



Response of Chili Pepper (*Capsicum spp.*) Cultivars Cultivated in Benin to Salt Stress at Germination Stage

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Authors' contributions

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

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ABSTRACT

Aims: In this study, salt resistance level of six chili (*Capsicum spp.*) cultivars including five local cultivars (Adologbo, Gbatakin, Pili-pili, Gbatakin d'Agbédranfo, TPS0251) and one imported variety (Démon) grown in Benin was evaluated at the germination stage.

Study Design: The experiment was laid out as a completely randomized design with four replications.

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Place and Duration of Study: The experiment was carried out in the Laboratory of Plant Physiology and Abiotic Stresses Study of University of Abomey-Calavi, Republic of Benin from September through October, 2016.

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 sixteen days incubation period. Four replicates of 40 seeds each were used.

Results: From day 2 to day 16, NaCl delayed seed germination rate proportionately to NaCl concentration except for cultivars TPS0251 and Démon. At the end of the 16 days, NaCl stress effects on seed germination of cultivars were significantly variable. No significant reduction was observed for cultivars Démon and TPS0251 whereas a significant decrease was observed for the four other cultivars with a significant difference among them. The average reduction due to NaCl stress was lower for cultivars Démon (0%) and TPS0251 (4.31%) and higher for cultivar Pili-pili (63.61%). Salt Tolerance Index was significantly variable according to the cultivar with the highest values for cultivar Démon (1.227) and TPS0251 (1.127) and the weakest values for cultivars Pili-pili (0.374) and Gbatakin d'Agbédranfo (0.46).

Conclusion: NaCl stress delayed seed germination and reduced the rate of final germination. Salt Tolerance Index was variable among the six cultivars: cultivars Démon and TPS0251 appeared to be the most salt resistant whereas Pili-pili and Gbatakin d'Agbédranfo appeared as the most salt sensitive at germination stage. For the first time, we demonstrated a variability of relative salinity resistance among local chili pepper cultivars at germination stage.

Keywords: *Capsicum* spp.; cultivars; germination; salt tolerance index; salt-resistance.

1. INTRODUCTION

Soil salinity is the major stress that adversely affects the overall metabolism of plant [1] and leads to agricultural land deterioration and production reduction [2]. There are large number of studies that deal with the effects of salinity on germination, seedling growth, plant growth and fruit yield in various crops [3-5]. Plant growth is affected by salinity at all stages of development, but sensitivity varies greatly at different stages [6-8]. Germination is a critical stage in the growth cycle of plant species; it determines plant establishment and final crop production [9]. Thus, the ability of plant seeds to germinate at high salt concentration in the soil is therefore very crucial [10]. Increasing salinity generally reduces germination of glycophytes [11,8,12,13] and the response is concentration dependent and also specific on species [14-17]. It has been reported that there is a substantial variation in salt sensitivity among cultivars of the same species [6,18,19]. Chili (*Capsicum annuum* L.) is a spice, a fruit vegetable widely grown in the world as it is very important in human food [20,21]. Pepper (*Capsicum annuum* L.) is one of the three important solanaceous vegetable crops grown for their fruits, which are consumed, either fresh or dried [22]. Chili pepper belongs to the crops grown throughout the world for their nutraceutical (nutritional and medicinal) and economic value [23]. In Benin, chili is the second market gardening crop besides tomato [24]. Its annual

production is about 47.162 tons and has never evolved over the last 10 years [24]. Moreover, except for research works on natural foes conducted in different production zones and the few agronomic assessment tests conducted on certain varieties [24], no other significant study has been conducted on chili in Benin. It is classified as moderately sensitive to salinity [25], and some adverse effects of salinity on this species have been reported [26-34]. In Benin, chili pepper is grown only for food [35] partially in the cultivable lands of the coastal areas where soil salinity and water irrigation constitute a reality. It was reported that salt stress decreases the kinetic and the final percentage of germination in chili [30]. However, despite a substantial amount of literature on responses of plants to salinity stress, little information is available on salt resistance of chili cultivars produced in Benin. Indeed, the present study aims to evaluate NaCl stress effects on seed germination of six chili 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 chili cultivars, produced in Benin were used including five local cultivars (Adologbo, Gbatakin, Pili-pili, Gbatakin d'Agbédranfo) and one imported cultivar (Démon). Local cultivars were

obtained from the Market Gardening Crops Program of the Benin National Institute for Agricultural Research (INRAB) whereas the imported variety 'Demon' was bought at 'Bénin Semences' a company that trades seeds.

