



## **Salt Resistance of Tomato (*Lycopersicon esculentum* Mill.) Cultivars Produced in Benin at Germination Stage**

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### **Authors' contributions**

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

### **Article Information**

DOI: 10.9734/IJPSS/2019/v28i230102

#### Editor(s):

(1) Dr. Davide Neri, Professor, Polytechnic University of Marche - Via Brece White, Ancona, Italy.

#### Reviewers:

(1) Schirley Costalonga, Universidade Federal do Espírito Santo, Brazil.

(2) Temegne Nono Carine, University of Yaoundé I, Cameroon.

(3) Dr. R. Mahalakshmi, India.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/49171>

**Original Research Article**

**Received 04 March 2019**

**Accepted 15 May 2019**

**Published 22 May 2019**

### **ABSTRACT**

**Aims:** In this research study, salt resistance level of seven tomato cultivars grown in Benin, namely Akikon, Tounvi; F1 Mongal, Petomech, Padma, TLCV 15 and Thorgal was evaluated at the germination stage.

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

**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 May to June, 2017.

**Methodology:** Seeds were submitted to treatment with four NaCl concentrations (0; 30; 60 and 90 mM NaCl) in Petri dishes. Seed germination was checked every day during ten days incubation period. Four replicates of 40 seeds each were used.

**Results:** NaCl reduced seed germination rate in all cultivars from day 2 to day 10 and the germination index proportionately to NaCl concentration. At the end of the 10 days, salt stress reduced the final germination percentages with a significant difference among cultivars: cultivars F1 Mongal followed by Akikon, Thorgal, TLCV15 and Tounvi were less affected in comparison with the two other cultivars. Salt Tolerance Index was significantly variable according to the cultivar with the highest values for cultivars F1 Mongal (1.086), Akikon (1.028), TLCV15 (1.005) and Tounvi (0.989) and the weakest value for cultivar Petomech (0.436).

**Conclusion:** NaCl stress delayed seed germination and reduced the rate of final germination. Salt Tolerance Index was variable among the seven cultivars. Based on this criterion, cultivars F1 Mongal, Akikon, TLCV15 and Tounvi were the most salt-resistant whereas Petomech was the most salt-sensitive at germination stage.

*Keywords: Germination; local cultivars; salinity; salt tolerance index; tomato.*

## 1. INTRODUCTION

Soil salinity is the major stress that reduced plant productivity mainly in arid and semi-arid climates [1]. Plant growth is affected by salinity at all stages of development, but sensitivity varies greatly at different stages [2-5]. Germination is a critical stage in the growth cycle of plant species which determines plant establishment and final crop production [6]. Thus, the ability of plant seeds to germinate at high salt concentration in the soil is therefore very crucial [7]. Increasing salinity generally reduces germination of glycophytes [4,8-10] and the response is concentration dependent and also specific on species [11-14]. It has been reported that there is a substantial variation in salt sensitivity among cultivars of the same species [2,5,15,16]. Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable plants in the world. It is consumed fresh, cooked or after processing: through canning, transformation into juice, pulp, paste, or a variety of sauces [17]. According to FAO [18], tomato is grown in 170 countries under various climates. Global production of tomato in 2017 was 182.3 million tons for a farmed land surface of 4.8 million hectares, an average yield of 37.6 tons per hectare [19]. The climate of Benin is favorable for its farming throughout the country. The South of Benin which provides the highest production, about 80% of the national production included the coastal zone which is most strongly affected by salinity [20]. The water used by market

gardeners located in the coastal areas for irrigation has high electrical conductivity and salinity [21]. It was reported that the salts presented in soil and irrigation water negatively affected tomato seed germination, plant growth and consequently reduced yields [22]. It is also well known that a large genetic variation of tolerance to salt level exists among tomato genotypes [23]. However, despite a substantial amount of literature on responses of tomato response to salinity stress, little information is available on salt resistance of tomato cultivars produced in Benin. This study aims to evaluate NaCl stress effects on seed germination of seven tomato varieties grown in Benin and compare the relative salt resistance level of these cultivars at the germination stage.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Seven cultivars including two local cultivars (Akikon and Tounvi) and five improved varieties (F1 Mongal, Petomech, Padma, Thorgal' and 'TLCV15) were used. Seeds of cultivars Akikon, Tounvi and TLCV15 were provided by the National Institute of Agricultural Research of Benin (INRAB) whereas those of the four over cultivars were bought from the society 'Bénin Semences'. The experiment was carried out at the Laboratory of Plant Physiology and Abiotic Stresses Study of University of Abomey-Calavi in Benin.

## 2.2 Methods

### 2.2.1 Experiment design

The experiment was laid out as a Completely Randomized Design (CRD) with four replications.

### 2.2.2 Methodology

Seeds (40) of each variety were incubated in 10 cm Petri dishes on one layer of filter paper moistened with 15 ml distilled water (check solution) and the same volume of water solutions of 30 - 90 mM NaCl at 30 mM intervals chosen according to the literature. The seeds were incubated in darkness at 26°C. Seed was considered as germinated when the radicle emerged from the seed coat. Seed germination was checked every two days during ten days incubation period.

