# Salt resistance of tomato (L*ycopersicon esculen*t*um* Mill.) cultivars produced in Benin at germination stage

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# 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.

Keys words : germination, local cultivars, salinity, salt tolerance index, tomato.

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#### 8 INTRODUCTION

9 Soil salinity is the major stress that reduced plant productivity mainly in arid and semi-arid climates [1]. 10 Plant growth is affected by salinity at all stages of development, but sensitivity varies greatly at 11 different stages [2-5]. Germination is a critical stage in the growth cycle of plant species which 12 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 13 14 reduces germination of glycophytes [4, 8-10] and the response is concentration dependent and also 15 specific on species [11-14]. It has been reported that there is a substantial variation in salt sensitivity 16 among cultivars of the same species [2, 5, 15-16]. Tomato (Lycopersicon esculentum Mill.) is one of 17 the most important vegetable plants in the world. It is consumed fresh, cooked or after processing: 18 through canning, transformation into juice, pulp, paste, or a variety of sauces [17]. According to FAO 19 [18], tomato is grown in 170 countries under various climates. Global production of tomato in 2017 was 20 182.3 million tons for a farmed land surface of 4.8 million hectares, an average yield of 37.6 tons per 21 hectare [19]. The climate of Benin is favorable for its farming throughout the country. The South of 22 Benin which provides the highest production, about 80% of the national production included the 23 coastal zone which is most strongly affected by salinity [20]. The water used by market gardeners 24 located in the coastal areas for irrigation has high electrical conductivity and salinity [21]. It was 25 reported that the salts presented in soil and irrigation water negatively affected tomato seed 26 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 27 28 substantial amount of literature on responses of tomato response to salinity stress, little information is 29 available on salt resistance of tomato cultivars produced in Benin. This study aims to evaluate NaCl 30 stress effects on seed germination of seven tomato varieties grown in Benin and compare the relative 31 salt resistance level of these cultivars at the germination stage.

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#### 33 2. MATERIAL AND METHODS

34 2.1. Plant material

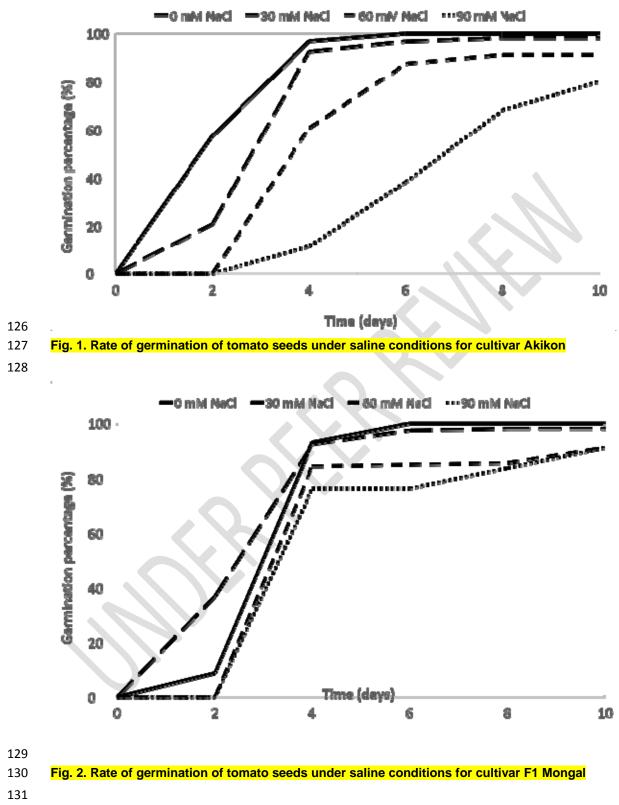
Seven cultivars including two local cultivars (Akikon and Tounvi) and five improved varieties (F1 35 36 Mongal, Petomech, Padma, Thorgal' and 'TLCV15) were used. Seeds of cultivars Akikon, Tounvi and 37 TLCV15 were provided by the National Institute of Agricultural Research of Benin (INRAB) whereas 38 those of the four over cultivars were bought from the society 'Bénin Semences'. The experiment was 39 carried out at the Laboratory of Plant Physiology and Abiotic Stresses Study of University of Abomey-40 Calavi in Benin. 41 42 2.2. Methods 43 2.2.1. Experiment design 44 The experiment was laid out as a Completely Randomized Design (CRD) with four replications. 45 46 2.2.2. Methodology 47 Seeds (40) of each variety were incubated in 10 cm Petri dishes on one layer of filter paper moistened 48 with 15 ml distilled water (check solution) and the same volume of water solutions of 30 - 90 mM NaCl 49 at 30 mM intervals chosen according to the literature. The seeds were incubated in darkness at 26°C. 50 Seed was considered as germinated when the radicle emerged from the seed coat. Seed germination 51 was checked every two days during ten days incubation period. 52 53 2.2.3. Measurements 54 Physiological parameters as germination kinetic, final germination percentage and germination index 55 were evaluated and compared among the seven tomato varieties. The means of salinity effect on 56 cultivars were determined in relation to indicate the salinity effect of these cultivars as well as the 57 salinity tolerance clues that were calculated with the final percentage of germination. The following 58 parameters as germination kinetic and final germination percentage were calculated according to [24]. <u>新闻中的第一</u>时间 59 60 With 61 Nj1, Nj2 ..... : Number of germinated seed in each day Final germination percentage =  $100 \ X \frac{\text{final number of germinated seeds}}{\text{initial number of seeds for germination}}$ 62 63

