Original Research Article

Evaluation of salinity resistance of amaranth (*Amaranthus cruentus* L.) mutant lines selected in Benin Republic

ABSTRACT

Aims: In this study, salt resistance level of nine amaranth (*Amaranthus cruentus*) mutant lines selected from Benin cultivar '*Locale*' was evaluated at young plants stage in comparison with the cultivar '*Locale*' used as control.

Study design: The experiment was laid out as a Completely Randomized Design with three replications.

Place and duration of study: The experiment was carried out in a screening house at University of Abomey-Calavi, City of Abomey-Calavi, Republic of Benin from May to June, 2020.

Methodology: Three-weeks old plants of the nine stable mutant lines and the control cultivar '*Locale*' were submitted in pots containing a mixture of potting soil and sand to four NaCl concentrations: 0; 100; 150 and 200 mM NaCl by irrigation every two days. Plant growth parameters were evaluated after two weeks.

Results Salt effect caused a reduction of young plant growth whatever the growth parameter considered with a significant disparity (p=.001) among genotypes. Growth of the control cultivar, lines 1, 11 and 15 was the most affected under salt stress whereas that of lines 18; 23 and 16 was the least affected. A significant difference (p=.01 or p=.001) was observed among the salt tolerance index of genotypes. The highest salt tolerance index was observed in lines 23 followed by lines 18 and 2; and the lowest in line 15 followed by line 17, line 10 and the control cultivar.

Conclusion: Some variability was observed among lines for their salt resistance. Lines 23, 18 followed by line 2 appeared as the most salt resistant whereas line 15, followed by lines 17, 10 and the control cultivar were the most salt sensitive. Further studies are necessary to determine the physiological and biochemical mechanisms involved in the lines' salt resistance.

***Key words**: Amaranthus cruentus,* cultivars discrimination, mutation induction, plant growth, NaCl.

Abbreviations

PH: Plant Height

LN: Leaf Number

SFM: Shoot Fresh Mass

SDM: Shoot Dry Mass

RL: Root Length

RFM: Root Fresh Mass

RDM: Root Dry Mass

1. INTRODUCTION

Salt stress is considered one of the main limiting factors affecting plant productivity worldwide and influences almost all aspects of plant biology [1]. Thus, increasing tolerance to salt stress in crop plants is necessary to increase the yield [2]. The effects of salinity on plant growth and yield are complex, and may result from a combination of toxic, nutritional, and osmotic factors [3]. Plant overall response to increasing NaCl dose appear to be species-specific [4]. In addition, within the same given species, a substantial variation in salt sensitivity may appear in cultivars or varieties as reported in several species of vegetable crops including amaranth [5,6] chili [7] tomato [8] African eggplant [9] and tossa jute [10]. The genus *Amaranthus* included species cultivated as leaf vegetables, pseudocereals, and ornamental plants. Plants of this genus exhibit a high nutritive value but also a fascinating ability to adapt to diverse harsh environments [11]. As a tropical leafy vegetable, it is acquiring increasing importance as a potential subsidiary food crop for its excellent quality of protein and endogenous micronutrients content [12, 13]. Vegetable crops are predominantly cultivated in the south of Benin, in urban and suburban areas and in the valley of *Oueme* [14]. In Benin, amaranth species are mainly cultivated as leaf vegetable in the arable lands of the coastal areas where soil and irrigation water's salinity are a real problem hindering crop production [15]. It has been demonstrated that there is variability in relative salt resistance among *Amaranthus cruentus* cultivars at young plant stage and that the most appreciated cultivar either by farmers or consumers, named Locale was the most salt sensitive among five available cultivars [6]. Thus, it would be interesting to improve this cultivar with the purpose of enhancing its salt resistance. It has been reported that genetic improvement of *Amaranthus* crops can be achieved by various techniques, such as classical breeding, mutagenesis and biotechnological approaches. The availability of simple and efficient techniques for inducing genetic variation, such as the use of radiation for inducing mutation and

selection for desired traits is an essential component of any plant breeding programme [16]. Mutagenesis is a simple and cost-effective technology [17, 18]. Induced mutations have been utilized to create genetic variability for the selection of mutant varieties with improved agronomic traits in several plant species [19, 20, 21, 22, 23]. Induced mutagenesis in well adapted and culturally accepted local cultivars can produce small genetic changes which will affect critical agronomic traits [24]. Mutation technology was used as a tool to create genetic variation in different amaranth genotypes with enhanced quality and quantity of grain or with improved drought tolerance [25, 16].