### 2.2.3 Measurements

Physiological parameters as germination kinetic, final germination percentage and germination index were evaluated and compared among the seven tomato varieties. The means of salinity effect on cultivars were determined in relation to indicate the salinity effect of these cultivars as well as the salinity tolerance clues that were calculated with the final percentage of germination. The following parameters as germination kinetic and final germination percentage were calculated according to Gandonou et al. [24].

$$\text{Germination kinetic} = \frac{Nj_1 + Nj_2 + \dots + Nj_n}{\text{Initial total number of seeds}}$$

With

$Nj_1, Nj_2, \dots$ : Number of germinated seed in each day

$$\text{Final germination percentage} = 100 \times \frac{\text{final number of germinated seeds}}{\text{Initial number of seeds for germination}}$$

Germination index was calculated as described by [25].

$$\text{Germination index} = \frac{X_1}{Y_1} + \frac{(X_2 - X_1)}{Y_2} + \dots + \frac{(X_u - X_{u-1})}{Y_u}$$

With

$X_1, X_2, \dots, X_u$ : Germination rate in day<sub>1</sub>; day<sub>2</sub>; .....; day<sub>u</sub>

$Y_1, Y_2, \dots, Y_u$ : Number of day

Salinity Tolerance Index (STI) was calculated using formula adapted from [26] based on final germination percentage.

$ITS = (Y_s \times Y_p) / (\bar{Y}_p)^2$  with:

$Y_s$ = measure of one replication under one NaCl concentration;

$Y_p$ = measure of one replication of the control;

$\bar{Y}_p$ = means of measures of all replications of the control.

## 2.3 Statistical Analysis

Data collected were statistically analyzed using the JMP software [27]. The means of each parameter for the seven cultivars were compared using one way analysis of variance.

## 3. RESULTS AND DISCUSSION

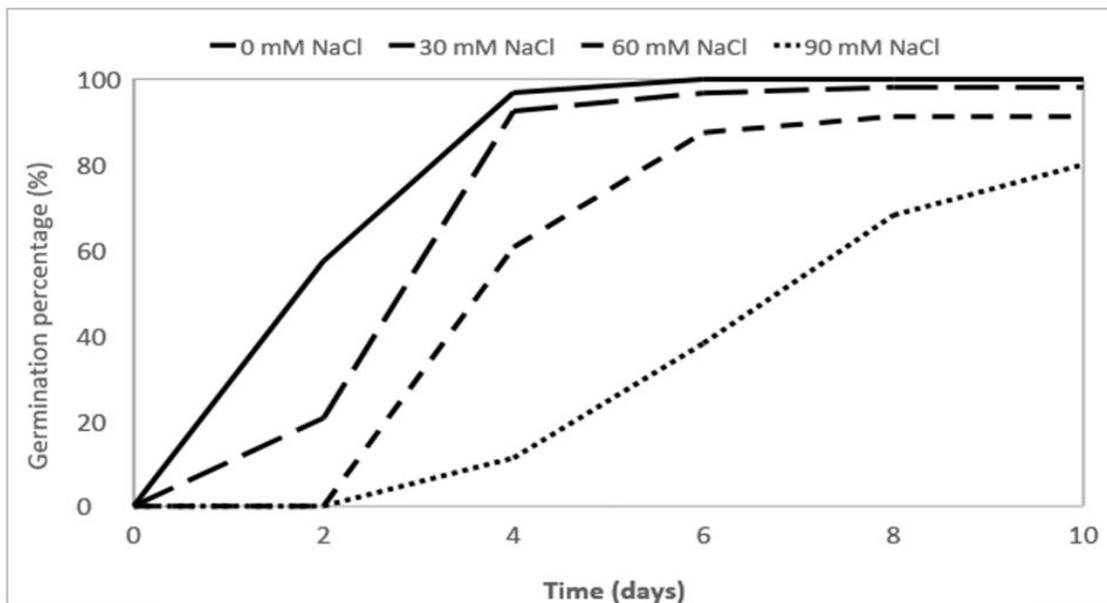
### 3.1 Effect of Salt Stress on Seed Germination Kinetics and Germination Index

Figs. 1; 2; 3; 4; 5; 6 and 7 showed respectively the effect of different concentrations of NaCl (0, 30, 60 and 90 mM) on the germination rate for cultivars Akikon, F1 Mongal, Tounvi, Petomech, Thorgal, TLCV15 and Padma after 2, 4, 6, 8 and 10 days.

