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Original Research

In Vitro Early Vegetative Growth of Tomato (*Solanum lycopersicum* L.) Cultivars Under Salt Stress

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Abstract

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and is considered an important vegetable worldwide. Its production has been challenged due to soil salinization and water shortages. Local tomato cultivars could be better adapted to salt stress. Based on this hypothesis, the present study was carried out to determine the variation in fourteen different tomato cultivars for salt tolerance. Seed germination, mean germination time, shoot length, and root length were examined under salinity stress (1.5 g L^{-1} NaCl). Plant growth and seed germination were severely affected by saline conditions. The results of this *in vitro* experiment showed that the seed germination of Yellow Milk was significantly increased (73.33%) however, Pink Jade and Red Jade cultivars were significantly decreased under the NaCl treatment. Similarly, among the fourteen tomato cultivars, the mean germination time of only Yellow Pearl and Black Current significantly increased. Moreover, the shoot length of eight tomato cultivars decreased compared with the control, while the highest shoot length (12.5 cm) was recorded in the case of Saint cultivar. The root length of Taiwan Red Saint, Purple Beauty, Yellow Milk, Red Pearl, Yellow Pearl, Pink Cooperative 906, Qinzu Shanghai 903, and Scarlet cultivars significantly increased, while the other five cultivars significantly decreased under NaCl treatment compared to control. It is concluded that NaCl stress significantly affects the vegetative growth of tomato cultivars under *in vitro* culture.

Keywords: salt stress, tomato, seed germination, cultivars, *In vitro*, abiotic stress

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Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family, originating from the Peru-Ecuador area, where its indigenous name was Tomati [1], and is considered an important vegetable in the world [2,3]. It is considered rich in vitamins, antioxidants, and some bioactive mechanisms that are very reliable and seem preventative for some diseases [1], such as blood pressure [4], and lung, breast, and stomach cancers [5,6]. The production and cultivated area of tomatoes have moderately improved throughout the world [7], and it was reported that ~5.08% of production has increased from the last three decades until the present. China is producing around (59.4 t ha^{-1}), but this has still not met the country's requirements. Cultivation of tomatoes on a large scale in China is still below par and cannot compete with countries like the United States (96.8 t ha^{-1}) and South Africa (75.5 t ha^{-1}). Inadequate crop yields are linked to the salinization of arable land, one of the major constraints that severely limits world production [8].

The production of tomatoes has been challenged by different factors in China and around the world, such as soil salinization, water shortages, and abiotic stresses [9, 10]. According to Munns & Tester [11], salinity affects around 800 mha of the land in the world, but they also suggest that salt stress leads to a decline in worldwide agriculture production. Salinity not only declines production, but also decreases agricultural land [12]. Tomatoes are sensitive to moderate levels of salt in the soil. Therefore, salinity is the main obstacle, which reduces food and increases land degradation in arid and semi-arid regions [13, 14]. Under controlled environmental conditions, tomato screening for salt tolerance becomes more active [15]. Tomatoes under saline conditions produce osmotically dynamic organic substances (mainly amino acids and sugars), which help to improve the salinity-mediated osmotic stress. In their review, Cuartero & Fernández-Muñoz [16] concluded that although there are several promising selection criteria, the complex physiology of salt tolerance and the variation between species make it difficult to identify a single criterion for evaluating a cultivar's responses to salinity.

Moderate salt concentrations in the soil can also make tomatoes sensitive. Genetically, tomato species are more dependent on salinity; meanwhile, research interest has increased in screening and breeding for salt tolerant tomato species for higher production [17, 18]. Under *in vitro* culture, tomato production is considered the most auspicious food crop due to the smaller number of chromosomes and wide-ranging acquaintance with tomato genetics. Through conventional breeding in tomatoes, extensive enhancement has already been made by exploiting the natural variation. However, this success in traditional breeding cannot increase or reach the tomato demand of consumers in the 21st century.

Based on the above discussion, it is imperative to screen various genotypes of tomatoes regarding their tolerance to salt stress. Using NaCl salt, a significant correlation has been observed between tomato calluses and whole plants [1]. Screening new cultivars regarding salt stress tolerance could enhance the growth and productivity of tomatoes. Based on this hypothesis, the present study was conducted under *in vitro* conditions on fourteen tomato cultivars (Black Currant, Pink Jade, Purple Beauty, Red Jade, Red Pearl, Saint, Taiwan Red Saint, Taiwan Yellow Saint, Yellow Milk, Yellow Pearl, Pink Cooperative 906, Pink Cooperative 908, Qinzu Shanghai 903, Scarlet) to study the germination percentage and growth parameters under NaCl treatments and to screen the best NaCl-tolerant variety based on the physiological and biological characteristics of fourteen tomato varieties. To our knowledge, no study has reported the screening of the mentioned tomato cultivars regarding their salt stress tolerance and their physiological and biochemical responses under salt stress.