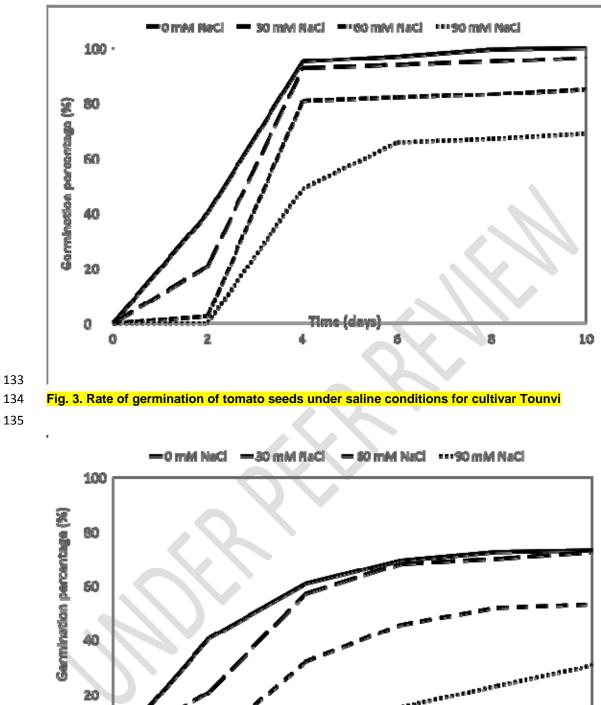
64 Germination index was calculated as described by [25].

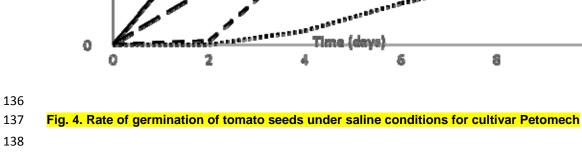
65	Germination index = $\frac{X_2}{Y_2} + \frac{(X_2 - X_3)}{Y_2} + \dots + \frac{(X_2 - X_{2-3})}{Y_2}$
66	With
67	$X_1$ ; $X_2$ ;; $X_u$ : Germination rate in day $_1$ ; day $_2$ ;; day $_u$
68	$Y_1$ ; $Y_2$ ;; $Y_u$ : Number of day
69	
70	Salinity Tolerance Index (STI) was calculated using formula adapted from [26] based on final
71	germination percentage.
72	ITS= (Ys x Yp)/(Yp) <sup>2</sup> with:
73	Ys= measure of one replication under one NaCl concentration;
74	Yp= measure of one replication of the control;
75	Yp= means of measures of all replications of the control.
76	
77	2.2.4. Statistical analysis
78	Data collected were statistically analyzed using the JMP software [27]. The means of each parameter
79	for the seven cultivars were compared using one way analysis of variance.
80	
81	3. RESULTS AND DISCUSSION
82	3.1. Effect of salt stress on seed germination kinetics and germination index
83	Figures 1; 2; 3; 4; 5; 6 and 7 showed respectively the effect of different concentrations of NaCl (0,
84	30, 60 and 90 mM) on the germination rate for cultivars Akikon, F1 Mongal, Tounvi, Petomech,
85	Thorgal, TLCV15 and Padma after 2, 4, 6, 8 and 10 days.
86	In absence of stress, the reaction of varieties were different: after 2 days, more than 50% of seeds
87	germinated for cultivars Thorgal (83.12%), TLCV15 (67.5%) and Akikon (57.40%) whereas less than
88	20% of seeds germinated for cultivars Padma (15.62%) and F1 Mongal (8.75%). Cultivars Tounvi
89	(40%) and Petomech (40.62%) presented intermediate values. Thus, the seed germination started
90	from the 2 <sup>nd</sup> day in the absence of stress for all cultivars. After 4 days, germination rate increased in all
91	cultivars and reached 96.87% for Akikon; 95% for TLCV15 and Tounvi; 93.12% for F1 Mongal;
92	88.75% for Thorgal ; 80.62% for Padma and 60.62% for Petomech. Thus, seed germination rate was
93	superior to 80% for all tomato cultivars after 4 days except for cv. Petomech (60.62%). After 6 days,
94	the percentages of seed germination were 100% for F1 Mongal and Akikon; 97.5% for TLCV15;