2.1.1 NaCl effect on seed germination

Seeds were incubated in 10 cm Petri dishes on one layer of filter paper moistened with 15 ml of water solutions of 0–120 mM NaCl at 30 mM intervals. Seed germination was checked every day during 16-day incubation in water and/or NaCl. Germination percentage was determined within 16 days incubation. The experiment was laid out as a completely randomized design with four replications (40 seeds each). The seeds were incubated in darkness at 26°C. A seed was considered as germinated when the radicle emerged from the seed coat according to Wouyou et al. [36].

Salt effect on seed germination was evaluated using germination kinetics and final germination rate. Cultivars were distinguished according to their salt resistance using two parameters:

- the average effect of NaCl defined as $100 \times [(FGP_{30} + FGP_{60} + FGP_{90} + FGP_{120})/FGP_0]/4$ with FGP the final germination percentage at each NaCl concentration used (0; 30; 60; 90 and 120 mM)
- Salt Tolerance Index (STI) calculated according to Baheri et al. [37].

2.2 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 "JMP Pro 12" software [38].

3. RESULTS AND DISCUSSION

3.1 Effect of Salt Stress 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, 10, 12, 14 and 16 days in the presence of NaCl at 0, 30, 60, 90 and 120 mM, respectively for cultivars Adologbo, Gbatakin, Pili-pili, TPS0251, Gbatakin

d'Agbédranfo and Démon. In absence of stress, the reaction of varieties were different: after 2 days, no seed of cultivars Adologbo, Pili-pili, TPS0251; Gbatakin d'Agbédranfo and Démon germinated whereas 12.5% of seeds germinated for cultivar 'Gbatakin'. After 4 days, germination started for the five cultivars and the percentages of seed germination were about 10.62%; 23.75%; 15.62%; 6.25%; 6.87% and 1.25%, respectively, for Adologbo; Gbatakin; Pili-pili; TPS0251; Gbatakin d'Agbédranfo and Démon. Thus, the germination started from the 2nd day or from 4 days in the absence of stress according to the cultivar. In a previous study, Sikha [39] reported that seed germination started in control at the 6th day for one pepper cultivar. This observation indicated that the germination start time depends on the cultivar. After 6 days, the percentages of seed germination were about 31.87%; 53.12%; 43.12%; 58.12%; 29.37% and 55.62% respectively, for Adologbo; Gbatakin; Pili-pili; TPS0251; Gbatakin d'Agbédranfo and Démon. After 8 days, the percentage of seed germination was 63.75% for Adologbo; 70% for Gbatakin and Pili-pili; 89.37% for TPS0251; 51.87% for Gbatakin d'Agbédranfo and 100% for Démon. After 10 days, the percentage of seed germination was 84.37% for Adologbo; 78.75% for Gbatakin; 86.25% for Pili-pili; 71.87% for Gbatakin d'Agbédranfo; 95% for TPS0251 and 100% for Démon. After 12 days, the percentages of seed germination were 91.25%; 84.37%; 91.87%; 96.87%; 76.87% and 100% respectively for Adologbo; Gbatakin; Pili-pili; TPS0251; Gbatakin d'Agbédranfo and Démon. Except for TPS0251, no progress was observed in the rate of seed germination of control for the cultivars after 12 days (from 12 days to the end of the experiment) and for variety 'Démon' germination rate becomes constant from the 8th day. In one pepper cultivar, Sikha et al. [39] reported that germination rate becomes constant from the 18th day indicating that this parameter in variable according to the cultivar.

