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Original Research

Interaction effect between humic acid and salicylic acid on seed germination and seedling growth of *Capsicum annuum* under salt stress

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ABSTRACT:

In order to investigate the interaction effects of humic acid and salicylic acid on seedling growth of sweet peppers under different salinity stresses, a factorial experiment was carried out based on complete randomized design with three replications. Salinity factor was at six levels (0, 30, 60, 90, 120 and 150 mM of NaCl), salicylic acid at three levels (0, 0.5 and 1 mM) and humic acid at two levels (0 and 1.5 g/L). Results showed that germination percentage and shoot length were significantly decreased by increasing salinity, but the highest root length, seedling fresh weight, germination rate and seedling vigor were recorded in 30 mM of NaCl. In all stress levels, germination parameters were increased by salicylic acid and humic acid, and a positive cum significant correlation was observed between germination rate with seedlings and root length. According to the results, it can be recommended for the pre -treatment of sweet pepper seeds with 1 mM salicylic acid and 1.5 g of humic acid to obtain resistant and uniform seedlings under salt stress conditions.

Keywords:

Correlation, Germination percentage, Seed germination, Seedling fresh weight.

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INTRODUCTION

Salinity is one of the most important factors that limit plant growth. The first effect of salinity is non-uniformity in seed germination and seedling growth (Grieve *et al*., 1992). Germination is one of the most sensitive stages in plant growth cycle because germination is important in determining the final plant density under stress conditions (Livington and De Jong, 1990). Creating resistance to environmental stress is one of the important strategy. Salicylic acid, as a group of phenolic compounds, has an aromatic ring attached to a hydroxyl group, which is known as an effective inducer in expressing of gene resistance (Khavari-nejad and Asadi, 2006). Salicylic acid has different physiological roles in plants, including plant growth (Khan *et al*., 2003), flowering induction, food absorption, stomatal movements (Larque, 1979), photosynthesis (Fariduddin *et al*., 2003) and germination (El-Tayeb, 2005; and Raskin 1992). Salicylic acid acts as a signal molecule and then activates the signal cascade pathway by ABA, hydrogen peroxide and calcium, which activates the synthesis of special protein kinases that activate further responses, like changes in gene expression. The response to these signals causes more changes in plant metabolism, which involves the activation and synthesis of antioxidants, the synthesis and accumulation of osmoprotectants such as proline under stress (Farooq *et al.,* 2009). Different researches have shown that pre-treatment of seeds with salicylic acid can reduce the harmful effects of salinity and improve seeds germination (El-Tayeb, 2005; Khodary, 2004; Singh and Usha, 2003; Bagheri kazem abadi *et al*., 1997).

Today, using of organic acids has been developed to improve the quality and quantity of crops, such as humic acid and fulvic acid from various sources like humus, peat, and so on (Sebahattin and Necdet, 2005). Several studies have reported that the use of low concentrations of humic acid improves plant growth (Malik and Azam, 1985; Jack and Evans, 2000). Azam and Mauk (1983) stated that 54 mg/L of humic acid increased significantly root and stem length in wheat. It has been reported that Rosselle (*Hibiscus sabdariffa*) can bear salinity up to 140 mM and maintain stamina root length by 80 mM of humic acid (Sabzevari *et al*., 2010). Also, it has been shown that the use of humic acid causes increasing germination parameters in barley (Asgharipour and Rafiei, 2011).

MATERIALS AND METHODS

In order to study the interaction effects of salicylic acid and humic acid on germination of sweet peppers under salt stress, a factorial experiment based on completely randomized design with three replications was carried out in central laboratory of Torbat-e Jam University on 2018. Salinity factor was at six levels (0, 30, 60, 90, 120 and 150 mM of NaCl), salicylic acid at three levels (0, 0.5 and 1 mM), and humic acid at two levels (0 and 1.5 g/L). The seeds of sweet California variety were used, which were previously disinfected. In each replicate, 25 seeds were used for each treatment. First, the seeds were divided into three groups and each group was exposed to dark conditions at different concentrations of salicylic acid at 10°C for 10 h. The seeds transferred to petri dishes containing Wattem filter paper, and then 5 mL of humic acid solution and salinity were added to them. Then they were placed at 28°C with 85% relative humidity in a germinator with a 12 h light period. The germination record was started after 2 mm of emergence. In this experiment, Germination Percentage (GP), root length, stem length, seed vigor index, Germination Rate (GR) and seedling fresh weight were investigated (Azad *et al*., 2017; Rohani *et al*., 2014).