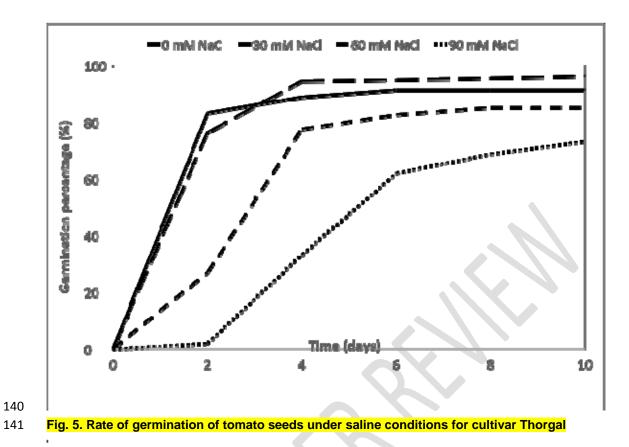
95 96.87% for Tounvi; 91.25% for Thorgal; 88.75% for Padma and 69.37% for Petomech. After 6 days, 96 no change was observed in germination rate for cultivars F1 Mongal, Akikon and Thorgal indicating 97 that these cultivars reached the maximum of their seed germination rate afer 6 days. For the four other cultivars, seed germination rate increased slowly until the 10<sup>th</sup> day corresponding to the end of 98 99 the experiment reaching 100% for cultivar Tounvi; 98.12% for TLCV15; 91.87% for Padma and 100 73.12% for Petomech. For three of the tested cultivars, germination rate becomes constant from the 101 6<sup>th</sup> day. Our results revealed a significant variability in the germination capacity of cultivars in the 102 absence of stress. Cultivars F1 Mongal, Akikon and Tounvi showed the highest germination capacity 103 whereas Petomech exhibited the lowest. NaCl stress effect resulted globally in a reduction of the 104 germination rate according to the time but cultivars behaved differently (Figures 1 to 7). A regular reduction was observed from the 2<sup>nd</sup> day for cultivars Akikon, Tounvi, Petomech and TLCV15. For 105 cultivar F1 Mongal, a slight increase was observed only at the 2<sup>nd</sup> day at 30 mM NaCl followed by a 106 107 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 wih a decrease for NaCl concentrations higher 108 109 than 30 mM. For culivar 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; 110 111 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, 112 113 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 114 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 115 116 salt stress conditions, always gave a precise tendency of the varieties studied [28]. Our results clearly 117 indicate that the tomato seeds of all the cultivars studied show good germination in the absence of salt 118 (control) or with low concentration of NaCl (30 mM) with germination kinetics ranging from 56.87 to 119 96.87% at four days of germination. When the salt concentration increases (60 to 90 mM NaCl), a 120 decrease in kinetics of germination occurs, between 84.37 and 3.75%. For low doses of NaCl (0 and 121 30 mM) and practically for all varieties, tomato seed germination started at the second day after 122 incubation. While for high doses of NaCl (60 and 90 mM), germination started after two days for 123 almost all varieties. Thus, salt stress delayed germination by slowing down its speed.