NaCl stress effect resulted in a variable response according to the cultivar and the time (Figs. 1 to 6). A regular reduction was observed for cultivar Gbatakin from 2nd day. Thus, the percentage of seed germination after 2 days shifts from 12.5% in absence of NaCl, to 10%; 4.37%; 0% and 0%, respectively at 30, 60, 90 and 120 mM of NaCl. Similar result was observed from the 4th day to the end of the experiment but these reductions were not significant. A regular reduction was observed for cultivars Pili-pili and Gbatakin d'Agbédranfo from 4th day. For cultivar

Pili-pili, the percentage of seed germination after 4 days shifts from 12.5% in absence of NaCl, to 10% ; 4,37% ; 0% and 0% respectively at 30, 60, 90 and 120 mM of NaCl while for cultivar Gbatakin d'Agbédranfo, the percentage of seed germination after 4 days shifts from 12.5% in absence of NaCl, to 10% ; 4,37% ; 0% and 0% at the same NaCl concentrations. The same trend was observed for the other times in both cultivars. The reductions observed were significant for both cultivars from 30 mM NaCl. For cultivar Adologbo cultivar, the reduction was visible until the 8th day mainly for the highest NaCl concentrations used ranging from 63.75% to 54.37% at 90 mM NaCl but these reductions were not significant. For cultivar Démon, a reduction was observed only between the 6th and the 8th day with no effect after. The germination percentage decreased from 100% to 97.5% ; 89.37% ; 92.5% and 91.25% at the 8th day respectively at 30, 60, 90 and 120 mM of NaCl. The reductions were not significant. For cultivar TPS0251, a stimulation of germination percentage was observed between the 2nd and the 8th day increasing from 89.37% to 96.87% ; 92.5% and 91.25% at the 8th day respectively at 30, 60 and 90 mM of NaCl, followed by a slight decrease after the 10th day. The changes observed were not significant. These observations indicated that salt stress generally delays seed germination mainly for cultivars

Gbatakin, Pili-pili and Gbatakin d'Agbédranfo. Similar results were reported in chili pepper [40].

The maximum seed germination percentage was obtained at the 12th day for all cultivars and NaCl concentrations except for Démon for which the maximum of seed germination percentage was obtained after 8 days for all NaCl concentrations used. This result indicates that NaCl effect on chili seeds final germination rate couldn't be studied before 12 days of stress. In three other chili pepper cultivars, Aloui et al. [41] studied NaCl effect on final germination after 14 days corroborating our findings. Similar trend was reported by Sikha et al. [39].

3.2 Effect of Sodium Chloride on Final Germination Percentage

In the absence of stress, the germination percentages after 16 days were 96.20% ; 84.40% ; 93.1% ; 98.12% ; 81.9% and 100% respectively for cultivars Adologbo ; Gbatakin ; Pili-pili ; TPS0251 ; Gbatakin d'Agbédranfo and Démon. Pili-pili presented the lowest germination percentage whereas Démon presented the highest germination percentage (Fig. 7). The cultivars tested in this study present different capacities of seed germination after 16 days in absence of salt stress. Similar trend was observed in different genotypes of chili [40].