In absence of stress, the reaction of varieties were different: after 2 days, more than 50% of seeds germinated for cultivars Thorgal (83.12%), TLCV15 (67.5%) and Akikon (57.40%) whereas less than 20% of seeds germinated for cultivars Padma (15.62%) and F1 Mongal (8.75%). Cultivars Tounvi (40%) and Petomech (40.62%) presented intermediate values. Thus, the seed germination started from the 2<sup>nd</sup> day in the absence of stress for all cultivars. After 4 days, germination rate increased in all cultivars and reached 96.87% for Akikon; 95% for TLCV15 and Tounvi; 93.12% for F1 Mongal; 88.75% for Thorgal; 80.62% for Padma and 60.62% for Petomech. Thus, seed germination rate was superior to 80% for all tomato cultivars after 4 days except for cv. Petomech (60.62%). After 6 days, the percentages of seed germination were 100% for F1 Mongal and Akikon; 97.5% for TLCV15; 96.87% for Tounvi; 91.25% for Thorgal; 88.75% for Padma and 69.37% for Petomech. After 6 days, no change was observed in germination rate for cultivars F1 Mongal, Akikon and Thorgal indicating that these cultivars

reached the maximum of their seed germination rate after 6 days. For the four other cultivars, seed germination rate increased slowly until the 10<sup>th</sup> day corresponding to the end of the experiment reaching 100% for cultivar Tounvi; 98.12% for TLCV15; 91.87% for Padma and 73.12% for Petomech. For three of the tested cultivars, germination rate becomes constant from the 6<sup>th</sup> day. Our results revealed a significant variability in the germination capacity of cultivars in the absence of stress. Cultivars F1 Mongal, Akikon and Tounvi showed the highest germination capacity whereas Petomech exhibited the lowest. NaCl stress effect resulted globally in a reduction of the germination rate according to the time but cultivars behaved differently (Figs. 1 to 7). A regular reduction was observed from the 2<sup>nd</sup> day for cultivars Akikon, Tounvi, Petomech and TLCV15. For cultivar F1 Mongal, a slight increase was observed only at the 2<sup>nd</sup> day at 30 mM NaCl followed by a decrease from the 4<sup>th</sup> day and for NaCl concentrations higher than 30 mM. For Thorgal, a slight increase was observed from the 4<sup>th</sup> day at 30 mM NaCl with a decrease for NaCl concentrations higher than 30 mM. For cultivar Padma, a slight increase was observed only at the 8<sup>th</sup> day at 30 mM NaCl. At 60 and 90 mM NaCl, no seed of cultivars Akikon, F1 Mongal

and Padma germinated at the 2<sup>nd</sup> day; similarly, no seed of cultivars Tounvi and Petomech germinated for the same time at 90 mM NaCl. From the 2<sup>nd</sup> day to the end of the experiment, cultivars behaved differently: For cultivars Padma, Tounvi, Petomech and F1 Mongal, the effect of salt stress was similar from the 2<sup>nd</sup> day to the end of the experiment. For cultivars Akikon, Thorgal and TLCV15, the effect of salt stress was more accentuated at the 2<sup>nd</sup> and 4<sup>th</sup> days than at the 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day. The kinetics of germination under salt stress conditions, always gave a precise tendency of the varieties studied [28]. Our results clearly indicate that the tomato seeds of all the cultivars studied show good germination in the absence of salt (control) or with low concentration of NaCl (30 mM) with germination kinetics ranging from 56.87 to 96.87% at four days of germination. When the salt concentration increases (60 to 90 mM NaCl), a decrease in kinetics of germination occurs, between 84.37 and 3.75%. For low doses of NaCl (0 and 30 mM) and practically for all varieties, tomato seed germination started at the second day after incubation. While for high doses of NaCl (60 and 90 mM), germination started after two days for almost all varieties. Thus, salt stress delayed germination by slowing down its speed.