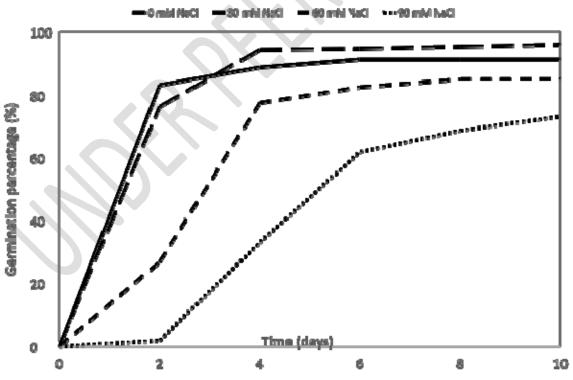
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143 Fig. 6. Rate of germination of tomato seeds under saline conditions for cultivar TLCV15

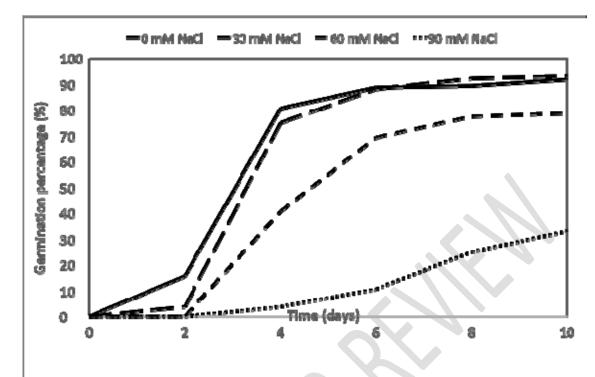




Fig. 7. Rate of germination of tomato seeds under saline conditions for cultivar Padma
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Table 1 showed the variation of the germination index of the tomato varieties according to the 149 150 treatments. In the absence of salt stress, the germination indexes were 0.434, 0.411, 0.392, 0.344, 151 0.336, 0.272 and 0.255 respectively for cultivars Thorgal, TLCV15, Akikon, Tounvi, F1 Mongal, 152 Petomech and Padma; Thorgal showed the highest germination index (0.434) whereas Padma 153 showed the weakest (0.255). The effect of sodium chloride (NaCl) resulted in an overall significant 154 reduction in the germination index, but the response of the seven cultivars differed. The decrease was 155 significant (P = .001) from 30 mM NaCl for cultivars TLCV15, Akikon, Tounvi and F1 Mongal whereas 156 it was significant (P = .001) from 60 mM NaCl for cultivars Padma, Petomech and Thorgal. Thus, the 157 germination index of cultivars Padma, Petomech and Thorgal was less affected by salt stress than that 158 of the four other cultivars. According to [29], the germination index quantifies the speed with which the 159 seeds perform their germination indicating whether the seeds quickly germinate and synchronously. 160 Our results revealed that salt stress significantly reduced the germination index in tomato cultivars 161 from 30 mM NaCl indicating that salt stress reduced from lower concentration the speed of seed 162 germination in four cultivars and consequently delayed germination. According to [30], this delay 163 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.

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

	Naci Concentrations (min)			
<b>Culivars</b>	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
<mark>AKIKON</mark>	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

NaCl Concentrations (mM)

171 Values are means  $\pm$ SE (n = 4). Means with different letters within a line were significantly different

172 173 (P = .001)