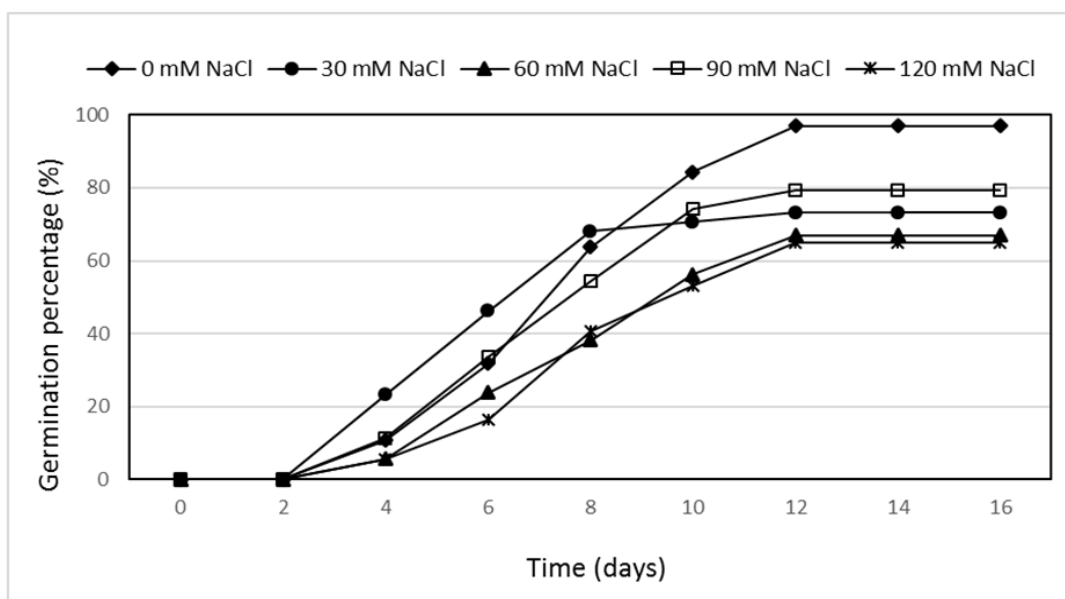


Fig. 1. Rate of germination of chili seeds under saline conditions for cultivar Adologbo

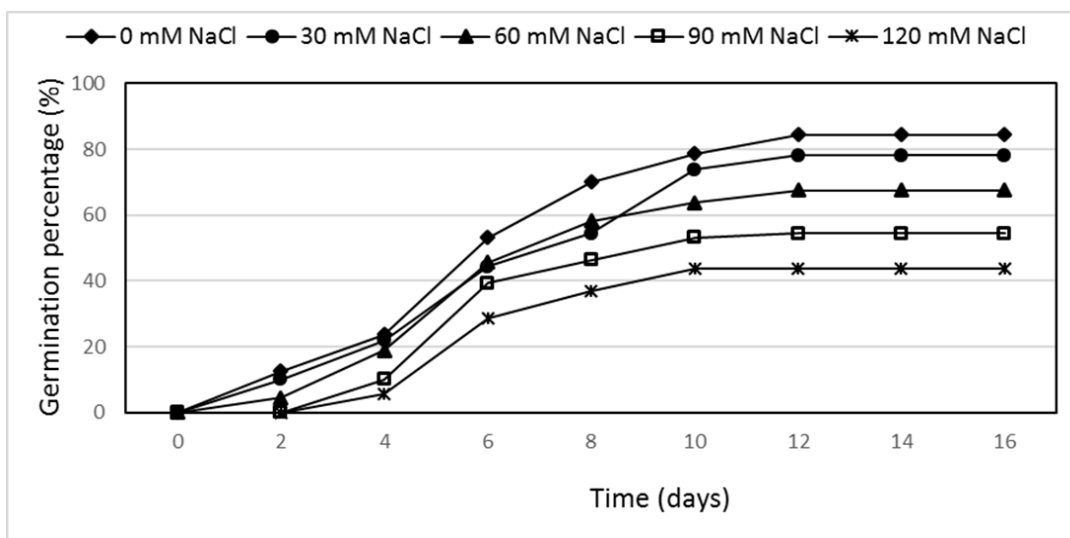


Fig. 2. Rate of germination of chili seeds under saline conditions for cultivar Gbatakin

