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Salinity Resistance of Six Amaranth (*Amaranthus* sp.) Cultivars Cultivated in Benin at Germination Stage

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Authors' contributions

This work was carried out in collaboration among all authors. Author AW designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AW and CBG managed the literature searches. Authors AW, CBG and DM contributed to the protocol writting and managed the analyses of the study. Author DM performed the statistical analysis. Authors JK, EK and FAK contributed to the protocol writting. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, salt resistance level of six amaranth (*Amaranthus* sp.) cultivars including five from *Amaranthus cruentus* species (AA-04-028, AA-04-017, Locale, Rouge and Red-Sudan) and one from *Amaranthus graecizans* species (Stem2-Sat2) cultivated in Benin was evaluated at the germination stage.

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Study Design: The experiment was laid out as a Randomized Complete Block Design (RCBD) with four replications.
Place and Duration of Study: The experiment was carried out in the Plant Physiology.
and Abiotic Stresses Study of University of Abomey-Calavi, Republic of Benin from September to October 2015.
Methodology: Seeds were submitted to treatment with five NaCl concentrations (0; 30; 60; 90 and 120 mM NaCl) in petri dishes. Seed germination was checked every day during the ten days incubation period. Germination percentage was determined within 10 days incubation. Four replicates of 50 seeds each were used.
Results: From day 2 to day 10, NaCl delayed seed germination rate proportionately to NaCl concentration. Salt stress reduced the rate of germination and the germination index in all cultivars. At the end of the 10th days, salt stress significantly decreased the rate of final germination for all cultivars investigated whatever the NaCl concentrations used, except for cultivar Red Sudan and Rouge which showed a slight stimulation of germination at 30 mM NaCl. However, the NaCl stress effects on seed germination of the six cultivars were significantly variable. The average reduction due to NaCl stress was 22.11%; 20.90%; 17.28%; 15.58%; 8.03% and 6.57% for AA-04-017, AA-04-028, Rouge, Locale, Stem2-Sat2 and Red-Sudan, respectively.
Conclusion: NaCl stress delayed seed germination and reduced the germination index and the rate of final germination. Among the six cultivars, Red-Sudan appeared to be the most salt resistant. For the first time, we demonstrated that there is a variability of relative salt-stress resistance among *Amaranthus cruentus* cultivars at germination stage.

Keywords: Amaranthus cruentus; cultivars; germination; salt-resistance.

1. INTRODUCTION

Salt stress is one of the major environmental constraints limiting agricultural productivity [1,2]. Plant growth is affected by salinity at all stages of development, but sensitivity varies greatly at different stages [3-5]. Germination is a critical stage in the growth cycle of plant species; it determines plant establishment and final crop production [6]. Thus, crop production in saline areas largely depends upon successful germination, seedling emergence and also on post germination development or seedling establishment [7]. Thus, increasing salinity generally reduces germination of glycophytes [5-11] and the response is concentration dependent and also specific on species [12-15]. It has been reported that there is a substantial variation in salt sensitivity among cultivars of the same species [2,16,17].

Amaranth *(Amaranthus* sp.*)* species are tropical leafy vegetable crops, acquiring increasing importance as a potential subsidiary food crop for their excellent quality of protein and micronutrients [18,19]. Vegetable crops are predominantly cultivated in the south of Benin, in urban and suburban areas and in the valley of Oueme [20]. Owing to its high nutritive value and a wide adaptability to diverse environments, amaranth has been considered a promising crop for marginal lands and semiarid regions [21,22] where the problem of salinity is acute [7]. In Benin, amaranth species are mainly grown in the cultivable lands of the coastal areas where soil and salinity related to irrigation water are a reality.

The prospects for future cultivation of salt-tolerant, high-yielding genotypes of amaranth are very encouraging [23]. However, despite a substantial amount of literature on responses of plants to salinity stress, little information is available on amaranth [23]. Moreover, there is almost no information about salt resistance within cultivars of a given amaranth species. Further, little work has focused on salt tolerance of amaranth cultivars produced in Benin.

Indeed, the present study aims at evaluating NaCl stress effects on seed germination of six amaranth cultivars grown in Benin and comparing the mean level of salt resistance of these cultivars at the germination stage.