$GP = 100(n/N)$

n: number of germinated seeds on the n^{th} day

N: total number of seeds

Seed vigor index = GP× seedling length

$GR = \sum N_i/T_i$ (Azad *et al*., 2017; Rohani *et al*., 2014).

In order to measure the fresh weight of seed-

Table 1. Analysis of variance of traits

S. No	S.O.V	df	Germination percentage (%)	Root length (mm)	Shoot length (mm)	Fresh weight (mg)	Germination rate	Seed vigor
1	Salinity (S)	5	482.22 **	3123.86**	413.12**	0.05211**	443.40**	518083.93**
2	Humic acid (H)	1	59.26 ns	172.04**	108.12**	0.00605**	92.52**	61750.44**
3	Salicylic acid (SA)	2	1525.69 **	505.78**	120.46**	0.00311**	112.23**	179948.15**
4	S×H	5	3.70 ns	21.96**	2.73ns	0.00041*	4.94ns	3356.44**
5	S×SA	10	66.25 **	12.70**	4.69**	0.00050**	5.59**	3906.73**
6	H×SA	2	14.12 ns	6.17 ns	4.10*	0.00003ns	1.93ns	4253.39*
7	S×H×SA	10	1.90 ns	2.60 ns	1.02ns	0.00001ns	1.12ns	446.40ns
8	Error	72	22.22	4.55	1.15	0.00017	4.91	545.37

** and * respectively significant of 1 and 5% of probability. ns= non significant

lings, five samples from each replicates were randomly selected and measured by digital scales with the accuracy of 0.0001. Analysis of variance was carried out using SAS software and Duncan's multiple range test calculated at 5% level of probability (Azad *et al*., 2017).

RESULTS AND DISCUSSION

Based on the results of the analysis of variance Table, it was found that simple and interaction effects of salinity and salicylic acid were significant at 1% probability level on all traits. Simple effects of humic acid showed significant effects on all traits at 1% probability level, except on germination percentage. Interaction effects of salinity and humic acid on root length, seedling fresh weight and seed vigor were significant. Also, the results showed that interaction effect of salicylic acid and humic acid only on stem length and seed vigor were significant at 5% probability level.

Germination percentage

Germination rate significantly decreased by increasing of salinity Table 2. The lowest seed germination was obtained in 150 mM treatment of NaCl, which decreased 14.49% compared to the control. The highest and lowest germination percentages were observed in the treatment of 1 mM salicylic acid without salinity and treatment of 150 mM salinity with untreated seeds,