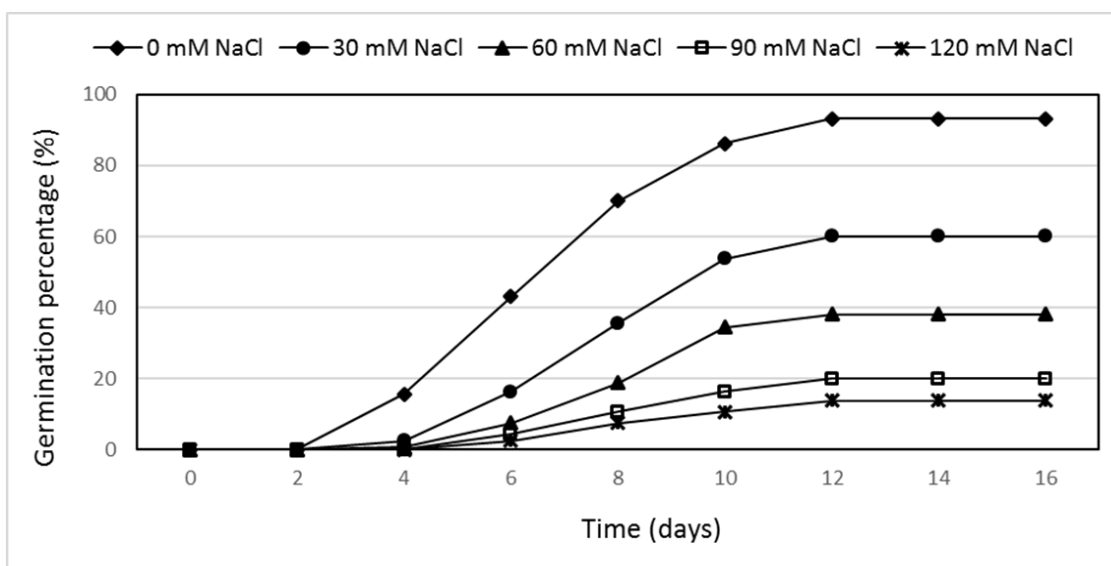


Fig. 3. Rate of germination of chili seeds under saline conditions for cultivar Pili-pili

Sodium chloride (NaCl) effect resulted generally in a reduction of the final germination percentage but the six cultivars showed different reactions (Fig. 7). No reduction of germination percentage was observed for cultivar Démon with a germination percentage of 100% at all NaCl concentrations used. Cultivar Adologbo exhibited a germination percentage of 73.12% ; 64.37% ; 74.37% and 68.75% at 30; 60; 90 and 120 mM of NaCl respectively, reduction significant only at 60 mM NaCl ($P = .05$). Cultivar Gbatakin showed a germination percentage of 78.12% ; 67.5% ;

54.37% and 43.75% at 30; 60; 90 and 120 mM of NaCl respectively, reduction significant from 60 mM NaCl ($P = .001$). For cultivar Pili-pili final germination percentage were 61.25% ; 38.75% ; 20% and 13.75% at 30; 60; 90 and 120 mM of NaCl respectively; the reduction observed was significant from 30 mM NaCl ($P = .001$). For cultivar TPS0251, final germination percentage were 97.5% ; 93.75% ; 93.12% and 91.25% at 30; 60; 90 and 120 mM of NaCl respectively; the reductions were not significant. For cultivar Gbatakin d'Agbédranfo, germination percentages

were 58.75%; 57.50%; 42.50% and 38.12% at 30; 60; 90 and 120 mM of NaCl respectively; the reductions were significant from 30 mM NaCl ($P = .001$). Thus, NaCl effect resulted in the reduction of the final germination percentages with a significant difference among cultivars:

cultivars Démon and TPS0251 were less affected followed by Adologbo in comparison with the three other cultivars. Thus, salinity induced a significant reduction in the percentage of final germination (after 16 days) in the cultivars tested except for cultivars Démon and TPS0251.