The objective of this study was to determine, among nine mutant lines of *Amaranthus cruentus*, those that were more salt resistant than the untreated control cultivar.

2. MATERIAL AND METHODS

2.1. Summary of mutation induction and lines stabilization processes

Seeds of cultivar '*Locale*' of *Amaranthus cruentus* were irradiated by γ radiation dose 200 Gy at IAEA in Vienna (Austria). The irradiated seeds were transferred to Benin, sown and self-pollinated at maturity using « single-seed descent » technique [26] at the experimental site of the International Institute of Tropical Agriculture (IITA / Benin, (latitude: N 6° 25' 260'' and longitude: E 2° 19' 682''; altitude: 15 meter above sea level) in the City of Abomey-Calavi (Republic of Benin) from November, 2018 to March, 2020. This city is located in the Gulf of Guinea which is characterized by a subequatorial bimodal climate with two dry seasons and two rainy seasons [27]. The annual rainfall varies between 1200 and 1500 mm/year and the temperature ranges from 24 to 30 °C [27]. At each self-pollinated generation, seedlings from individual selected plant were grown in 3 rows on a 3 m long and 1.5 m wide plot. Five plots were used per line. Plants were spaced 50 cm apart within rows and 50 cm between rows with a total of 18 plants per plot. Among those 18 plants grown per plot, the 5 well developed and phenotypically close based on plant height, number of branches and leaves production were identified and their flowers were covered from appearance, against external pollen with envelope made up of tracing paper. At seeds maturity stage, seeds were harvested per plant per line and dried. Seedlings from one of the five selected plants per plot per line were transplanted into one plot at the next generation. This process was continued until generation M6 seeds. Nine amaranth mutant lines from generation M6 were analyzed for their relative salt resistance status in this study with the cultivar 'Locale' used for mutation induction and called control cultivar (CC).

2.2. Experimental conditions and design

The experiment was carried out in a screening house at the University of Abomey-Calavi (Republic of Benin) in the city of Abomey-Calavi. The experiment was laid out in a Completely Randomized Design (CRD) with three replications (three pots) and three plants per replication (per pot) (9 plants per treatment). Pots (11.3 cm diameter and 14 cm) were filled with 3 kg mixture of potting soil and sandy loam soil 50:50. The experiment was conducted as described by [28]. Salt treatments consisted of plant irrigation every two days with solution of four NaCl concentrations: 0, 100, 150 or 200 mM NaCl (CAS n°7647-14-5). The experiment was evaluated after two weeks exposure to salt stress.

2.3. Data collected

Plant height, leaf number, root length, shoot and root fresh and dry matters were measured at the end of treatment. For dry matter determination, fresh samples were transferred to an oven at 80 °C for 72 hours. Salt tolerance index (STI) was determined for each growth parameter for the ten genotypes using the modified formulae of Tabatabaei et al. [29].

2.4. Statistical analysis

The data collected were processed using descriptive statistics utilizing an Excel spreadsheet and presented in the form of tables and graphs. The analysis of the main effects of salt stress was based on the variance analysis. Means were compared utilizing Students, Newman and Keuls (SNK) test. Statistical analyses were performed using JMP Pro 12 software [30]. For all parameters, each value was presented in the form of mean ± standard error with a reading of three independent samples per treatment.

3. RESULTS

3.1. Overall reaction of the nine lines and the control cultivar with regard to salt stress

Salt stress reduced plant growth as the NaCl concentration increase (fig. 1). The two-ways analysis of variance revealed a significant effect of salt stress (p=.0.01) for all the growth parameters taken into account, a significant difference among genotypes (p=.0.01) and a significant salt x lines (genotypes) interaction (p=.0.01) (Table 1). With a significant interaction between both factors for all growth

parameters considered, the effect of salt stress on growth should be studied by considering each genotype at a time.