Experimental

This study was conducted at the postgraduate laboratory of the Department of Life Science of Engineering, Southwest University of Science and Technology, China, from April to September 2019. Fourteen tomato cultivars, free from insects and diseases, were collected from the local farmers market of Longmen town and the Funcheng district of Mianyang city, in the Sichuan province of China. Seeds were brought in sealed bags and kept at room temperature. Laboratory-grade sodium chloride (NaCl) and sucrose used in this experiment were purchased from Chengdu Jinshan, and the chemical reagent and a medium not containing sucrose and agar were purchased from Shanghai Bio-way Technology.

Preparation of Medium

Murashige and Skoog (MS) media were prepared for seed germination and seedling growth, containing different macro-elements and organic substances. For preparing 1 liter of MS media, ~ 4.74 g MS media was weighed along with 30 g of sucrose and 8 g of agar, and then 700 mL of distilled water was added to the enamel vessel and placed into a thermal furnace for a few minutes to melt the agar. After that, the volume was up to 1 liter, and the pH adjusted to 5.8~6.0 with HCl and NaOH. Two types of MS media were prepared, one containing only the MS media component, and to the other, 1.5 mg L^{-1}, NaCl was added.

Sterilization of Tomato Seeds

Seeds of fourteen tomato cultivars (Black Currant, Pink Jade, Purple Beauty, Red Jade, Red Pearl, Saint, Taiwan Red Saint, Taiwan Yellow Saint, Yellow Milk, Yellow Pearl, Pink Cooperative 906, Pink Cooperative 908, Qinzu Shanghai 903, Scarlet) were surface sterilized with 70% ethanol for 1 minute, followed by 0.1%

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mercuric chloride ($HgCl_2$) solution for 10 minutes, and washed thoroughly with distilled water five times. Before seed inoculation, the UV light was turned on for 20 minutes to sterilize the interior and contents to prevent contamination. Then, five seeds were inoculated into each culture bottle containing 50 ml of solid medium [19], and nine bottles were applied as replicates for each tomato cultivar (each replicate with 3 bottles, 15 seeds). A control was used without containing NaCl in the MS medium. All cultures were maintained at 25±2 °C under white fluorescent light at an irradiance of 125 or 114 µmol m^{-2} s^{-1}, with a 16 hrs (light) and 8 hrs (dark) photoperiod.

Plant Growth

The seed germination percentage was counted according to the seedling evaluation procedure [20]. Seeds with a radicle size of 2 mm were considered germinated. The germination percentage was calculated after twenty days of inoculating. Similarly, mean germination time was observed from the start of *in vitro* culture, in this case, germination was defined when coleoptiles and plumules grew more than 5 mm in length. After 10 days, mean germination time was calculated by using the relation

$$MGT = \Sigma (n \times d) / N$$

where MGT is mean germination time, n is the number of seeds germinated on day d, and N is the total number of germinated seeds on the tenth day [21]. After four weeks, shoot length and root length were calculated after removing excess water by placing them on dry paper. The fresh weight of the dry shoot was also measured. The dry weight of the shoot and root was determined by placing tomato seedlings in Petri dishes and placing them in an oven at 65 °C for 24 hours.

Statistical Analysis

The collected data was used for statistical analysis using the Statistical Package for Social Sciences (SPSS) version 23. A student "T-test" was applied among the control and treatment groups to check the significance level at 0.05 and 0.01 probabilities.

Results

Seed Germination Percentage

The NaCl effect on the seed germination percentage of fourteen tomato cultivars is presented in (Table 1). The results of seed germination inside the table show that, compared to the control, Yellow Milk was significantly promoted, however, Pink Jade and Red Jade cultivars were significantly inhibited under the NaCl treatment. However, the other eleven tomato cultivars were non-significantly inhibited under NaCl treatment compared to the control. The seed germination percentage of Yellow Milk cultivars statistically performed higher at 73.33% compared to the control and Red Pearl, which was statistically inhibited at 67.67% compared to the control under NaCl treatment compared to the other fourteen cultivars.