### 174 3.2. Effect of salt stress on final germination percentage

175 In the absence of stress, the germination percentages after 10 days were 100%; 98.12%; 100%; 176 91.25%; 100%; 73.12% and 91.87% respectively for cultivars F1 Mongal, TLCV15, Akikon, Thorgal, 177 Tounvi, Petomech and Padma. Petomech presented the lowest germination percentage whereas F1 178 Mongal, Akikon, and Tounvi presented the highest germination percentage (Fig. 8). In a previous 179 study, [22] reported a percentage of final germination of approximately 91% for the same cultivar. The 180 difference could be due to seeds freshness. Globally, the cultivars tested in this study presented 181 variable capacity of seed germination after 10 days in absence of salt stress as reported in cultivars of 182 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 187 significant reduction (P = .05 or .01) for the six other cultivars. In a previous study, [22] reported a 188 significant reduction of final germination percentage from 17 mM NaCl for the same culivar. For 189 Akikon, Thorgal, TLCV15 and Tounvi, the reduction of final germination percentage was significant 190 only at the highest NaCl concentration used (90 mM) whereas for Petomech and Padma the reduction 191 was significant from 60 mM NaCI. Thus, NaCI reduced the final germination percentages with a 192 significant difference among cultivars: cultivars F1 Mongal followed by Akikon, Thorgal, TLCV15 and 193 Tounvi were less affected in comparison with the two other cultivars. The reduction of final germination 194 percentage by NaCl is a common behavior in several plant species. Our results revealed that final 195 germination percentage was more affected at the higher NaCl concentrations corroborating the report 196 of [22]. The reduction was significant and more accentuated in cultivars Petomech and Padma in 197 comparison with cultivars F1 Mongal and the four other cultivars indicating variability in the response 198 of the seven cultivars tested to salt stress as reported in other tomato cultivars [22]. The same trend 199 has been reported in several species including rice [15], durum wheat [40] sugar beet [41], chili [5] and 200 amaranth [38]. The percentage of final germination is suggested to be the best way to identify the 201 saline concentration that presents the physiological limit of germination of tomato seeds [42]. All seeds 202 have not the same ability to tolerate desiccation due to salinity [43]. It is noted that at this stage the 203 cultivars which express a weak germination under salt stress are 'Padma' and 'Petomech'. It is a 204 temporary condition and viable seeds cannot germinate even under favorable conditions; this way is 205 characterized by the absence of metabolic activity, plant development and plant growth [44]. 206 According to [45], the decrease in the germination rate of seeds subjected to salt stress is due to an 207 osmotic dormancy process developed under these stress conditions.

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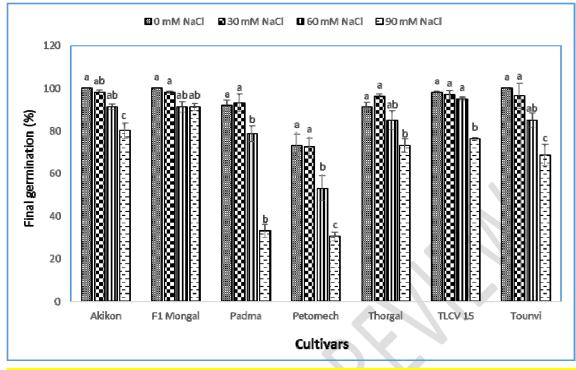


Fig. 8. Effect of different concentrations of NaCl salinity on final germination percentage of
 seven tomato cultivars

- 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)
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# 215 3.3. Salt tolerance index of cultivars

216 Table 2 showed the salt tolerance index of the seven tomato cultivars tested. A significant difference 217 (P = .05) was observed among cultivars. F1 Mongal, Akikon, TLCV15 and Tounvi presented the 218 highest Salt. Tolerance Index respectively 1.086; 1.028; 1.005 and 0.989, followed by Thorgal 219 (0.887) whereas Petomech (0.436) followed by Padma (0.715) presented the weakest Salt Tolerance 220 Index. The STI was based on the final germination percentage reflecting the effect of salt stress from 221 the beginning to the end of the experiment. This criterion was used by [5] to classify chili pepper 222 cultivars according to their salt resistance level. Based on this criterion, cultivars F1 Mongal, Akikon, 223 TLCV15 and Tounvi appeared as the most salt resistant at germination level whereas Petomech 224 followed by Padma appeared as the most salt sensitive; Thorgal was intermediate.

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228	Table 2. Salt tolerance Index (STI) of seven tomato cultivars at germination stage

	Cultivars				
	F1 MONGAL AKIKON TLCV15 TOUNVI THORGAL PADMA PETOMECH				
	<b>STI</b> 1.086a 1.028a 1.005a 0.989a 0.887ab 0.715b 0.436c				
229	Means with different letters were significantly different ( $P = .05$ )				
230	CONCLUSION				
231 232	The results reported in this research study show that the tomato is susceptible to NaCl stress at the				
233	germination stage. It revealed that NaCl salinity delayed seed germination and reduced the				
234	percentage of final germination in tomato cultivars. It pointed out the variability of relative salinit				
235	resistance for some tomato cultivars at germination stage including local cultivars. Among the seve				
236	cultivars, F1 Mongal, Akikon, TLCV15 and Tounvi were the most salt resistant at germination stag				
237	whereas Petomech was the most salt sensitive.				
238					
239	ACKNOWLEDGEMENTS				
240	The authors thank Mr. Patrice Amoussou for the reading and correcting of the manuscript.				
241	COMPETING INTERESTS				
242	There is no competing interest related to this manuscript.				
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