Figure 1: Plant of mutant lines of *Amaranthus cruentus* (line 2) under different NaCl concentrations

Table 1. Results of two-ways analysis of variance of growth of nine mutant lines of *Amaranthus cruentus* and the control cultivar after two weeks of culture under different NaCl concentrations

Growth parameters	Stress	genotype	Interaction (Stress x genotype)
PH	105.80***	28.38***	2.73***
LN	54.63**	6.59**	2.23**
SFM	138.08***	24.04***	3.24***
SDM	149.42***	42.04***	7.50***
RL	63.06***	4.75***	2.02**
RFM	29.53***	13.32***	1.99**
RDM	59.34***	12.49***	3.18***

: difference significant at p=.01; *: difference significant at p=.001

3.2. Effect of NaCl on plant aerial part growth

3.2.1. NaCl effect on plant height

Salt stress reduced plant height in lines and the control cultivar (image . 1). NaCl induced a significant (p=0.001) reduction of plant height in the control cultivar and mutant lines except lines 18 and 23. The reduction was significant from 100 mM NaCl for lines 10, 15 and 16 whereas it was significant from150 mM for lines 1, 2, and 17 and the control cultivar. The plant height reduction was significant only at 200 mM NaCl for line 11. Thus, salt effect on plant height inhibition was more increased in lines 10, 15 and 16 than the other lines; lines 18 and 23 were the least affected, followed by line 11.

Image 1: Plant height of mutant lines of *Amaranthus cruentus* under different NaCl concentrations (n = 3; vertical bars are standard errors). Values within cultivar with same letters are not significantly different at p=.001.

3.2.2. NaCl effect on leaf number

NaCl induced a significant (p=.001) reduction in leaf number in the control cultivar and mutant lines except lines 2, 16, 17 and 18 (Fig. 2). The reduction was significant from 150 mM NaCl for lines 1, 11 and 15 whereas it was significant only at 200 mM NaCl for lines10, 23 and the control cultivar. Thus, leaf number reduction under salt stress was more increased in lines 11 and 15 than the other lines; lines 2, 16, 17 and 18 were the least affected.

3.2.3. NaCl effect on shoot fresh mass

NaCl induced a significant (p=.001) reduction of shoot fresh mass growth in the control cultivar and all mutant lines (fig. 3). The reduction was significant from 100 mM NaCl for lines 2, 10, 15 and 17 whereas it was significant from 200 mM for lines 11, 18 and 23. Shoot fresh mass reduction was significant at 150 mM NaCl for line 1; 16 and the control cultivar. Shoot fresh mass inhibition was more increased in lines 2; 10; 15 and 17 than the other lines; lines 11; 18 and 23 were the least affected.

Figure 2: Leaf number of mutant lines of *Amaranthus cruentus* under different NaCl concentrations (n = 3; vertical bars are standard errors). Values within cultivar with same letter are not significantly different at p=.001.

Figure 3: Shoot Fresh Mass of mutant lines of *Amaranthus cruentus* cultivars under different NaCl concentrations (n = 3; vertical bars are standard errors). Values within cultivar with same letter are not significantly different at p=.001.

3.2.4. NaCl effect on shoot dry mass

NaCl induced a significant (p=.001) reduction of shoot dry mass in the control cultivar and all mutant lines (fig. 4). The reduction was significant from 100 mM NaCl for lines 2; 10, 15, 16 and 17 whereas it was significant was significant only at 200 mM NaCl for lines 1; 11; 18 and 23 and the control cultivar. These results showed that salt effect on shoot dry mass inhibition was more accrued in lines 2; 10, 15, 16 and 17 than the other lines and the control cultivar.

Figure 4: Shoot Dry Mass of mutant lines of *Amaranthus cruentus* under different NaCl concentrations (n = 3; vertical bars are standard errors). Values within line with same letter are not significantly different at p=.001.

3.3. Effect on NaCl on roots growth

NaCl induced a significant reduction (p=.001) of root length in the control cultivar and mutant lines except lines 2; 16 and 23 (table 2). The reduction was significant from 100 mM NaCl for line 18 whereas it was significant only at 200 mM NaCl for lines 10; 15 and 17. Root length reduction was significant from 150 mM NaCl for lines 1, 15 17, 23 and the control cultivar. Thus, salt effect on root

length inhibition was more accrued in lines 18 than the other lines; lines 2; 16 and 23, followed by lines 10; 15 and 17 were the least affected.