2. MATERIALS AND METHODS

2.1 Plant Material

Six amaranth cultivars, produced in Benin were used (AA-04-028, Locale, AA-04-017, Rouge, Stem2 Sat2 and Red Sudan), including five cultivars of *Amarnthus cruentus* L. (AA-04-028, Locale, AA-04-017, Rouge and Red Sudan) and

one of *Amaranthus graecizans* (Stem2-Sat2). Species identification was done by the team at the National Herbarium of Benin. Cultivars are obtained from the Market Gardening Crops Program of the Benin National Institute for Agricultural Research (INRAB).

2.2 NaCl Effect on Seed Germination

Seeds were incubated in 10 cm Petri dishes on one layer of filter paper moistened with 15 ml distilled water or the same volume of water solutions of 30–120 mM NaCl at 30 mM intervals. Seed germination was checked upon every day during 10 days incubation in water and/or NaCl. Germination percentage was determined within 10 days incubation. The experiment was laid out as a Randomizes Complete Block Design (RCBD) with four replications (40 seeds each). The seeds were incubated in darkness at 26℃. A seed was considered as germinated when the radicle emerged from the seed coat.

Salt effect on seed germination was evaluated using germination kinetics, germination index and final germination rate which were the main parameters generally used to evaluate salinity effect at germination stage. Germination kinetics and germination rates were expressed according to the method used by 24. Germination index (GI) was calculated according to EBC method used by 25.

$$IG = 10 \times (n_{24} + n_{48} + n_{72})/(n_{24} + 2n_{48} + 3n_{72})$$

where:

n_{24}, n_{48}, n_{72} – numbers of germinated seeds at 24, 48, and 72 h

2.3 Statistical Analysis

For each concentration and each cultivar, 40 seeds were used with four replications. The analysis of the main effects of cultivars and stress intensity was based on 1-way analysis of variance (ANOVA). Means were compared utilizing Student, Newman and Keuls test. Analysis was performed using GenStat discovery [26].

3. RESULTS AND DISCUSSION

3.1 Effect of Sodium Chloride on Seed Germination Kinetics

Figs. 1, 2, 3, 4, 5 and 6 present the effect of NaCl on seed germination rate after 2, 4, 6, 8 and 10 days in the presence of NaCl at 0, 30, 60, 90 and 120 mM, respectively for cultivars Locale, Rouge, Red-Sudan, AA-04-028, AA-04-017 and Stem2-Sat2. In absence of stress, the reaction of varieties were different: after 2 days, 66% of the seeds of Red-Sudan germinated, whereas for cultivars AA-04-028, AA-04-017 and Locale, respectively 92, 92 and 94% of seeds germinated during the same period; this rate was about 95.5 and 96% for Rouge and Stem2-Sat2. After 4 days, the percentages of seed germination were about 94.5, 95, 94, 95.5, 68.5 and 96%, respectively, for AA-04-028, AA-04-017, Locale, Rouge, Red-Sudan and Stem2-Sat2. No progress was observed in the rate of seed germination of control for the six cultivars after 4 days (from 4 days to the end of the experiment). NaCl stress effect resulted in a reduction of bud germination speed, visible for all varieties. Indeed, a reduction of the percentages of bud was observed for all varieties at the various NaCl concentrations used as well after 2, 4, 6, 8 as after 10 days (Figs. 1 to 6). For AA-04-028, the percentage of bud germination after 2 days shifts from 92% in absence of NaCl, to 78, 75, 15.5 and 1%, respectively at 30, 60, 90 and 120 mM of NaCl. Similar observations have been made concerning cultivars AA-04-017, Locale, Rouge, Red-Sudan and Stem2-Sat2 where seed germination percentage respectively shifts from 92, 94, 95.5, 66 and 96% on the control to 3, 0, 0, 1 and 1.5% at 120 mM of NaCl. Similar tendencies were observed for 4, 6 and 8 days. These observations indicated that salt stress delays seed germination for all cultivars.

The maximum seed germination percentage was obtained at the 6th day for all cultivars and NaCl concentrations. This result indicates that NaCl effect on amaranth seeds final germination rate could be studied after 6 days of stress.