**Fig. 1. Rate of germination of tomato seeds under saline conditions for cultivar Akikon**

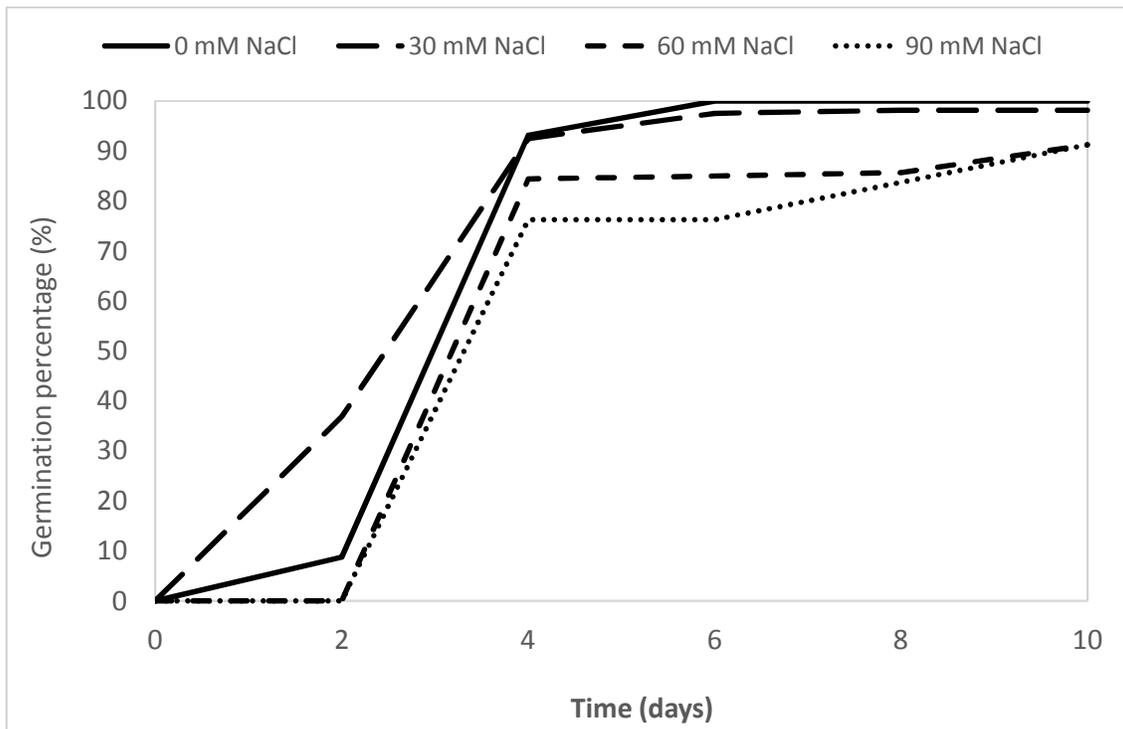


Fig. 2. Rate of germination of tomato seeds under saline conditions for cultivar F1 Mongal

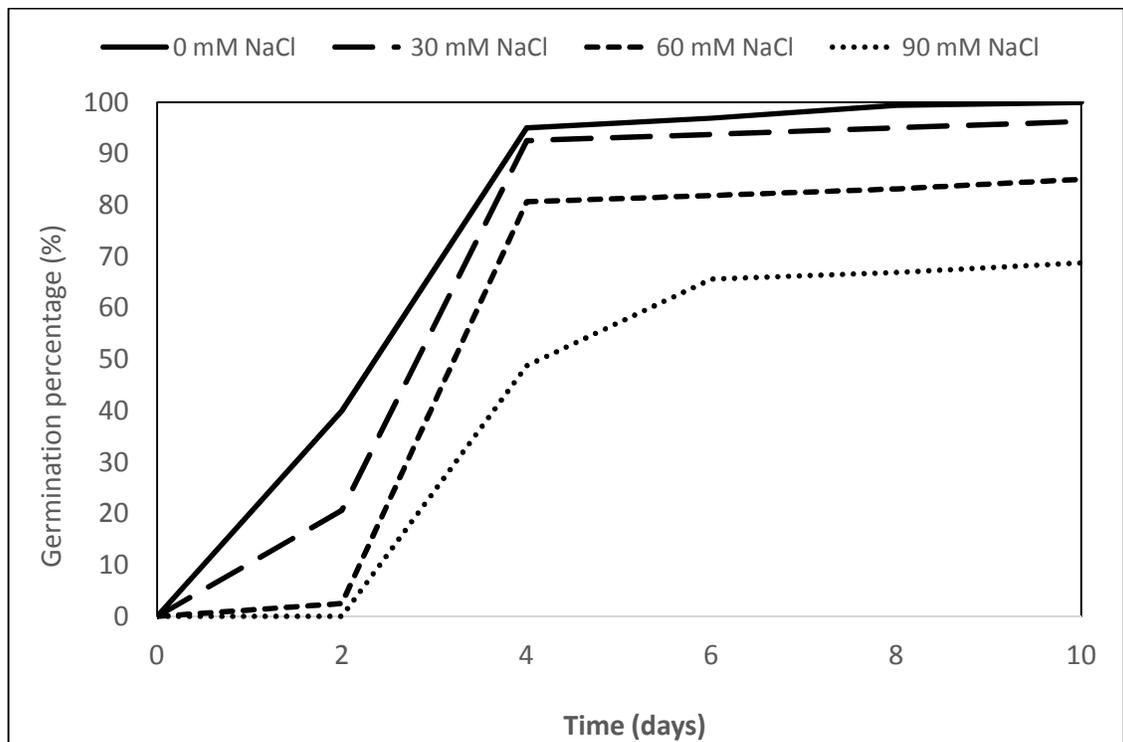


Fig. 3. Rate of germination of tomato seeds under saline conditions for cultivar Tounvi