For root fresh mass, NaCl induced a significant reduction (p=001) of root fresh mass in the control cultivar and mutant lines except lines 10; 17; 18 and 23 (table 1). The reduction was significant from 100 mM NaCl for the control cultivar and line 2 whereas it was significant only at 200 mM NaCl for line 1 and 11. Root fresh mass reduction was significant from 150 mM NaCl for lines 15 and 16. The root length was significant affected only at 200 mM NaCl for line 1 and 11. Thus, salt effect on root fresh mass inhibition was more accrued in the control cultivar and line 2 than the other lines; lines 10; 17; 18 and 23, followed by lines 1 and 11 were the least affected.

For root dry mass, NaCl induced a significant reduction (p=.001) in the control cultivar and mutant lines except for lines 16 and 18 (table 1). The reduction was significant from 100 mM NaCl for lines 11; 15 and 17 and the control cultivar, whereas it was significant only at 200 mM NaCl for lines 1 and 23. The root dry mass reduction was significant from 150 mM NaCl for lines 10. Thus, salt effect on root dry mass inhibition was more accrued in the control cultivar and lines 11; 15 and 17 than the other lines; lines 16 and 18, followed by 23 and 1 were the least affected.

In general, the growth reduction due to NaCl salt stress was not significant for 4 and 3 of the 8 growth parameters studied respectively for lines 18 ; 23 and 16 whereas this reduction was significant for all the 8 or 7 growth parameters for lines 1; 11; 15 ; the control cultivar and line 10. These results showcased that lines 18; 23 and 16 were the least affected by salinity whereas lines 1; 11 and 15 were the most affected followed by line 16 and the control cultivar.

3.4. Salt tolerance index of lines

There is a significant difference (p=.01 or p=.001) among lines for their salt tolerance index for the seven growth parameters evaluated (table 3). For plant height, line 23 (0.87) and line 18 (0.82) showed the highest values whereas line 15 (0.58) followed by line 1 (0.65) and the control cultivar (0.63) presented the weakest values. The other lines showed intermediary values. For leaf number, lines 18 (1.03) and 23 (0.80) showed the highest value whereas line 15 (0.59) presented the weakest value. The other lines showed intermediary values. For shoot fresh mass, lines 18 (0.75) and 23 (0.67) showed the highest value whereas line 15 (0.30) presented the weakest value. The other lines showed intermediary values. For shoot dry mass, line 18 (1.02) followed by line 23 (0.82) showed the

highest values whereas line 15 (0.28) followed by line 10 (0.41) presented the weakest values. The other lines showed intermediary values. For root length, followed by line 2 (1.07) showed the line 23 (0.93) whereas lines highest values followed by 17 11 (0.49) (0.57) (0.55) and 18 weakest presented the other lines values. The intermediary showed root fresh values. For (0.76) and 23 mass, lines 18 the highest (0.69) showed whereas lines values (0.33), the 17 (0.31), 10 (0.34), lines 16 control cultivar (0.36) and 1 (0.36) presented the weakest ones. The other lines showed intermediary values.

Table 2: Effect of different NaCl concentrations (0, 100; 150 and 200 mM) on root length (RL), fresh (RFM) and dry masses (RDM) of control cultivar and nine mutant lines of *Amaranthus cruentus* after two weeks of stress: *Values are means* ±SE (n = 3).