The varieties tested in this study present different capacities of seed germination in absence of salt stress. Similar trend was observed in other genotypes of amaranth [23]. With 82.5%, cultivar Red-Sudan presents the weakest capacity of seed germination, in comparison with the five other cultivars. Sodium chloride (NaCl] concentrations used in this study affect the kinetics of seed germination mainly until the 4th day corroborating the results reported in sugar cane [24]. Similar results were reported in four cultivated species of amaranth [27].

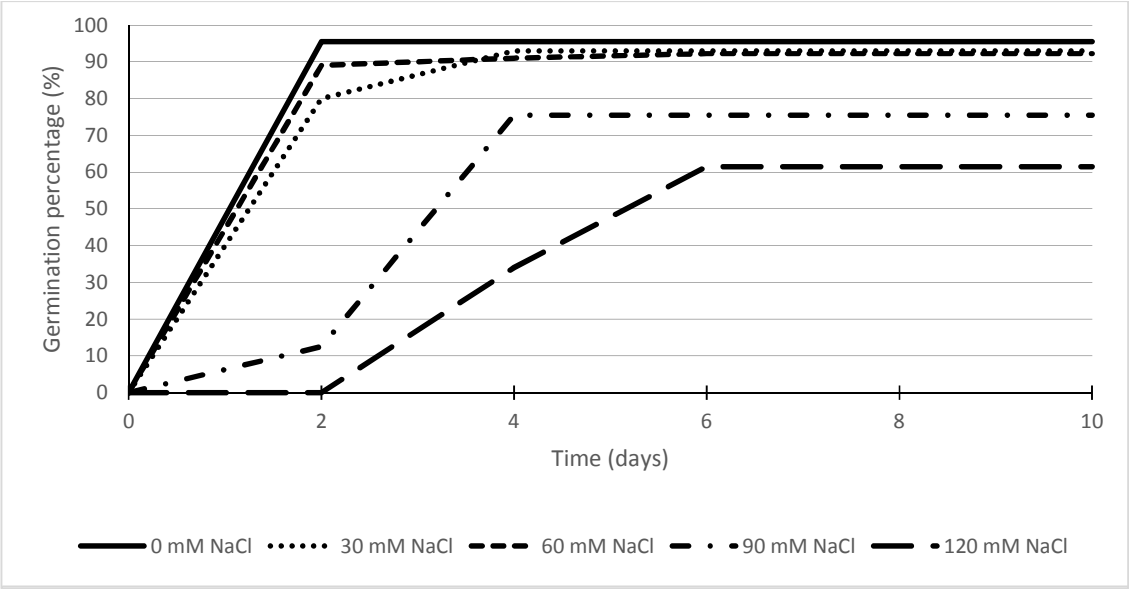

Fig. 1. Rate of germination of amaranth seeds under saline conditions for cultivar Locale