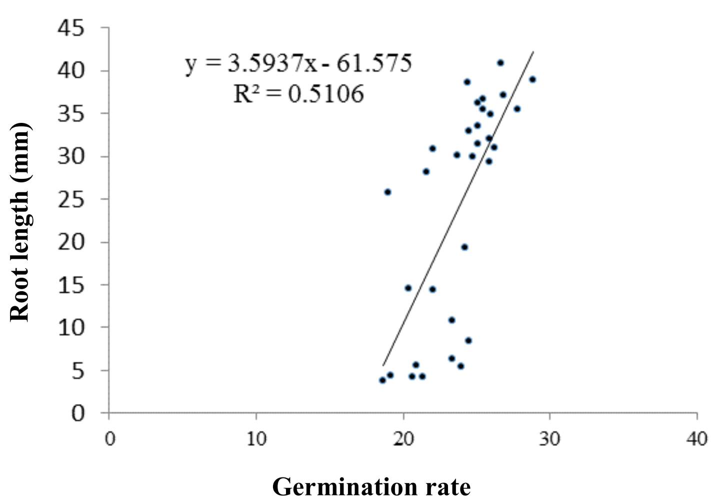

Figure 1. Correlation between germination rate and root length

Table 2. Mean comparison for simple effects of treatment on traits

S. No	Treatment	Level	Germination percentage (%)	Root length (mm)	Shoot length (mm)	Fresh weight (mg)	Germination rate	Seed vigor
1	Salinity	0	97.78 ±3.52	34.85 ± 3.57	15.69 ± 2.43	0.153±0.02	27.44±2.88	494.89±64.63
		30	89.72 ±7.76	40.74 ± 5.37	14.53± 2.94	0.179±0.03	27.07 ±3.38	499.99±106.57
		60	88.61 ±6.82	33.44 ± 4.79	13.19±2.43	0.141±0.02	25.13 ±3.47	415.89±80.84
		90	85.04 ±5.69	29.28 ± 2.57	11.44 ±1.80	0.135±0.01	23.79 ±2.45	347.33±50.75
		120	85.28 ± 8.99	12.54 ± 4.63	6.50 ±1.79	0.072±0.02	17.64 ±2.16	165.74±64.86
		150	83.61 ± 9.36	7.94 ± 3.54	3.57±1.77	0.037±0.01	15.70 ±1.61	98.72±48.04
2	Humic acid	0	87.59 ± 7.99	25.20±12.25	9.82±4.35	0.110±0.05	21.87±4.29	313.17±158.38
		1.5	89.07 ± 9.12	27.73±13.63	11.83±4.50	0.130±0.07	23.72±5.51	360.99±180.61
3	Salicylic acid	0	80.97 ± 8.43	22.63±12.12	8.87±4.35	0.110±0.05	21.10±5.27	261.80±142.83
		0.5	90.69 ± 6.67	26.63±13.06	11.11±4.50	0.121±0.06	22.66±4.65	347.38±169.61
		1	93.33 ± 4.63	30.13 ±12.29	12.49±5.25	0.128±0.05	24.62±5.43	402.07±172.32

respectively (Table 3). In all levels of stress, salicylic acid treatment increased seed germination, which was higher at 1 mM, except for 60 mm NaCl (Table 3). It has been reported that salinity stress reduces germination percentage in radishes (Rohani *et al*., 2014) and *Hibiscus sabdariffa* (Azad *et al*., 2017). Reduction of germination percentage under salt stress has been generally reported due to the lack of ethylene production during water absorption (Orlovsky *et al*., 2011; Chang *et al*., 2010). In this experiment, it was determined that germination was significantly increased by salicylic acid. The obtained results are similar to the study by Sharikova *et al*. (2003).

Root length

As given in Table 2, the highest root length was obtained in 30 mM NaCl, which it increased 16.9% compared to the control, and after increasing salt stress, root length significantly decreased. Interaction effects of salinity and humic acid (Table 4) showed that the highest root length was obtained in 30 mM salinity combination with the seeds treated by humic acid, which increased 29.28% compared to the control and the lowest amount was obtained at 150 mM salinity and whit out

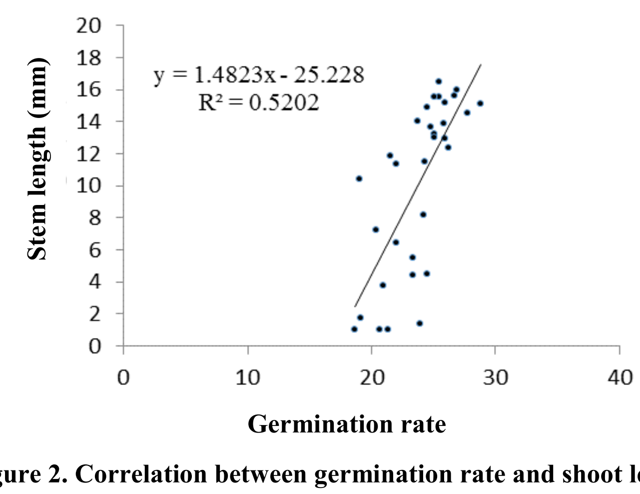

Figure 2. Correlation between germination rate and shoot length

Table 3. Mean comparison for interaction effects of salinity and salicylic acid on traits