		RL	RFM	RDM
CC	00 mM	9.13±0.46a	0.14±0.08a	0.02±0.92a
	100 mM	7.66±0.66ab	0.07±0.07b	0.01±0.33b
	150 mM	4.73±0.34b	0.05±0.11b	0.01±0.49b
	200 mM	5.06±1.23b	0.03±0.06b	0.01±0.00b
L1	00 mM	8.00±0.57a	0.31±0.12a	0.02±0.00a
	100 mM	7.30±1.04ab	0.27±0.03a	0.01±0.00ab
	150 mM	4.75±0.27bc	0.21±0.01ab	0.01±0.00ab
	200 mM	4.00±0.00c	0.04±0.00b	0.00±0.13b
L2	00 mM	9.16±0.92a	0.60±0.06a	0.05±0.00a
	100 mM	8.20±0.35a	0.37±0.04b	0.03±0.00b
	150 mM	6.56±0.53a	0.29±0.05b	0.02±0.00b
	200 mM	9.33±0.66a	0.25±0.01b	0.02±0.00b
L10	00 mM	8.75±0.72a	0.40±0.13a	0.04±0.01a
	100 mM	6.75±0.72ab	0.26±0.05a	0.02±0.00ab
	150 mM	6.60±0.46ab	0.08±0.01a	0.01±0.00b
	200 mM	5.75±0.05b	0.16±0.02a	0.00±0.00b
L11	00 mM	10.50±1.44a	0.36±0.04a	0.05±0.00a
	100 mM	7.00±0.00ab	0.35±0.00a	0.03±0.00b
	150 mM	4.90±0.80b	0.24±0.03a	0.02±0.00bc
	200 mM	3.25±1.38b	0.07±0.01b	0.01±0.00c
L15	00 mM	9.00±0.00a	0.55±0.06a	0.04±0.00a
	100 mM	7.90±0.00a	0.33±0.00ab	0.01±0.00b
	150 mM	6.33±1.20ab	0.30±0.03b	0.01±0.00b
	200 mM	4.10±0.05b	0.18±0.07b	0.01±0.00b
L16	00 mM	8.55±0.99a	0.33±0.02a	0.02±0.00a
	100 mM	7.33±1.01a	0.22±0.03ab	0.02±0.00a
	150 mM	5.70±1.15a	0.18±0.2b	0.01±0.00a
	200 mM	4.53±0.03a	0.15±0.03b	0.01±0.00a
L17	00 mM	9.33±1.76a	0.63±0.13a	0.06±0.00a
	100 mM	6.53±0.54ab	0.35±0.04a	0.03±0.00b
	150 mM	5.00±0.00ab	0.27±0.08a	0.02±00b
	200 mM	4.40±0.60b	0.34±0.01a	0.02±0.00b
L18	00 mM	10.00±0.57a	0.25±0.05a	0.02±0.00a
	100 mM	7.00±0.57b	0.22±0.00a	0.03±0.00a
	150 mM	6.66±0.33b	0.26±0.02a	0.02±0.00a
	200 mM	3.50±0.50c	0.21±0.02a	0.01±0.00a
L23	00 mM	6.66±0.88a	0.46±0.08a	0.04±0.00a
	100 mM	5.46±0.48a	0.43±0.09a	0.03±0.00ab
	150 mM	4.06±0.53a	0.38±0.06a	0.02±0.00ab
	200 mM	4.66±0.33a	0.21±0.04a	0.01±0.00b

Means with different letters within column for each line are significantly different (p=.01 or p=.001).

For root dry mass, lines 18 (1.10) followed by L23 (0.86) and L2 (0.73) showed the highest values whereas lines 11 (0.24), L15 (0.30) and L10 (0.33) presented the weakest values. The other lines showed intermediary values Thus, based on the salt tolerance index, lines 23 and 18 showed the highest salt tolerance index for 7 and 6 growth parameters respectively. These two lines appeared as the most salt resistant, followed by line 2 which presented the highest STI for two parameters. In

contrast, line 15 presented the weakest salt tolerance index for five growth parameters and appeared then as the most salt sensitive followed by the control cultivar and line 17 which presented the weakest STI for two parameters.

Table 3. Salt tolerance index of nine mutant lines of *Amaranthus cruentus* and the control cultivar after two weeks of stress: *Values are means* ±SE (n = 3).
Means with different letters within column for each line are significantly different (p=.001)