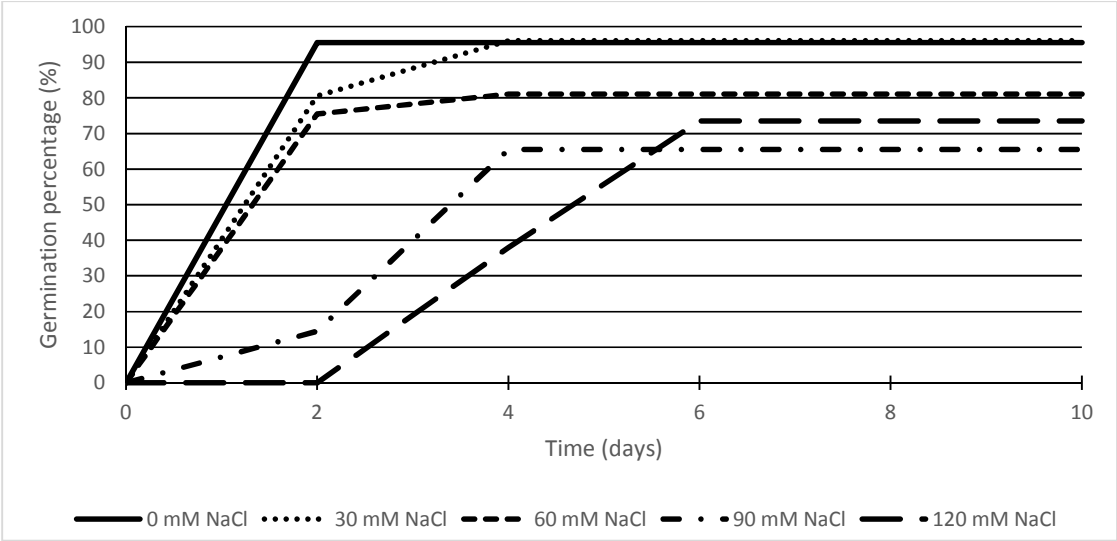

Fig. 2. Rate of germination of amaranth seeds under saline conditions for cultivar Rouge

3.2 Effect of Sodium Chloride on Germination Index

In the absence of stress, the germination index were 9.05; 9.28; 8.60; 7.20; 8.15; and 7.65 respectively for cultivars Stem2-Sat2; Locale; Rouge; AA-04-017; AA-04-028 and Red Sudan. AA-04-017 presented the lowest germination index (7.20) (Table 1) whereas Locale showed the highest germination index (9.28). Sodium chloride (NaCl) effect resulted in a reduction of the germination index in all cultivars tested. The same trend was reported in various species such as *Lathyrus sativus* and *Pisum sativum* var. abyssinicum [28], maize [29], hot pepper [30], barley [31]. However, the six cultivars showed different response (Table 1). Cultivar Locale exhibited a germination index of 6.43; 5.40; 3.75 and 3.30; Srem2-Sat2 6.88; 5.15; 4.23 and 4.03; Rouge 6.50; 5.48; 3.83 and 3.30 respectively at 30; 60; 90 and 120 mM of NaCl. For these three cultivars, the reduction of germination index due to NaCl was high and significant (P =0.001) from 30 mM NaCl (Table 1). For cultivars Red Sudan

and AA-04-017, germination index were respectively 6.33; 5.19; 4.33; 2.65 and 6.2; 5; 4.05; 3.73 respectively at 30; 60; 90 and 120 mM of NaCl. The reduction of germination index due to NaCl for these two cultivars was low and significant only from 60 mM NaCl (P =0.001). For cultivars AA-04-028, the germination index were 6.45; 5.28; 3.88; 3.65 respectively at 30; 60; 90 and 120 mM of NaCl. For this cultivar, the reduction of germination index due to NaCl was intermediary and significant from 30 mM NaCl (P =0.001). Thus, cultivar Red Sudan and AA-04-017 were less affected by NaCl in comparison to the five other cultivars used; these cultivars appeared as the most salt resistant among the six cultivars evaluated based on germination index. Cultivars Locale appeared as the most salt sensitive based on this variable. Using germination index, [28] have distinguished the more salt sensitive *Pisum sativum* var. abyssinicum from the less salt sensitive *Lathyrus sativus.*