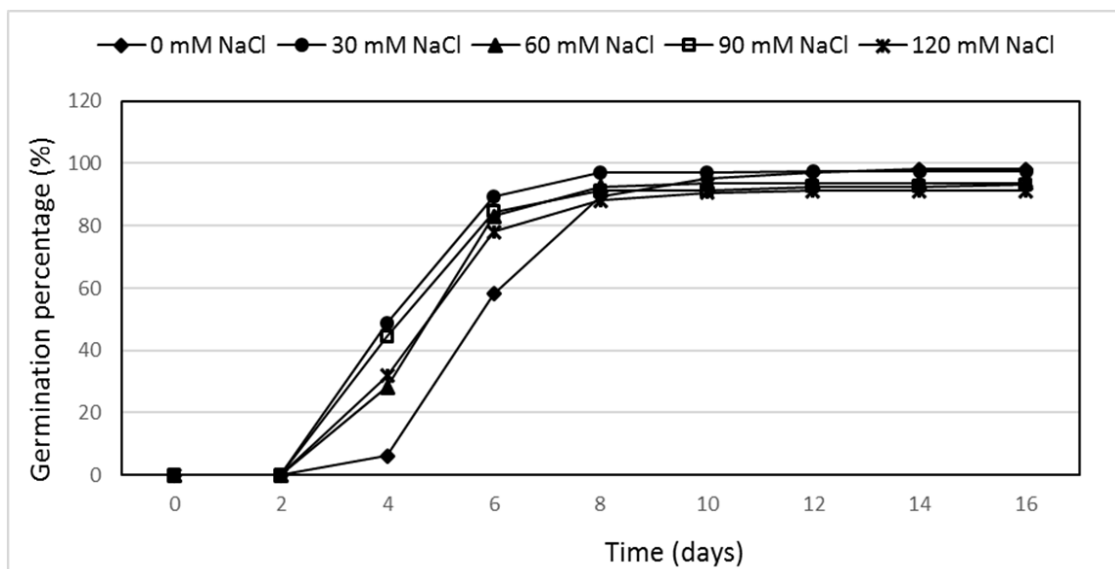


Fig. 4. Rate of germination of chili seeds under saline conditions for cultivar TPS0251

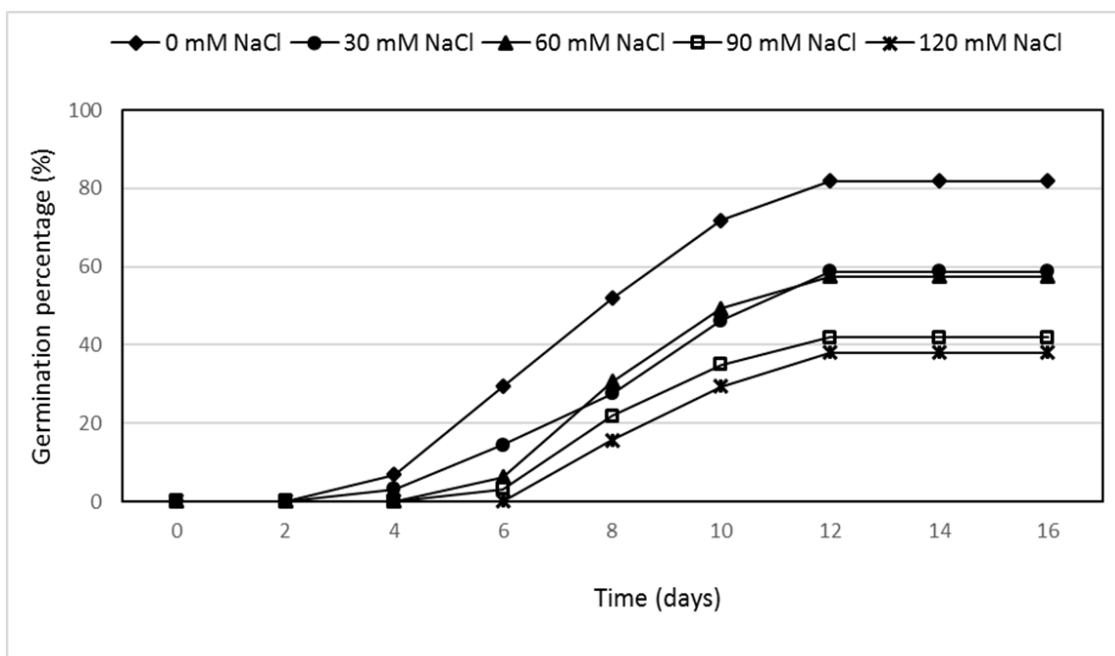


Fig. 5. Rate of germination of chili seeds under saline conditions for cultivar Gbatakin d'Agbédranfo