S. No	Salinity	Salicylic acid	Germination percentage (%)	Root length (mm)	Shoot length (mm)	Fresh weight (g)	Germination rate	Seed vigor
1	0	0	95.83±4.92	30.30±1.60	13.38±1.19	0.143±0.01	26.68±3.55	418.00±22.23
		0.5	98.33±2.58	36.33±1.10	15.70±0.86	0.147±0.01	26.76±1.73	511.88±25.03
		1	99.17±2.04	37.90±0.97	18.00±2.28	0.169±0.03	28.90±2.97	554.57±36.99
2	30	0	80.00±0.01	36.74±3.88	12.05±1.25	0.160±0.03	25.82±2.74	390.28±31.72
		0.5	93.33±4.08	40.08±3.42	14.20±2.11	0.188±0.02	26.48±3.13	507.10±56.46
		1	65.83±3.76	45.40±5.04	17.33±2.53	0.190±0.02	28.89±3.90	602.60±85.33
3	60	0	80.04±0.02	29.23±3.35	11.28±1.48	0.123±0.01	22.38±1.39	324.11±32.78
		0.5	94.17±2.04	34.70±4.61	13.08±2.10	0.149±0.02	25.90±3.55	450.32±53.71
		1	91.67±4.08	36.40±3.96	15.21±2.03	0.150±0.02	27.10±3.46	473.25±52.72
4	90	0	80.02±0.05	26.13±1.00	9.70±1.08	0.125±0.02	22.11±1.56	286.64±14.82
		0.5	83.33±2.58	30.30±1.41	13.00±1.19	0.140±0.01	22.42±0.72	360.83±22.47
		1	91.67±4.08	31.40±0.98	11.63±1.25	0.140±0.02	26.84±0.85	394.52±26.86
5	120	0	75.00±7.75	8.92±1.07	5.10±1.44	0.076±0.01	15.23±2.03	105.34±20.05
		0.5	83.33±2.58	10.25±0.27	6.30±1.40	0.059±0.03	18.18±0.22	145.99±11.80
		1	92.50±2.74	18.45±2.79	8.10±1.20	0.080±0.02	19.50±0.55	245.88±34.79
6	150	0	75.00±7.75	4.48±0.48	1.68±0.60	0.030±0.01	14.37±1.71	46.42±9.20
		0.5	86.67±9.31	8.13±2.11	4.36±0.59	0.041±0.02	16.24±1.09	108.15±25.98
		1	89.17±3.76	11.20±3.30	4.67±1.87	0.40±0.01	16.50±1.22	141.59±38.64

humic acid. Abdul Qados (2011) reported that plant organ enlargement under low salt concentrations is probably due to activation of osmotic adjustment in plant cells. Root length increased in all treatments with humic acid at all stress levels, which was significant at 30, 60 and 150 mM salt (Table 4). Azad *et al*. (2017) reported that humic acid increased root length of roselle seed under salt stress. Stephan and Charles (1994) also reported a positive effect of humic acid on wheat roots growth. Interactions effect of salinity and salicylic acid are shown in Table 3. The highest and lowest root lengths were obtained in 30 mM NaCl in comparison with 1 mM salicylic acid, and 150 mM NaCl and without salicylic acid respectively. Increasing root length in the presence of salicylic acid has also been reported in the similar studies (Serraj and Sinclair, 2002; Taheri *et al*., 2017). As given in Table 3, the root length decreased at concentrations above 30 mM NaCl, which could be due to the decrease in water absorption (Katergi *et al*., 1994). Also, it has been reported that water removal from cells under salt stress can cause growth reduction (Puppala *et al*., 1999). Salicylic acid application can play a role in cytokinin and auxin regulation, which increase cell division in root meristem and cell elongation (Serraj and Sinclair, 2002). Results also showed that there is a positive and high correlation (r = 0.73) between germination rate and root length (Figure 1).

Stem length

Simple effects of treatments shown in Table 2 prescribes that, stem length was significantly decreased under salinity stress. The least amount of stem length was obtained at 150 mM NaCl, which decreased 24.27% relative to the control. It was determined that

Table 4. Mean comparison for interaction effect of salinity and humic acid on traits

S. No	Salinity (mM)	Humic acid (g/L)	Root length (mm)	Fresh weight (mg)	Seed vigor
1	0	0	34.04±3.65	0.143±0.019	472.14±56.44
		1.5	35.69±3.48	0.163±0.018	517.49±67.35
2	30	0	37.47±3.73	0.161±0.020	452.02±76.26
		1.5	44.01±4.82	0.195±0.018	547.96±114.63
3	60	0	32.24±4.21	0.137±0.017	386.17±67.01
		1.5	34.64±5.26	0.144±0.013	445.62±86.08
4	90	0	28.88±2.62	0.130±0.015	332.75±46.96
		1.5	29.68±2.64	0.140±0.013	361.92±52.18
5	120	0	12.29±4.77	0.067±0.016	156.21±62.90
		1.5	12.79±4.92	0.077±0.017	175.27±69.14
6	150	0	6.33±2.03	0.032±0.007	79.73±31.09
		1.5	9.54±4.08	0.042±0.001	117.70±55.92