Table 1. Effect of NaCl on seed germination of fourteen tomato cultivars under *in vitro* culture

Tomato cultivars	Seed germination rate (%)		Inhibitory effect of NaCl (%)	Student t-Test between CK and Treatment	
	Control	NaCl treatment		t value	*p*-value
Yellow Milk#	32.20±20.09	73.33±15.28 a	-127.74*	2.8229	0.0477
Red Pearl#	73.90±16.71	61.67±10.41 ab	16.55	1.0764	0.3423
Pink Jade#	47.77±22.67	60.00±15.00 a-c	-25.61	0.7795	0.4793
Saint#	69.43±12.94	60.00±32.79 a-c	13.59	0.4635	0.6671
Yellow Pearl#	70.57±27.70	56.67±28.43 a-c	19.70	0.6065	0.5769
Purple Beauty#	61.67±7.30	55.00±10.00 a-d	10.81	0.9328	0.4037
Qinzu Shanghai 903	61.10±11.69	46.67±12.58 a-e	23.62	1.4556	0.2192
Pink Cooperative 906	62.20±10.18	41.67±16.07 b-e	33.01	1.8693	0.1349
Red Jade#	24.43±10.19	40.00±10.00 b-e	-63.71	1.8884	0.1320
Scarlet	43.33±5.77	36.67±15.28 b-e	15.38	0.7071	0.5185
Taiwan Yellow Saint#	71.67±15.91	36.67±12.58 b-e	48.84	2.7483	0.0515
Taiwan Red Saint#	50.57±8.21	33.33±7.64 c-e	34.08	2.6613	0.0563
Black Currant#	35.00±3.48	29.44±12.95 de	15.88*	3.4441	0.0262
Pink Cooperative 908	55.00±8.66	23.33±2.89 e	57.58**	6.0083	0.0039

#Cherry tomato; *Show the significant difference between the control and the treatment (n=3, $p \leq 0.05$); **Show the highly significant difference between the control and the treatment (n=3, $p \leq 0.01$); Negative value in inhibitory effect means promotion action of NaCl. The value of NaCl treatment followed with the same small letter means no significant difference among cultivars ($p \leq 0.05$)

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Table 2. Effect of NaCl on mean germination time of tomato cultivars under *in vitro* culture

Tomato cultivars	Mean germination time (days)		Inhibitory effect of NaCl (%)	Student t-Test between CK and Treatment	
	Control	NaCl treatment		t value	*p*-value
Saint#	5.30±0.47	5.20±0.23 e	0.10	0.3299	0.7580
Pink Cooperative 906	5.69±0.41	5.28±0.69 e	0.41	0.8840	0.4266
Red Jade#	7.33±2.52	5.83±1.61 de	1.50	0.8701	0.4334
Taiwan Red Saint#	6.56±0.27	5.89±0.54 de	0.67	1.1932	0.1251
Qinzu Shanghai 903	6.96±0.40	6.55±0.14 cd	0.41	1.6729	0.1697
Pink Jade#	7.23±1.78	6.62±0.97 cd	0.61	0.5248	0.6275
Black Currant#	5.49±0.23	6.74±0.51 b-d	-1.25*	3.8535	0.0182
Purple Beauty#	6.79±0.80	7.32±0.98 a-c	-0.52	0.7165	0.5133
Red Pearl#	7.39±0.58	7.60±0.65 a-c	-0.20	0.4055	0.7059
Yellow Pearl#	8.74±0.08	7.97±0.24 ab	0.76**	5.2032	0.0065
Scarlet	8.14±1.28	8.09±0.53 a	0.05	0.0583	0.9563
Taiwan Yellow Saint#	7.95±1.21	8.17±0.29 a	-0.22	0.3022	0.7776
Pinc Cooperative 908	7.56±0.12	7.50±0.23 a-c	0.06	0.2531	0.5843
Yellow Milk#	10.00±0.23	8.42±0.18 a	1.58	0.0315	0.1689

#Cherry tomato; *Show the significant difference between the control and the treatment (n=3, $p \leq 0.05$); **Show the highly significant difference between the control and the treatment (n=3, $p \leq 0.01$); Negative value in inhibitory effect means promotion action of NaCl. The value of NaCl treatment followed with the same small letter means no significant difference among cultivars ($p \leq 0.05$)

Table 3. NaCl effect on shoot length of fourteen tomato cultivars under *in vitro* culture