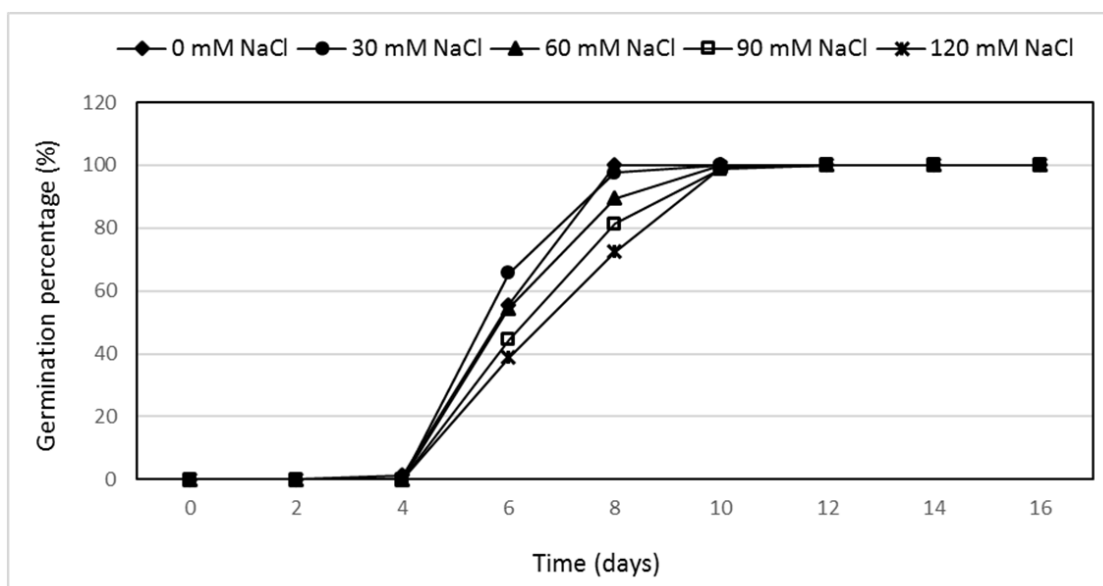


Fig. 6. Rate of germination of chili seeds under saline conditions for cultivar Démon

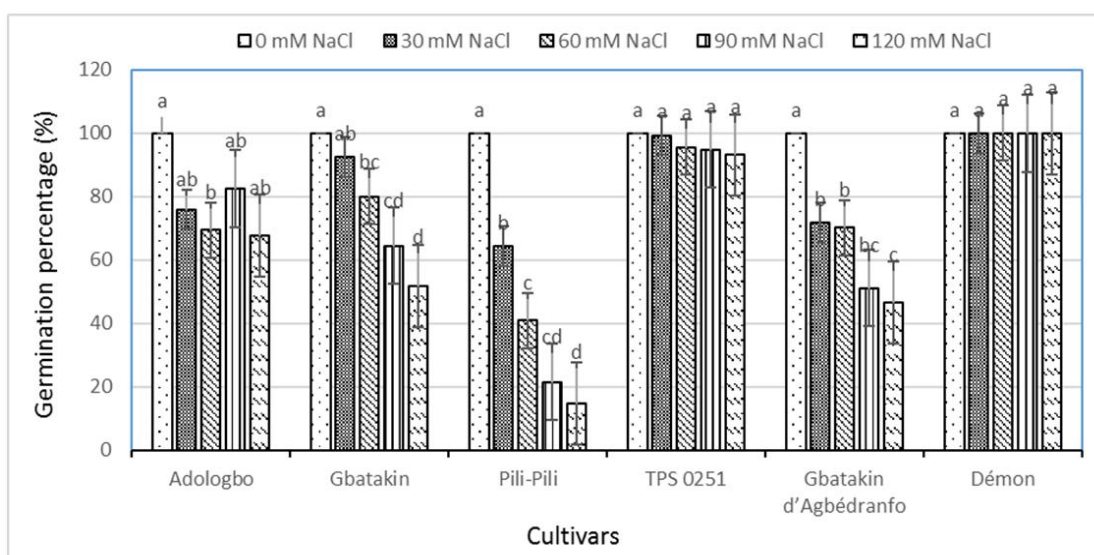


Fig. 7. Effect of different concentrations of NaCl salinity on final germination percentage of six chili cultivars (Adologbo; Gbatakin; Pili-pili; TPS0251; Gbatakin d'Agbédranfo and Démon)
 Vertical bars are standard error of means of four replications. Averages followed by the same letter do not differ by the SNK test ($P = .05$ or $.001$)

