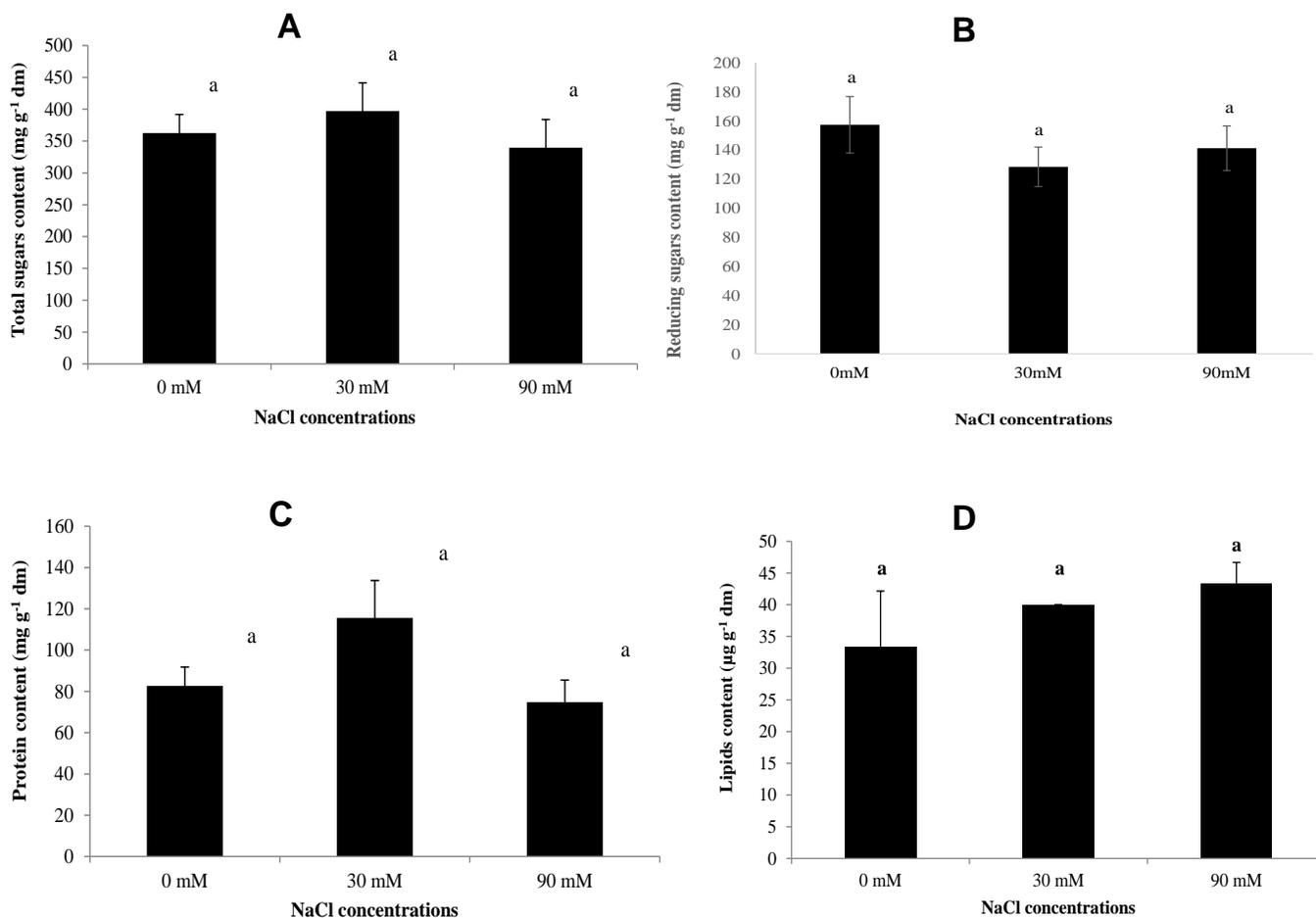


Figure 1. Effect of different NaCl concentrations (0, 30 and 90 mM) on leaf macronutrients contents of *A. cruentus* cv. Locale after four weeks of stress. Values are means \pm SEs, n = 3. (A) Total sugars, (B) Reducing sugars, (C) Proteins and (D) Lipids. Means with different letters are significantly different ($p \leq 0.05$).

Table 1. Effect of different NaCl concentrations (0, 30 and 90 mM) on leaf mineral contents (potassium, calcium and iron: mg g⁻¹ dm) of *A. cruentus* cv. 'Locale' after four weeks of stress.