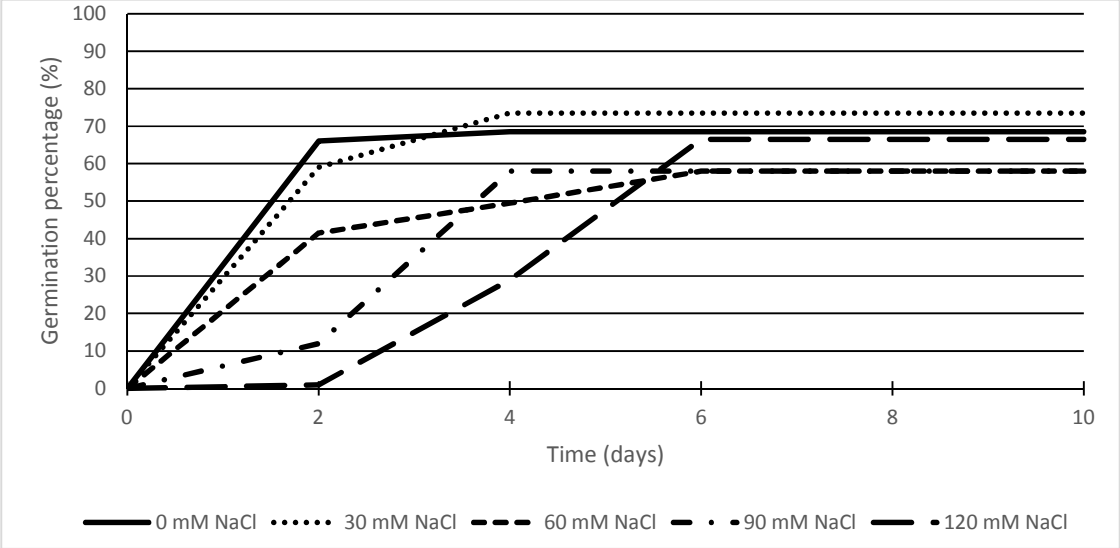

Fig. 3. Rate of germination of amaranth seeds under saline conditions for cultivar Red-Sudan

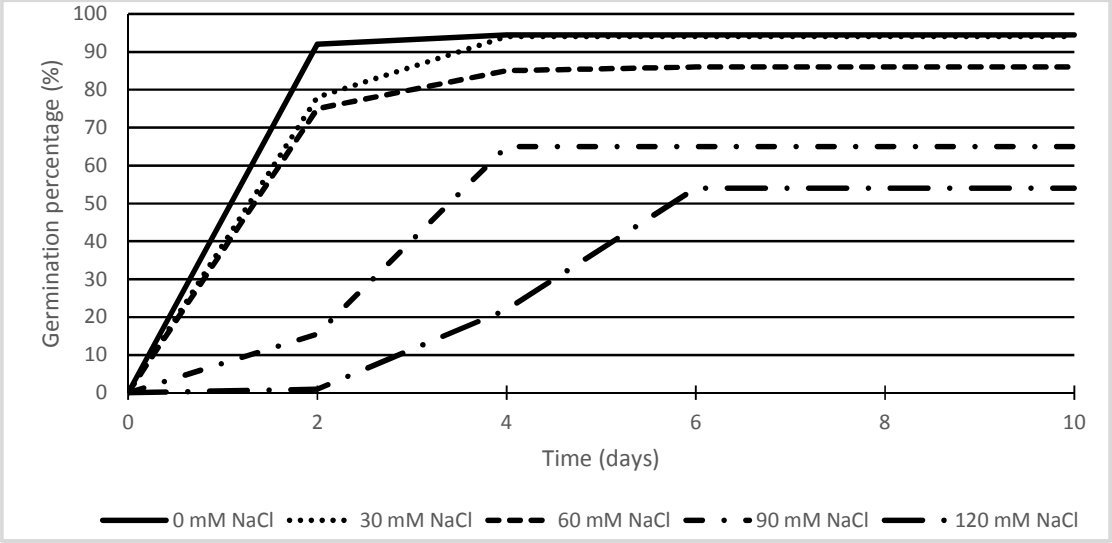

Fig. 4. Rate of germination of amaranth seeds under saline conditions for cultivar AA-04-028