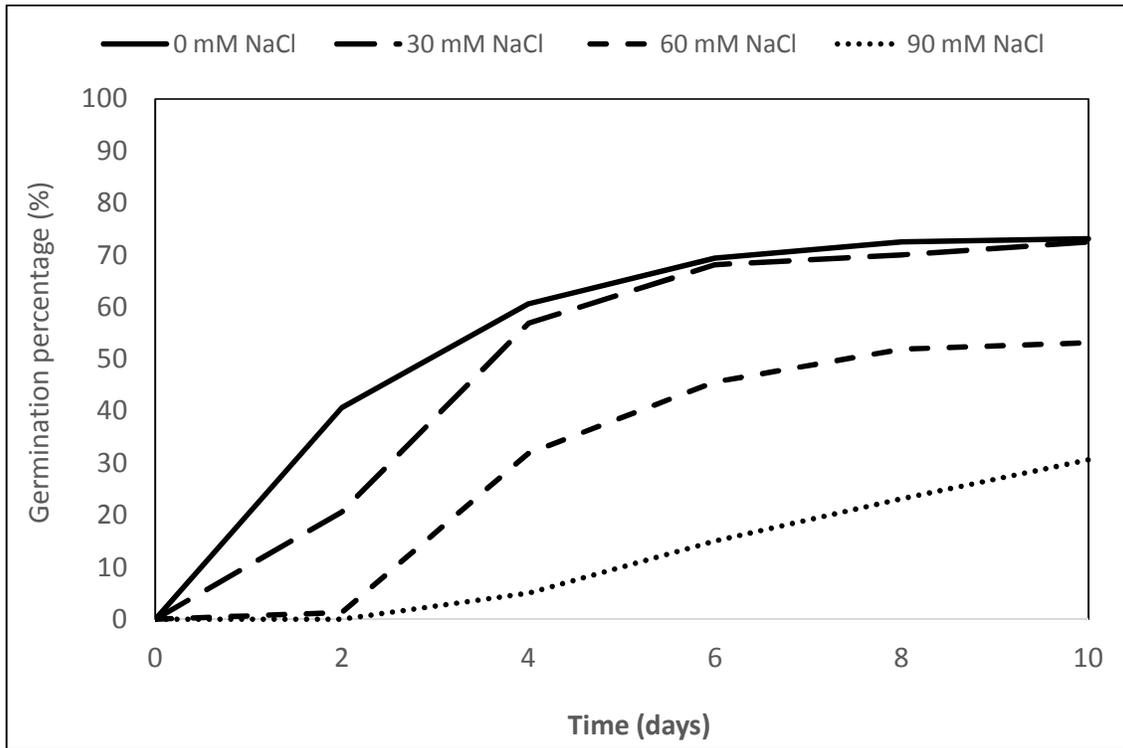


Fig. 4. Rate of germination of tomato seeds under saline conditions for cultivar Petomech

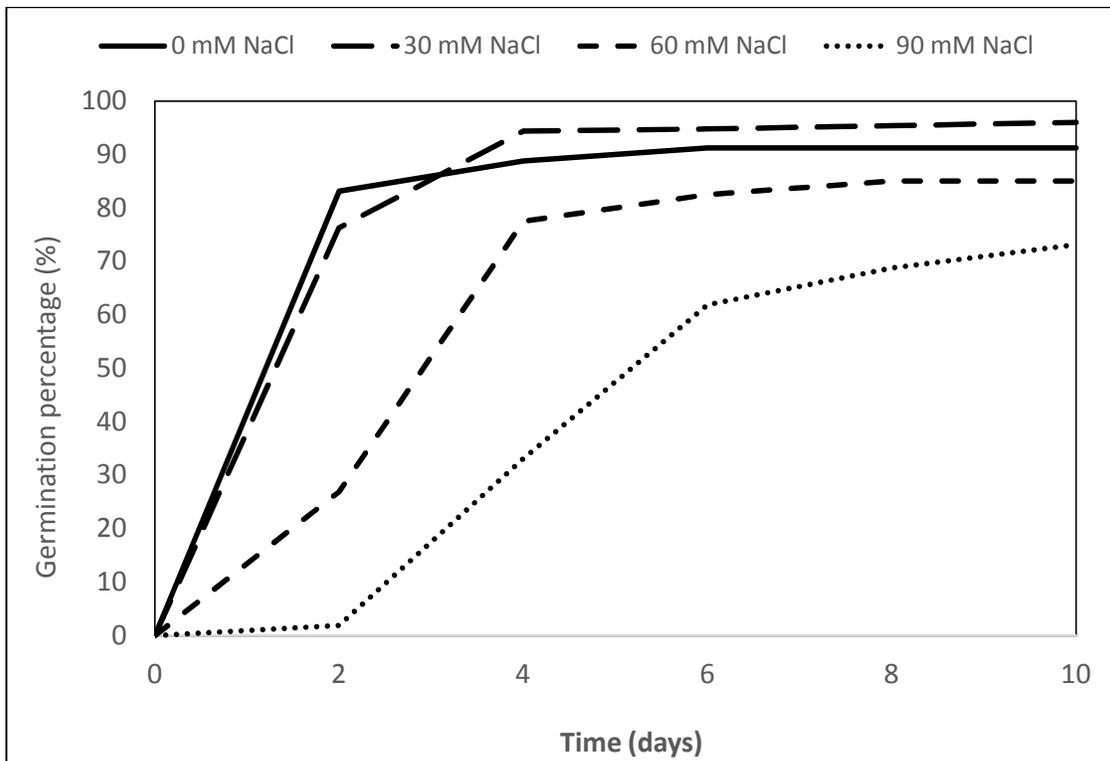


Fig. 5. Rate of germination of tomato seeds under saline conditions for cultivar Thorgal