NaCl (mM)	Potassium (K ⁺)	Calcium (Ca ²⁺)	Iron (Fe)
0	39.76 \pm 2.72 ^a	20.26 \pm 2.33 ^a	0.297 \pm 0.013 ^a
30	47.74 \pm 3.36 ^a	21.88 \pm 1.92 ^a	0.358 \pm 0.012 ^b
90	45.61 \pm 4.25 ^a	18.83 \pm 2.03 ^a	0.284 \pm 0.004 ^a

Values are means \pm SEs, n = 3. Means with different letters within a column were significantly different ($p \leq 0.05$).

for total sugars (Figure 1A) and proteins (Figure 1C) followed by a slight non-significant decrease at 90 mM NaCl. For reducing sugars, a non-significant decrease was observed at 30 and 90 mM NaCl (Figure 1B), whereas for lipids, a non-significant increase was observed at 30 and 90 mM NaCl (Figure 1D). Thus, NaCl effect on leaf total sugars, reducing sugars, proteins and lipids contents remained not significantly affected by the NaCl concentrations used.

NaCl effect on mineral concentration

NaCl effect on leaf potassium, calcium and iron contents is shown in Table 1. A non-significant increase was observed at 30 and 90 mM NaCl for potassium, whereas a similar observation was made at 30 mM NaCl followed by a non significant decrease at 90 mM NaCl for calcium. For iron, a significant increase ($p < 0.05$) was noted at 30 mM NaCl followed by a non significant decrease at 90

Table 2. Effect of different NaCl concentrations (0, 30 and 90 mM) on leaves vitamins contents ($\mu\text{g g}^{-1}\text{fm}$) of *A. cruentus* cv. 'Locale' after four weeks of stress.

NaCl (mM)	Vitamin A	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin C
0	0.190±0.014 ^a	0.244±0.014 ^a	3.725±0.001 ^a	2.053±0.022 ^a	6.4±0.1 ^{ab}
30	0.254±0.020 ^a	1.057±0.046 ^b	3.755±0.004 ^b	2.054±0.02 ^a	4.10±1 ^a
90	0.666±0.036 ^b	1.62±0.146 ^c	3.839±0.004 ^c	2.054±0.052 ^a	7.5±0.05 ^b

Means with different letters within a column are significantly different ($p \leq 0.001$).

mM NaCl (Table 1). Thus, NaCl effect on leaf micronutrient contents was significant only for iron at 30 mM NaCl.

NaCl effect on vitamins concentrations

NaCl effect on leaf vitamins concentrations is shown in Table 2. A non-significant increase was observed at 30 mM followed by a significant increase ($p < 0.001$) at 90 mM NaCl for vitamin A, whereas a non-significant decrease was noted at 30 mM NaCl followed by a non significant increase at 90 mM NaCl for vitamin C in comparison with the control (0 mM NaCl). For vitamins B1 and B2, a significant increase ($p < 0.001$) was observed for 30 mM NaCl. For vitamin B3, a slight non-significant increase was observed in the presence of NaCl. Thus, NaCl effect on leaf vitamins contents increase was significant for vitamins A, B1 and B2.

DISCUSSION

Vegetables are known to contain nutritional properties provided by sugars, proteins, lipids, minerals, antioxidants, vitamins, etc. (Prasad et al., 2014). According to these authors, increasing environmental stresses has strong influence on the concentration of bioactive compounds while affecting the valuable constituents of vegetables which are getting deteriorated day by day. The results revealed no modification in total sugars, reducing sugars, proteins and lipids concentrations of leaves. In other vegetables, a positive effect of salt stress on these nutrients was reported. It is the case in watermelon with an increase in fructose, glucose, sucrose and total soluble solids contents in fruits by salt stress (Colla et al., 2006) and in tomato with an increase in sugar and organic acids contents of fruits (Dorais et al., 2001). Minerals play a vital role in plant and animal metabolism (Zargar et al., 2015). Among these minerals, potassium (K), calcium (Ca) and iron (Fe) are three of the main one in leafy vegetables. The results revealed no effect of NaCl on K^+ and Ca^{2+} concentrations in amaranth leaves. Similar results were reported in watermelon fruit for K^+ (Colla et al., 2006) and cucumber fruit for Ca^{2+} (Trajkova et al., 2006). As reported in other