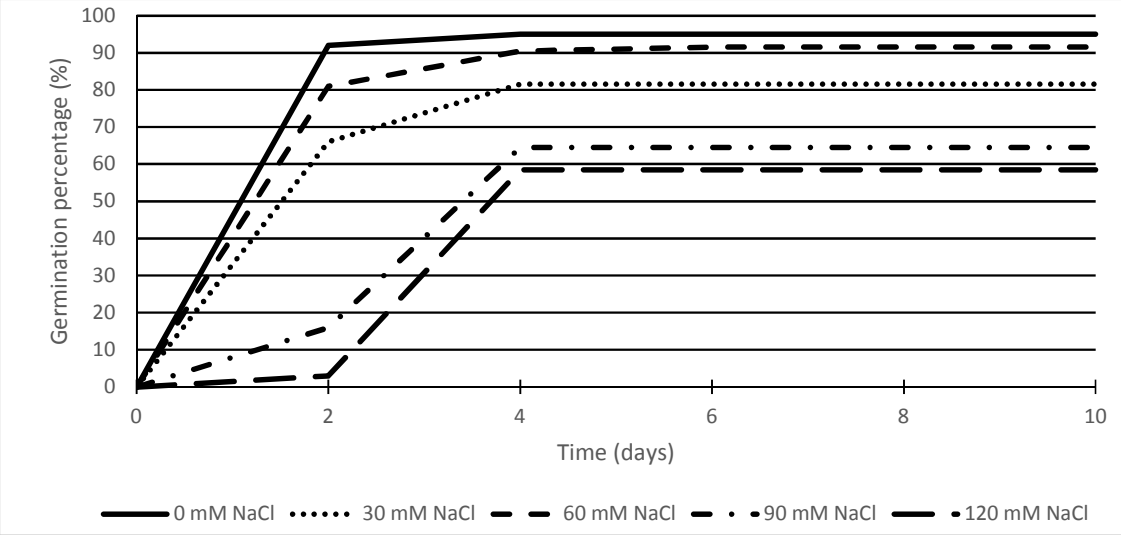

Fig. 5. Rate of germination of amaranth seeds under saline conditions for cultivar AA-04-017

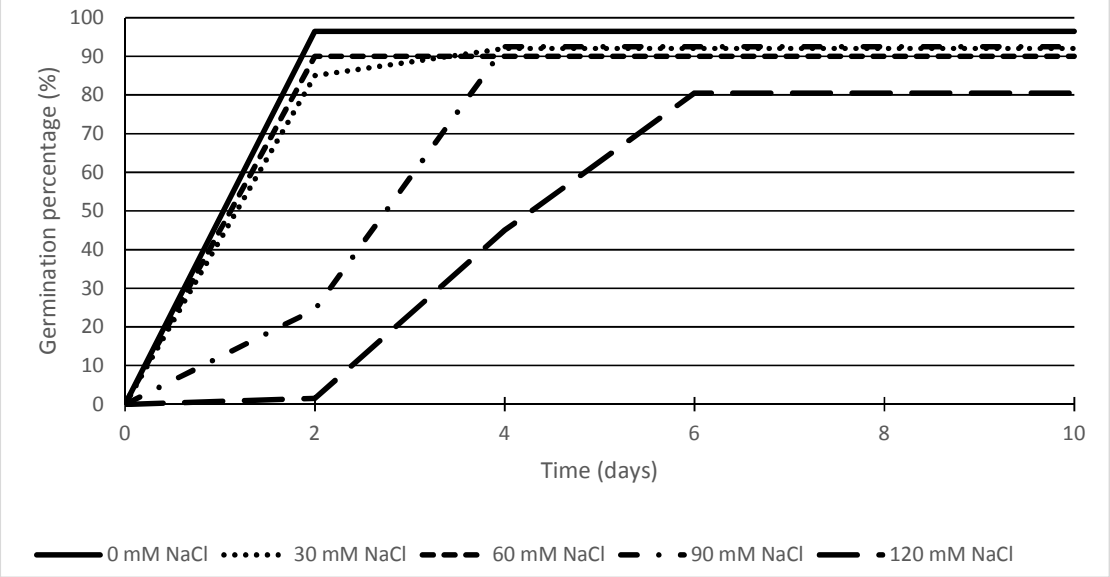

Fig. 6. Rate of germination of amaranth seeds under saline conditions for cultivar Stem2-Sat2

3.3 Effect of Sodium Chloride on Final Germination Rate

In the absence of stress, the germination percentages after 10 days were 96.5%; 95.5%; 95; 5%; 95; 94.5% and 68.5% respectively for cultivars Stem2-Sat2; Locale; Rouge; AA-04-017; AA-04-028 and Red Sudan. Red-Sudan presented the lowest germination percentage (Fig. 7). Sodium chloride (NaCl) effect resulted globally in a reduction of the final germination percentage but the six varieties showed different response (Fig. 7). Cultivar AA-04-017 exhibited a germination percentage of 96.31; 85.79; 67.89 and 61.58%; AA-04-028 94; 86; 65 and 54% at 30; 60; 90 and 120 mM of NaCl respectively, reduction significant from 30 mM NaCl (P =0.001) for AA-04-017 and from 90 mM NaCl for AA-04-028. For cultivar Stem2-Sat2, germination percentages were 92.5; 92; 90 and 80.5% at 30; 60; 90 and 120 mM of NaCl respectively; the reduction was significant only at 120 mM NaCl (P =0.05). For cultivars rouge and Red-Sudan, a slight (non significant) stimulation