The reduction of final germination percentage by NaCl has been reported in several species including cabbage [12]; barley [42], sugar cane [43] and amaranth [12,36]. In three chili pepper cultivars, Aloui et al. [41] reported a reduction of final germination percentage by increasing NaCl concentrations after 14 days. Similar findings were reported in other pepper cultivars after 10

days [44] and 15 days [10]. However, according to Smith and Cobb [45], the priming of one chili pepper cultivars' seeds with NaCl concentrations ranging from 10 to 300 mM did not significant affect the final germination percentage. Generally, final germination percentage was more significantly affected at the higher NaCl concentrations corroborating the report of

Table 1. Germination percentage of six pepper cultivars Adologbo, Gbatakin, Pili-pili, TPS 0251, Gbatakin d'Agbédranfo and Démon as affected by average effect of NaCl

Parameters	Cultivars						
		Adologbo	Gbatakin	Pili-pili	TPS 0251	Gbatakin d'Agbédranfo	Démon
Germination rate (%)	0 NaCl	100	100	100	100	100	100
	+ NaCl	76.88	72.22	36.39	95.69	64.02	100

Table 2. Salt tolerance Index of six chili cultivars (Adologbo; Gbatakin; Pili-pili; TPS0251; Gbatakin d'Agbédranfo and Démon) at germination stage under five NaCl concentrations (0; 30; 60. 90 and 120 mM)

Cultivars	Démon	TPS0251	Adologbo	Gbatakin	Gbatakin d'Agbédranfo	Pili-pili
STI	1,227±0,003 ^a	1,127±0,016 ^a	0,782±0,033 ^b	0,629±0,038 ^{bc}	0,460±0,27 ^c	0,374±0,057 ^{de}

Values are means ± standard errors. Averages followed by the same letter do not differ by the SNK test ($P = .001$).

Aloui et al. [41]. The reduction was significant and more accentuated in cultivar Pili pili followed by Gbatakin d'Agbédranfo, Gbatakin and Adologbo in comparison with cultivars Démon and TPS0251 indicating a variability in the response of the six cultivars tested to salt stress as reported in over chili pepper cultivars [40]. The same trend has been reported in several species including rice [18]; durum wheat [11]; sugar beet [46] and amaranth [36].

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 1) according to [36,43]. The reduction of germination percentage due to the average effect of salt stress was lower for cultivars Démon (0%) and TPS0251 (4.31%) and higher for cultivar Pili-pili (63.61%). This reduction was average for cultivars Adologbo (23.12%), Gbatakin (27.78%) and Gbatakin d'Agbédranfo (35.98%).

3.3 Salt Tolerance Index (STI) of the Chili Cultivars

Salt Tolerance Index was significantly variable according to the cultivar ranging from 0.374 for Pili-pili to 1.227 for Démon (Table 2). STI were about 1.127; 0.782; 0.629 and 0.460 for cultivars TPS0251; Adologbo; Gbatakin and Gbatakin d'Agbédranfo. Thus, cultivar Démon presented the highest Salt Tolerance Index followed by TPS0251 whereas cultivars Pili-pili' and Gbatakin d'Agbédranfo presented the weakest Salt Tolerance Index.

Generally, salinity reduced significantly the percentage of final germination and the reduction was significant and more accentuated in cultivar Pili-pili followed by Gbatakin d'Agbédranfo, Gbatakin and Adologbo in comparison with cultivars Démon and TPS0251.

4. CONCLUSION

This study showed that NaCl salt stress delayed seed germination and reduced the percentage of final germination in chili pepper cultivars. It underlined the variability of relative salt-stress resistance for some chili pepper cultivars at germination stage including five local cultivars. Among the six cultivars tested, cultivars Démon and TPS0251 were the most salt resistant at germination stage whereas Pili-pili and Gbatakin d'Agbédranfo appeared as the most salt sensitive. For the first time, we showcased a variability of salt resistance among local chili cultivars at germination stage with cultivar TPS0251 as the most salt resistant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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