Tomato cultivars	Shoot length (cm)		Inhibitory effect of NaCl (%)	Student t-Test between CK and Treatment	
	Control	NaCl treatment		t value	*p*-value
Saint#	13.00±0.55	12.50±1.27 a	3.85	0.6218	0.5678
Taiwan Red Saint#	10.86±0.85	12.40±0.84 ab	-14.11	1.9765	0.1425
Purple Beauty#	9.26±2.90	12.30±0.95 ab	-32.73	1.7197	0.1606
Yellow Milk#	0.95±0.17	12.00±1.03 ab	-1156.34**	14.1570	0.0008
Red Pearl#	10.05±0.41	10.28±0.38 a-c	-2.29	0.7029	0.5208
Yellow Pearl#	6.74±3.81	10.21±1.79 a-c	-51.46	1.4252	0.2272
Red Jade#	11.33±4.04	10.20±3.20 a-c	10.00	0.3806	0.7229
Pink Cooperative 906	9.13±1.20	9.89±1.23 bc	-8.25	0.7557	0.4919
Qinzu Shanghai 903	8.64±0.80	9.02±0.24 cd	-4.40	0.7800	0.4790
Scarlet	7.50±1.50	8.49±0.88 c-e	-13.20	0.9823	0.3816
Black Currant#	9.29±0.41	8.14±1.57 c-e	12.34	1.2232	0.2884
Pink Jade#	10.26±2.68	8.03±1.82 c-e	21.70	1.1878	0.3006
Taiwan Yellow Saint#	8.22±0.85	7.01±1.53 de	14.72	1.1931	0.2988
Pink Cooperative 908	8.61±2.45	6.44±1.17 e	25.13	1.3748	0.2412

#Cherry tomato; **Show the highly significant difference between the control and the treatment (n=3, $p \leq 0.01$); Negative value in inhibitory effect means promotion action of NaCl. The value of NaCl treatment followed with the same small letter means no significant difference among cultivars ($p \leq 0.05$)

Mean Germination Time (MGT)

The Mean Germination time (MGT) of fourteen tomato cultivars after NaCl treatment compared to the control is presented in (Table 2). The results indicated that the MGT of Yellow Pearl and Black Current significantly increased under NaCl treatment compared to the control and the other twelve cultivars. Similarly, Taiwan Yellow Saint, Yellow Milk, Saint, and Pink Cooperative 906 showed a non-significant difference under NaCl treatment compared to the control. The results also showed that the Yellow Milk cultivar showed the longest time (8.42 days for MGT) and Saint showed the shortest (5.20 days for MGT) under NaCl stress compared to other cultivars.

Shoot Length

The *in vitro* culture results of the shoot length of fourteen tomato cultivars after NaCl treatment compared to the control are presented in Table 3. The shoot length

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Table 4. Effect of NaCl on the root length of fourteen tomato cultivars under *in vitro* culture

Tomato cultivars	Root length (cm)		Inhibitory effect of NaCl (%)	Student t-Test between CK and Treatment	
	Control	NaCl treatment		t value	p-value
Red Jade#	7.88±2.10	13.96±4.64 a	-77.08	2.0633	0.1080
Taiwan Red Saint#	7.72±1.11	8.72±2.54 b	-12.58	0.6189	0.5695
Saint#	9.34±1.22	8.67±0.88 b	7.14	0.7627	0.4881
Purple Beauty#	6.07±1.11	7.36±1.25 bc	-21.73	1.3344	0.2530
Pink Jade#	6.31±0.92	7.27±1.57 bc	-15.32	0.9190	0.4101
Pink Cooperative 906	6.19±2.19	7.27±1.07 bc	-17.44	0.7655	0.4866
Black Currant#	7.81±1.70	6.76±0.86 b-d	13.40	0.9503	0.3958
Red Pearl#	7.20±1.71	6.08±0.54 b-d	15.51	1.0756	0.3427
Yellow Milk#	11.00±0	6.07±0.29 b-d	44.76**	29.3122	0.0012
Qinzu Shanghai 903	3.83±0.54	5.28±0.83 cd	-38.03	2.5321	0.0645
Taiwan Yellow Saint#	5.35±1.14	5.25±1.72 cd	1.87	0.0838	0.9373
Yellow Pearl#	3.93±1.72	5.25±0.45 cd	-33.53	1.2810	0.2694
Pink Cooperative 908	6.40±3.23	4.25±0.73 d	33.56	1.1239	0.3239
Scarlet	4.35±0.79	4.16±0.95 d	4.37	0.2643	0.8046

#Cherry tomato; **Show the highly significant difference between the control and the treatment (n=3, $p \leq 0.01$); Negative value in inhibitory effect means promotion action of NaCl. The value of NaCl treatment followed with the same small letter means no significant difference among cultivars ($p \leq 0.05$)

of fourteen tomato cultivars under NaCl stress accounted that the shoot length of Saint, Red Jade, Black Current, Pink Jade, Taiwan Yellow Saint, and Pink Cooperative 908 was non-significantly decreased compared to control under NaCl treatment. Moreover, the shoot length of the other eight cultivars was significantly stimulated under NaCl treatment. The Yellow Milk cultivars show a highly significant difference compared to the control. The results also indicate that the highest shoot length was detected on Saint (12.50 cm) cultivars whereas, the lowest (6.44 cm) was observed at Pink Cooperative 908 compared to the control under NaCl treatments.