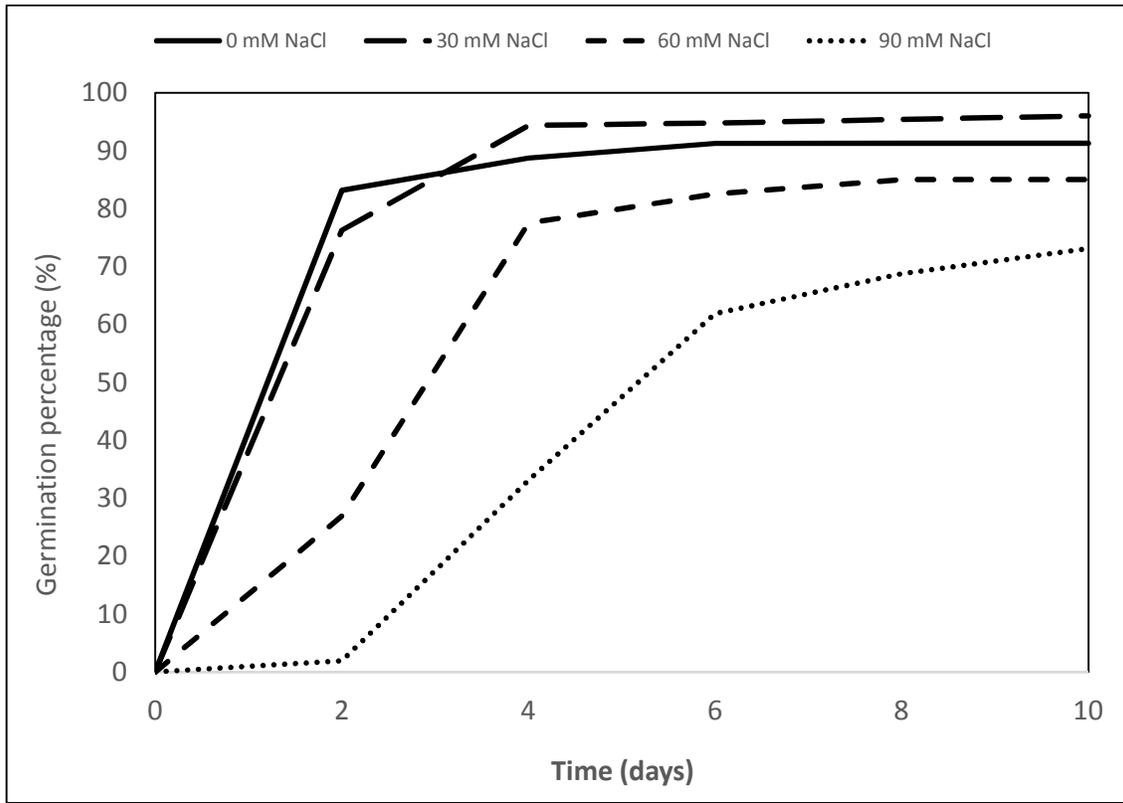


Fig. 6. Rate of germination of tomato seeds under saline conditions for cultivar TLCV15

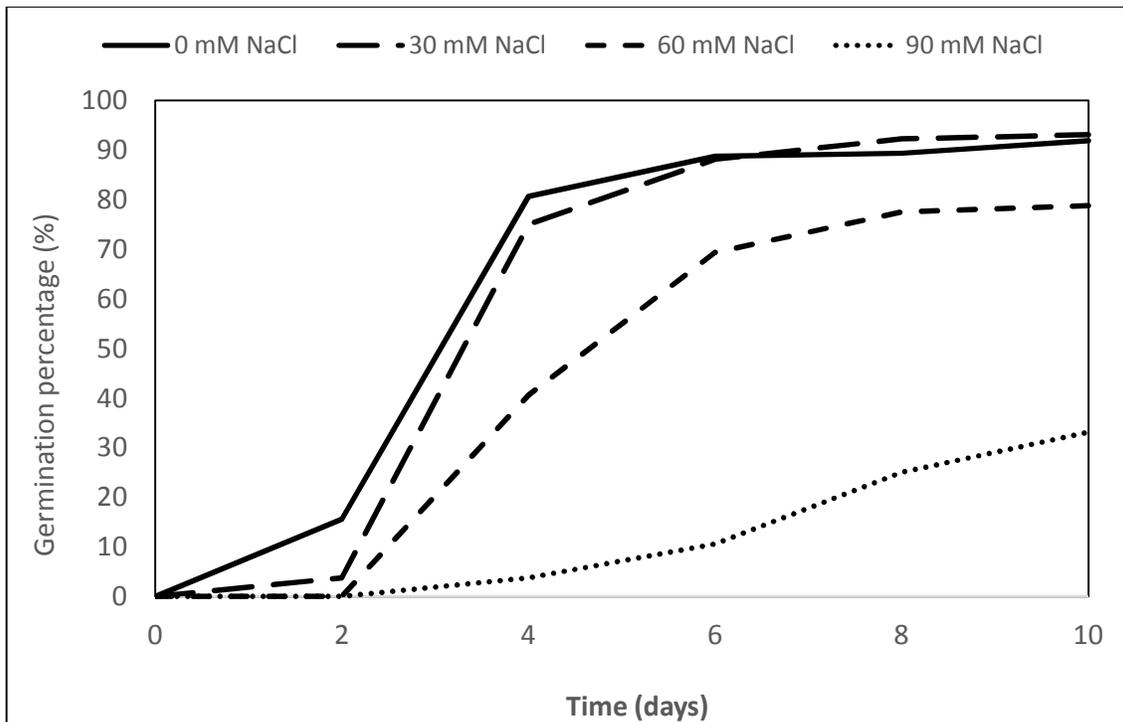


Fig. 7. Rate of germination of tomato seeds under saline conditions for cultivar Padma