	Growth parameters						
Lines	**HP**	**LN**	**SFM**	**SDM**	**RL**	**RFM**	**RDM**
CC	0.63±0.05bc	0.77±0.06ab	0.47±0.07ab	0.61±0.07bcd	0.63±0.06bcd	0.34±0.06c	0.40±0.08bc
L1	0.65±0.06bc	0.68±0.09ab	0.43±0.12ab	0.49±0.13abc	0.76±0.09abcd	0.36±0.06c	0.44±0.09bc
L2	0.74±0.03abc	0.78±0.05ab	0.47±0.04ab	0.71±0.07abc	1.07±0.08a	0.49±0.08ab	0.73±0.11ab
L10	0.67±0.07abc	0.67±0.05ab	0.41±0.05ab	0.41±0.04cd	0.86±0.04abc	0.33±0.05c	0.33±0.07c
L11	0.78±0.06abc	0.71±0.06ab	0.61±0.10ab	0.75±0.11abc	0.49±0.07d	0.40±0.05bc	0.24±0.05c
L15	0.58±0.02c	0.59±0.05b	0.30±0.03b	0.28±0.02d	0.67±0.07bcd	0.25±0.07bc	0.30±0.04c
L16	0.68±0.04abc	0.78±0.07ab	0.50±0.07ab	0.51±0.05bcd	0.66±0.05bcd	0.36±0.05c	0.59±0.10bc
L17	0.73±0.02abc	0.77±0.05ab	0.49±0.05ab	0.50±0.02bcd	0.55±0.05cd	0.31±0.06c	0.40±0.03bc
L18	0.82±0.05a	1.03±0.08a	0.75±0.07a	1.02±0.05a	0.57±0.06cd	0.76±0.24a	1.10±0.29a
L23	0.87±0.02a	0.80±0.06a	0.67±0.07a	0.82±0.09ab	0.93±0.09ab	0.69±0.09a	0.86±0.14ab
Prob >F	**0.0005**	**0.0046**	**0.0064**	**<0.0001**	**<0.0001**	**0.0007**	**<0.0001**

4. DISCUSSION

Salt stress caused an inhibition on plant root and shoot growth. Such observation is commonly reported in several amaranth genotypes including *Amaranthus cruentus* genotypes [31, 32, 33, 34, 35, 36, 37].

Our results also revealed that the growth parameters used were differently affected by salinity according to the mutant line indicating that the effect of salinity on mutant lines depend on the growth parameters taken into account as previously reported in several vegetable species including amaranth [6]. This statement suggests that difference among lines was genetically controlled. Results showed that lines 18; 23 and 16 were the least affected by salinity whereas lines 1; 11 and 15 were the most affected followed by line 16 and the control cultivar. Moreover, lines 23 and 18 showed the highest salt tolerance index followed by line 2 whereas line 15 presented the weakest salt tolerance index followed by the control cultivar and line 17. These results revealed certain variability in the response to salt stress of the nine mutant lines. Moreover, some of the mutant lines were less affected than the control

cultivar with the highest salt tolerance index indicating that the induced mutation process used was efficient to produce useful variability related to response to salt stress within lines from a salt sensitive cultivar. Induced mutations have been utilized for creation of genetic variability for the selection of mutant varieties with improved agronomic traits [19, 20, 21, 22, 23]. Therefore it is logical to consider that the mutation technology used in this study created a genetic variation in *Amaranthus cruentus* usefull to generate lines with improved salinity tolerance as reported in *A. tricolor* for drought tolerance [38]. Combining plant growth and salt tolerance index, lines 23 and 18 followed by line 2 appeared as the most salt resistant whereas line 15, followed by line 17, line 10 and the control cultivar were the most salt sensitive. According to Gandonou et al. [39], the effect of salt stress on plants depends on three interacting components: i) dehydration of the cells in response to the low external water potential, ii) nutritional imbalance caused by the interference of saline ions with essential nutrients and iii) toxicity due to the high accumulation of Na^+ and Cl^- in the cytoplasm. It would be important to evaluate which of these three components played a main role in growth reduction under salinity stress of the tested mutant lines.

5. CONCLUSION

This study showcased that there is variability in the response of the nine amaranth mutant lines to NaCl salt stress at young plant stage. We noticed lines with high salt tolerance from other with low salt tolerance. Among the nine mutant lines evaluated, lines 23 and 18 followed by line 2 appeared as the most salt resistant whereas lines 15 followed by line 17, line 10 and control cultivar appeared as the most salt sensitive. Thus, lines 23, 18 and 2 can be used as donors in amaranth breeding program for salt tolerance.

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