of germination was observed at 30 mM NaCl followed by non significant reduction for Red-Sudan (58; 58 and 66.5%, respectively at 60; 90 and 120 mM NaCl) and a significant reduction (*P* =0.001) for Rouge from 60 mM NaCl. For cultivar Locale, the reduction of germination percentage was significant (*P* =0.001) from 90 mM NaCl (93; 92.5; 75.5 and 61.5% respectively at 30; 60; 90 and 120 mM of NaCl). Thus, cultivars Red-Sudan was not significantly affected by salt concentrations used; this cultivar appeared as the most salt resistant among the six cultivars evaluated followed by Stem2-Sat2. Cultivars AA-04-017 and Rouge were the most salt sensitive; cultivars Locale and AA-04-028 showed an intermediate reaction.

Table 1. Germination index of six amaranth cultivars (Stem2-Sat2, Locale AA-04-028; AA-04-017 and Red-Sudan) as affected by different concentrations of NaCl

NaCl (mM)	Cultivars					
	Red-Sudan	Stem2-Sat2	Locale	Rouge	AA-04-028	AA-04-017
0	7.65±0,15a	9.05±0,28a	9.28±0,25a	8.85±0,32a	8.15±0,29a	7.2 ±0,14a
30	6.33±0,07ab	6.88±0,07b	6.43±0,05b	6.5±0,06b	6.45±0,03b	6.23±0,13a
60	5.19±0,14bc	5.35±0,08c	5.40±0,08c	5.48±0,1c	5.28±0,07c	5.0±0,07b
90	4.33±0,62c	4.15±0,16d	3.75±0,06d	3.83±0,02d	3.88±0,06d	4.05±0,13bc
120	2.65±0.89c	4.03±0,61d	3.30±0 e	3.30±0e	3.65±0, 2d	3.73±0,42c

Values are means±SE (n = 4). Means with different letters within a column were significantly different (P = 0.001)