Table 1 showed the variation of the germination index of the tomato varieties according to the treatments. In the absence of salt stress, the germination indexes were 0.434, 0.411, 0.392, 0.344, 0.336, 0.272 and 0.255 respectively for cultivars Thorgal, TLCV15, Akikon, Tounvi, F1 Mongal, Petomech and Padma; Thorgal showed the highest germination index (0.434) whereas Padma showed the weakest (0.255). The effect of sodium chloride (NaCl) resulted in an overall significant reduction in the germination index, but the response of the seven cultivars differed. The decrease was significant ( $P = .001$ ) from 30 mM NaCl for cultivars TLCV15, Akikon, Tounvi and F1 Mongal whereas it was significant ( $P = .001$ ) from 60 mM NaCl for cultivars Padma, Petomech and Thorgal. Thus, the germination index of cultivars Padma, Petomech and Thorgal was less affected by salt stress than that of the four other cultivars. According to [29], the germination index quantifies the speed with which the seeds perform their germination indicating whether the seeds quickly germinate and synchronously. Our results revealed that salt stress significantly reduced the germination index in tomato cultivars from 30 mM NaCl indicating that salt stress reduced from lower concentration the speed of seed germination in four cultivars and consequently delayed germination. According to [30], this delay corresponds to the time required for the seeds to set up mechanisms enabling it to adjust its internal osmotic pressure. In other cultivars of tomato, [17] and [31] reported that increasing salt concentrations in the plant cultivation medium delayed the speed of germination. Similar results were reported in chickpeas [32], wheat [33] and pepper [34,35]. Our results also revealed a significant difference among cultivars about the effect of salt stress in their germination index corroborating the observations of [33,35] respectively in wheat and pepper.

### 3.2 Effect of Salt Stress on Final Germination Percentage

In the absence of stress, the germination percentages after 10 days were 100%; 98.12%; 100%; 91.25%; 100%; 73.12% and 91.87% respectively for cultivars F1 Mongal, TLCV15, Akikon, Thorgal, Tounvi, Petomech and Padma. Petomech presented the lowest germination percentage whereas F1 Mongal, Akikon, and Tounvi presented the highest germination percentage (Fig. 8). In a previous study, [22] reported a percentage of final germination of approximately 91% for the same cultivar. The

difference could be due to seeds freshness. Globally, the cultivars tested in this study presented variable capacity of seed germination after 10 days in absence of salt stress as reported in cultivars of different vegetable species including lettuce [36], cabbage [37], amaranth [38] and chili [5].

Salinity induced a reduction of the final germination percentage but the seven cultivars showed different response. Two ways ANOVA revealed a significant effect ( $p=.001$ ) of NaCl, cultivars and interaction cultivars x NaCl, allowing us to consider cultivar by cultivar with one way ANOVA (Fig. 8). No significant reduction of germination percentage was observed for cultivar F1 Mongal whereas a significant reduction ( $P = .05$  or  $.01$ ) for the six other cultivars. In a previous study, [22] reported a significant reduction of final germination percentage from 17 mM NaCl for the same cultivar. For Akikon, Thorgal, TLCV15 and Tounvi, the reduction of final germination percentage was significant only at the highest NaCl concentration used (90 mM) whereas for Petomech and Padma the reduction was significant from 60 mM NaCl. Thus, NaCl reduced the final germination percentages with a significant difference among cultivars: cultivars F1 Mongal followed by Akikon, Thorgal, TLCV15 and Tounvi were less affected in comparison with the two other cultivars. The reduction of final germination percentage by NaCl is a common behavior in several plant species. Our results revealed that final germination percentage was more affected at the higher NaCl concentrations corroborating the report of [22]. The reduction was significant and more accentuated in cultivars Petomech and Padma in comparison with cultivars F1 Mongal and the four other cultivars indicating variability in the response of the seven cultivars tested to salt stress as reported in other tomato cultivars [22]. The same trend has been reported in several species including rice [15], durum wheat [39] sugar beet [40], chili [5] and amaranth [38]. The percentage of final germination is suggested to be the best way to identify the saline concentration that presents the physiological limit of germination of tomato seeds [41]. All seeds have not the same ability to tolerate desiccation due to salinity [42]. It is noted that at this stage the cultivars which express a weak germination under salt stress are 'Padma' and 'Petomech'. It is a temporary condition and viable seeds cannot germinate even under favorable conditions; this way is characterized by the absence of metabolic activity, plant

development and plant growth [43]. According to [44], the decrease in the germination rate of seeds subjected to salt stress is due to an osmotic dormancy process developed under these stress conditions.