plants, salt stress generally resulted in K^+ and/or Ca^{2+} decrease (Stamatakis et al., 2003; Trajkova et al., 2006; Abdelhamid et al., 2013). The results revealed a significant increase of iron content in the presence of 30 mM NaCl followed by a non significant decrease at 90 mM NaCl. In faba bean, Abdelhamid et al. (2013) reported that NaCl decreased shoot iron content. The increase or decrease of potassium and calcium concentration, and the decrease of iron content of amaranth leaves at 90 mM NaCl was not significant, indicating that NaCl salt stress did not reduce mineral concentrations in leaves of amaranth. Studies of salt stress effect on vitamin A content of vegetables are scarce. Carotene concentrations are commonly determined as these vegetable yellow pigments are converted to vitamin A in the human body and are accordingly refer to as provitamin A (Ratnakar and Rai, 2013). Amaranth species are known to be a rich β -carotene source (Gopalan et al., 1971). In the present investigation, vitamin A content in the leaves of *A. cruentus* showed a significant increase with increasing NaCl concentration in the growth medium. In unicellular green alga *Dunaliella*, several authors reported that increasing salt concentration induced increment in β -carotene (Pisal and Lele, 2005; Sarmad et al., 2007; Rad et al., 2011). However, other authors reported a decrease in β -carotene content under salt stress in several species or genera including *Paulownia imperialis* and *Paulownia fortunei* (Ayala-Astorga and Alcaraz-Melendez, 2010), *Chlorella* (Fathi and Asem, 2013) and *Amaranthus polygamous* (Ratnakar and Rai, 2013). B vitamins are the precursors of essential metabolic cofactors but are prone to destruction under stress conditions (Hanson et al., 2016). It is therefore *a priori* reasonable that stressed plants suffer B vitamin deficiencies and certain stress symptoms are metabolic knock-on effects of these deficiencies. Studies on environmental stresses effects on B vitamins in plants are very scarce. In general, the foods that contain carbohydrates as a major source of energy contain a higher level of thiamine (vitamin B1) which is required for carbohydrate metabolism (Gopalan et al., 1971). Thiamine can function to alleviate environmental stresses in plants, as it can directly act as an antioxidant (Tunc-Ozdemir et al., 2009). The results reveal that thiamine content in the leaves of *A. cruentus* increased with salt concentration. Similar trend was

observed in maize under salt stress (Rapala-Kozik et al., 2008) and *Arabidopsis thaliana* seedlings (Tunc-Ozdemir et al., 2009). However, Ratnakar and Rai (2013) observed a decrease of thiamine content in *A. polygamous* leaves under NaCl salinity stress. Riboflavin (vitamin B2) is essential for the metabolism and proper utilization of energy, carbohydrates, proteins and fats (Ratnakar and Rai, 2013). It is also essential for several oxidative processes occurring inside the cell. Green leafy vegetables are a good source of riboflavin (Ratnakar and Rai, 2013). In the present study, a significant increase in riboflavin concentration was recorded in *A. cruentus* leaves under NaCl salt stress. In leaves of *A. polygamous*, Ratnakar and Rai (2013) reported a decrease in riboflavin concentration under NaCl stress. Niacin (nicotinic acid or vitamin B3) is a water-soluble vitamin. Nicotinamide is the derivative of niacin and used by the body to form the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Over 400 enzymes require the niacin coenzymes, NAD and NADP, mainly to accept or donate electrons for redox reactions (Penberthy and Kirkland, 2012). NAD functions most often in energy-producing reactions involving degradation (catabolism) of carbohydrates, fats, proteins and alcohol. The niacin coenzyme, NAD, is the substrate (reactant) for at least four classes of enzymes that separate the nicotinamide moiety from NAD and transfer ADP-ribose to acceptors. The results revealed a non-significant increase in niacin content in *A. cruentus* leaves under NaCl salt stress. Ascorbic acid (vitamin C) is an essential nutrient which occurs widely in crop foods products, especially in fresh fruits and green leafy vegetables (Ratnakar and Rai, 2013). It is a small, water soluble, antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008). Vitamin C also helps in absorption of dietary iron by keeping it in the reduced form (Ratnakar and Rai, 2013). The current results revealed that in the leaves of *A. cruentus* cv. 'Locale', the ascorbic acid content was not significantly affected by NaCl. In other amaranth species (*A. polygamous*), Ratnakar and Rai (2013) observed a decrease of ascorbic acid content with increase of salt concentration. The same tendency was reported also in wheat (Seth et al., 2007; Mandhania et al., 2010), and in *Linum usitatissimum* plants (Emam and Helal, 2008). However, the increase of ascorbic acid contents under salt stress was reported in tomato fruits (Stamatakis et al., 2003; Kim et al., 2008; Gautier et al., 2010) and in other plants including barley (*Hordeum vulgare*) (Sarwat and El-Sherif, 2007) and *Cicer arietinum* cv. Abrodhi (Mishra et al., 2009). Generally, it is well known that during the onset and development of salinity-stress within a crop plant, major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected, leading to quality and yield losses in most crops (Hasegawa et al., 2000; Hagemann

and Erdmann, 1997; Hayashi and Murata, 1998). However, there are several crops with an inherent capacity to withstand salinity-stress, which allows for stable vegetable production and significantly contributes to palatability and food functionality (Sato et al., 2006). Thus, there are several reports on the application of salinity-stress for improvement of the quality of vegetables such as tomato fruits (Auerswald et al., 1999), spinach (Makabe and Tanii, 2008) and strawberry (Keutgen and Pawelzik, 2008). The results of the present study revealed that salt stress improved leaves nutritional quality by mainly increasing some vitamins concentrations.

Conclusion

This study indicated that increasing NaCl concentrations did not reduce total sugars, reducing sugars, proteins, lipids, potassium, calcium, vitamin B3 and vitamin C concentrations in leaves of *A. cruentus*, but rather increased vitamins A, B1 and B2 concentrations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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