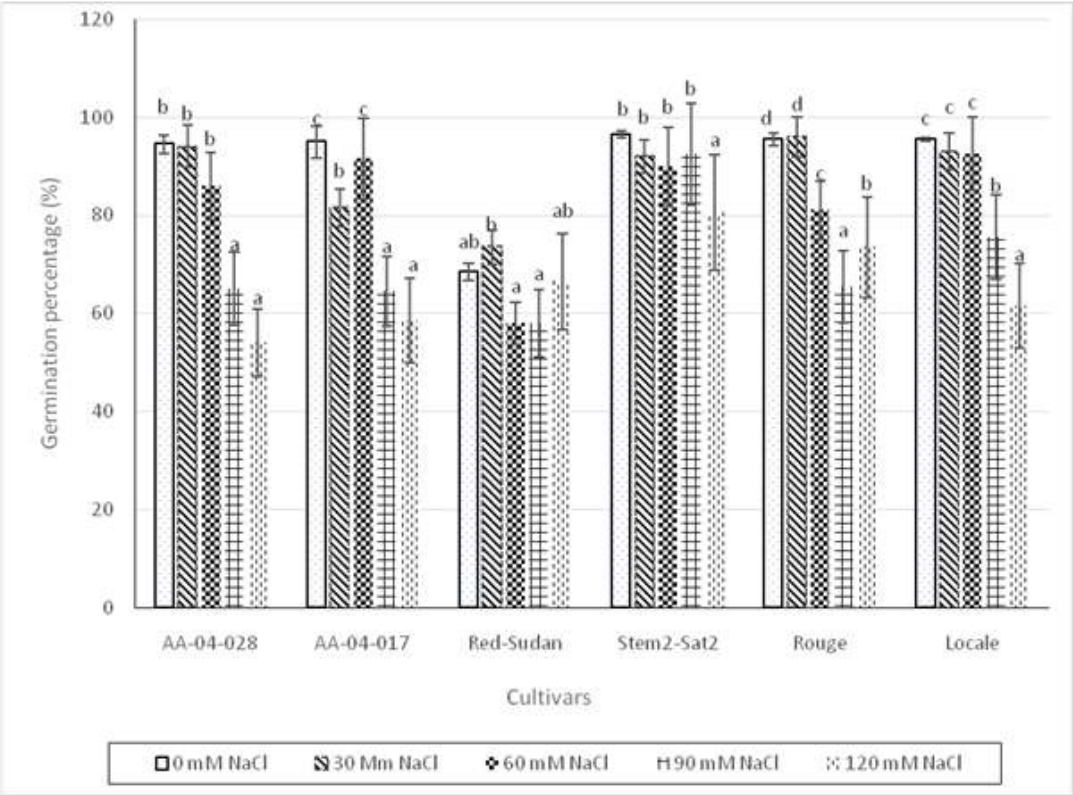

Fig. 7. Effect of different concentrations of NaCl salinity on final germination percentage of six amaranth cultivars (cultivars: AA-04-028 AA-04-017; Red-Sudan; Stem2-Sat2; Rouge and Locale)

Vertical bars are standard error of means of four replications; Means with different letters were significantly different (*P* = 0.001)

Table 2. Germination percentage of six amaranth cultivars (Stem2-Sat2, Locale AA-04-028; AA-04-017 and Red-Sudan) as affected by average effect of NaCl

Parameters		Cultivars					
		Red-Sudan	Stem2-Sat2	Locale	Rouge	AA-04-028	AA-04-017
Germination rate (%)	0 NaCl	100	100	100	100	100	100
	+ NaCl	93.43	91.97	84.42	82.72	79.10	77.89

Salinity induced a reduction in the percentage of final germination (after 10 days) in the cultivars tested except for cultivars Red-Sudan and Rouge at 30 mM NaCl. The reduction of final germination percentage by NaCl has been reported in durum wheat [9], sugar beet [32], cabbage [10]; barley [33], sugar cane [24] and various genotypes of amaranth [10,23,34]. In the recent study, [35] have reported that the lower NaCl concentration treatments (30 mM) promoted seed germination of amaranth. Apparently, it is not the case for all the six cultivars used in our study. Their conclusion was probably due to the fact that they just used one cultivar in their study. Generally, final germination percentage was more significantly affected at the higher NaCl concentrations corroborating the report of [35]. The reduction was significant and more accentuated in cultivars Rouge and AA-04-017 followed by Locale and AA-04-028 in comparison with cultivars Stem2-Sat2 and Red-Sudan indicating a variability in the response of the six cultivars tested to salt stress. The same trend has been reported in several species including rice [16]; durum wheat [9]; sugar beet [32]; barley [33] and sugar cane [24]. On the basis of our results related to germination rate, cultivars Red-Sudan and Stem2-Sat2 appeared to be more salt resistant at germination stage using final germination rate than AA-04-017; cultivars AA-04-028; Locale and Rouge were intermediary. Among the five *Amaranthus cruentus* cultivars, Red-Sudan appeared as the most salt resistant cultivar.

For a comparison of cultivars on the basis of their germination rate in the presence of NaCl, the average percentage of germination in the presence of NaCl was calculated as the average of the four values obtained in the presence of the four NaCl concentrations (30; 60; 90 and 120 mM) (Table 2).

0 NaCl, control; + NaCl, presence of NaCl: data in presence of NaCl were expressed as the average of the four values obtained in the presence of the four NaCl concentrations (30; 60. 90 and 120 mM) expressed in percentage of that of control

The reduction of germination percentage due to the average effect of salt stress was lower for cultivars Red Sudan (6.57%) and Stem2 Sat2 (8.03%) and higher for cultivars AA-04-017 (22.11%) and AA-04-028 (20.90%). This reduction was intermediary for cultivars Locale (15.58%) and Rouge (17.28%).

Globally Red Sudan appeared as the most salt resistant cultivar at germination stage whatever the variable taking into account whereas the response of stem2Sta2 and AA-04-017 depends on the considered variable. Thus, the salt resistance of amaranth cultivars at germination stage depends on the variable taking into account.

4. CONCLUSION

This study indicated that NaCl salt stress delayed seed germination and reduced germination index and the percentage of final germination in amaranth, particularly in *Amaranthus cruentus* cultivars. It underlined for the first time the variability of relative salt-stress resistance for some *Amaranthus cruentus* cultivars at germination stage according to the variable taking into account. Among the six cultivars tested, cultivar Red-Sudan was the most salt resistant at germination stage whatever the variable considered.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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