Root Length

The effect of NaCl on the root length of fourteen tomato cultivars under *in vitro* culture compared to control is presented in (Table 4). The statistical data revealed that the Saint, Taiwan Red Saint, Purple Beauty, Yellow Milk, Red Pearl, Yellow Pearl, Pink Cooperative 906, Qinzu Shanghai 903, and Scarlet cultivars were significantly increased, and the other five cultivars were observed to significantly decrease compared to the control under NaCl treatment. However, Yellow Milk cultivars were highly significantly decreased compared to other cultivars, whereas the highest root length was observed in Red Jade (13.96 cm), which was significantly higher than the other 13 cultivars, and the lowest (4.16 cm) compared to fourteen tomato cultivars.

Discussion

The reproduction, seed germination, and vegetative growth of tomatoes are moderately sensitive to salinity, which hinders the optimum productivity of tomatoes worldwide [22, 23]. Genetic variation in the species has already been used to improve salt tolerance in some modern crop cultivars [24]. In this study, we determined the tolerance of the fourteen tomato cultivars to salinity stress. Under *in vitro* culture, the experiment indicated 1.5 mg L^{-1} NaCl in the media solution. The results of the current study indicate that the seed germination of Yellow Milk was significantly promoted along with Red Jade and Pink Jade, however, the other eleven cultivars were non-significantly inhibited under NaCl treatment compared to control. This decrease in germination may be due to partially osmotic or less toxicity reported by others [25-27]. They reported that a delay or decrease in seed germination is caused by less osmotic potential and ion toxicity (Na^+, Cl^{-1}, etc.), which also affect enzyme activity. This type of result was also explained by other scientists [28-30]. When we compared 14 tomato cultivars, the MGT of Yellow Milk, Taiwan Yellow Saint, and Scarlet cultivars performed well over the other 11 cultivars under NaCl stress. However, it is also assumed that the higher concentration of salt reduces the water potential in the medium, which hinders water absorption by germinating seeds and thus reduces germination [5]. These results are in line with the result of Shahid et al. [31], which suggested that NaCl stress decreases the germination rate of various crop plants compared to control. Similarly, Adilu & Gebre [32] observed that the germination time of tomato seeds decreased as the salinity level increased.

The impact of salinity on the mean germination time varies in different cultivars of the same species [33, 34], which supports the findings of this study. The decrease in seed germination time in tomatoes may also depend on the genetic background of the tomatoes [23]. This study also indicates that among fourteen tomato cultivars, the shoot lengths of Saint, Red Jade, Black Current, Pink Jade,

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Taiwan Yellow Saint, and Pink Cooperative 908 cultivars decreased non-significantly compared with control. The highest shoot length was observed at Saint, and the lowest was detected at Pink Cooperative 908 compared to the control. Similarly, the Yellow Milk cultivar was observed to have the highest significant inhibitory effect compared to the fourteen cultivars. The decrease could depend on the variety, genetic influences, and some of the hand practices. Our experiment also suggests that the root length of Yellow Milk was significantly increased compared to other cultivars. Similarly, the root length of nine cultivars non-significantly decreased among fourteen tomato cultivars. These results conformed to other results reported by several authors [35-37]. Many other scientists have also reported that the physiological parameters like shoot length and root length of tomato cultivars were reduced in the saline condition [17]. Naseer et al. [38] have also reported that NaCl significantly reduced the tomato physiological parameters at 50 and 100 mM NaCl compared to the control.

Conclusions

In the present study, the germination and growth parameters of almost all the tested cultivars were severely affected by saline conditions (1.5 g L^{-1} NaCl). Seed germination of Yellow Milk was significantly increased, but that of the Pink Jade and Red Jade cultivars significantly decreased under the NaCl treatment. Similarly, among the fourteen tomato cultivars, the mean germination time of only Yellow Pearl and Black Current significantly increased. Moreover, the shoot length of eight tomato cultivars was decreased compared with the control, while the highest shoot length was recorded in the case of Saint Cultivar. The root length of Taiwan Red Saint, Purple Beauty, Yellow Milk, Red Pearl, Yellow Pearl, Pink Cooperative 906, Qinzu Shanghai 903, and Scarlet cultivars was significantly increased, while that of the other five cultivars significantly decreased under NaCl treatment compared to control. In conclusion, NaCl stress significantly affects the germination and growth parameters of tested tomato cultivars under *in vitro* culture.

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Conflict of Interest

The authors declare no conflict of interest.

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