### 3.3 Salt Tolerance Index of Cultivars

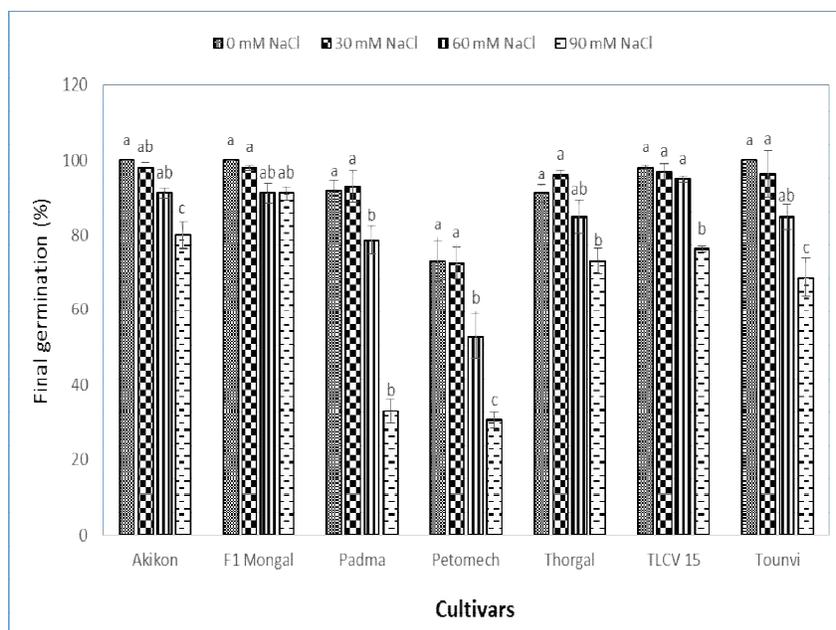
Table 2 showed the salt tolerance index of the seven tomato cultivars tested. A significant difference ( $P = .05$ ) was observed among cultivars. F1 Mongal, Akikon, TLCV15 and Tounvi presented the highest Salt. Tolerance Index respectively 1.086; 1.028; 1.005 and

0.989, followed by Thorgal (0.887) whereas Petomech (0.436) followed by Padma (0.715) presented the weakest Salt Tolerance Index. The STI was based on the final germination percentage reflecting the effect of salt stress from beginning to the end of the experiment. This criterion was used by [5] to classify chili pepper cultivars according to their salt resistance level. Based on this criterion, cultivars F1 Mongal, Akikon, TLCV15 and Tounvi appeared as the most salt resistant at germination level whereas Petomech followed by Padma appeared as the most salt sensitive; Thorgal was intermediate.

**Table 1. Germination index of seven cultivars of tomato as affected by salt stress**

Cultivars	NaCl Concentrations (mM)			
	0	30	60	90
THORGAL	0.434±0.013 a	0.423±0.007 a	0.272±0.007 b	0.144±0.006 c
TLCV15	0.411±0.003 a	0.327±0.003 b	0.222±0.002 c	0.145± 0.007 d
AKIKON	0.392±0.010 a	0.300±0.007 b	0.235±0.003 c	0.122± 0.007 d
TOUNVI	0.344±0.019 a	0.262±0.012 b	0.212±0.013 bc	0.154±0.011 c
F1 MONGA	0.336±0.010 a	0.263±0.004 b	0.206±0.007 c	0.185±0.006 c
PETOMECH	0.272±0.020 a	0.215±0.024 a	0.113±0.015 b	0.039±0.004 c
PADMA	0.255±0.009 a	0.222±0.015 a	0.159±0.007 b	0.039±0.007 c

Values are means ±SE (n = 4). Means with different letters within a line were significantly different ( $P = .001$ )



**Fig. 8. Effect of different concentrations of NaCl salinity on final germination percentage of seven tomato cultivars**

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  $.001$ )

**Table 2. Salt tolerance index (STI) of seven tomato cultivars at germination stage**

STI	Cultivars						
	F1 MONGAL	AKIKON	TLCV15	TOUNVI	THORGAL	PADMA	PETOMECH
	1.086a	1.028a	1.005a	0.989a	0.887ab	0.715b	0.436c

Means with different letters were significantly different ( $P = .05$ )

#### 4. CONCLUSION

The results reported in this research study show that the tomato is susceptible to NaCl stress at the germination stage. It revealed that NaCl salinity delayed seed germination and reduced the percentage of final germination in tomato cultivars. It pointed out the variability of relative salinity resistance for some tomato cultivars at germination stage including local cultivars. Among the seven cultivars, F1 Mongal, Akikon, TLCV15 and Tounvi were the most salt resistant at germination stage whereas Petomech was the most salt sensitive.

#### ACKNOWLEDGEMENTS

The authors thank Mr. Patrice Amoussou for the reading and correcting of the manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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