stem length increased 20.43% in seeds heated by humic acid compared to the control. Interactions effects of salicylic acid and salinity (Table 3), salicylic acid application increased stem length in all levels of salinity stress than control. This increment was more at 1 mM. Also, 1 mM salicylic acid caused a significant growth in stem length at 150, 120 and 30 mM, compared to 0.5 mM salicylic acid. Mean comparison of interaction effects between humic acid and salicylic acid as given in Table 5. The maximum stem length was obtained in seeds treated by 1mM salicylic acid and 1.5 g humic acid. Rohani *et al.* (2017) reported the humic acid application significantly increased stem length in radish under salt stress. Other researchers that reported humic acid have positive effects on increasing stem length (Kauser *et al*., 1985; Stephan and Charles, 1994; Paksoy *et al*., 2010; Sabzevari *et al*., 2010). Water absorption generally decreases under salt stress which subsequently leads to the reduction of hormones secretion, enzymes activities and plant growth. It is also stated that length stem decrease can be associated with reduced or none transmission of food from cotyledons (Ardalan, 2011). In this experiment, there was a high correlation (r = 0.71) between stem length with germination rate Figure 2.

Fresh weight

The maximum fresh seedling weight was obtained at 30 mM NaCl in combination with humic acid

Table 5. Mean comparison for interaction effect of humic acid and salicylic acid on traits

S. No	Humic acid (g/L)	Salicylic acid (mM)	Shoot length (mm)	Seed vigor
1	0	0	8.14±4.21	247.92±137.54
		0.5	10.20±4.36	324.97±163.97
		1	11.11±4.58	366.61±157.22
2	1.5	0	9.59±4.44	275.67±150.58
		0.5	12.01±4.56	369.78±176.86
		1	13.87±5.64	437.53±183.71

Table 4, and then, with increasing salinity stress, a decreasing trend was observed in the fresh weight of seedlings. Increasing seedling fresh weight at low levels of salinity can be due to the addition of more water to dissolve and reduce ions concentrations that accumulated in vacuoles (Munns, 2002). This results is similar to the other findings of Abdul Qados (2011) and Dantus *et al*. (2005). The maximum fresh weight was obtained at 30 mM NaCl with salicylic acid (Table 3), there was no significant difference between salicylic acid concentrations. The minimum of it was recorded at 150 mM NaCl with none of the treated seeds.

Germination rate

As given in Table 2, humic acid had no significant effect on germination percentage, but germination rate was significantly increased. These results were as same as the studies of Azad *et al*. (2017); Koo (2006) and Sabzevari *et al*. (2010). It was reported that the action of humic material is not recognizable for germination, but it can be due to hormone production especially GA (Gibberellic Acid) and better absorption of nutrients. Down trend of germination rate was observed by salinity increasing, so that the lowest of it was recorded at 150 mM NaCl without salicylic acid (Table 3). At all levels of salinity, salicylic acid application increased germination rate, so that the highest amount of it was obtained at 1 mM salicylic acid without salinity. Wang *et al*. (2006) reported that increasing germination rates in seeds were treated by salicylic acid could be due to its role in increasing levels of auxin and cytokinin hormones. It has been reported that salicylic acid prevent catalytic activity, reducing catalase activity leads to increased activity of hydrogen peroxide, which can improve germination (Nun *et al*., 2003). Salicylic acid also probably stimulates germination through gibberellin biosynthesis (Shah, 2003).

Seed vigor

The highest seed vigor was obtained at 30 mM NaCl with humic acid and then decreased with increasing salinity stress (Table 4). There was no significant difference between 0 and 30 mM NaCl (Table 2). The lowest amount of seed vigor was obtained at 150 mM NaCL and without humic acid (Table 4). Azad *et al*. (2017) also reported that humic acid increases stem vigor in roselle under salt stress. The highest seed vigor was obtained at 30 mM NaCl in combination with 1 mM salicylic acid, which increased 44.13% compared to the control and the lowest amount of it was obtained at 150 mM of that salicylic acid compound with untreated seeds. Ghasemi and Khoramivafa (2012) also reported salicylic acid increased seed vigor under salt stress. Results showed Table 5 that the highest amount of seed vigor was obtained in combination of seeds treated with humic acid and 1 mM salicylic acid, which can be due to the participation of salicylic acid and humic acid in producing plant hormones, that play an important role in the germination and vegetative growth of seedlings.

CONCLUSION

Based on the results, it seems that 1 mM salicylic acid and 1.5 g of humic acid for the pre-treatment of sweet pepper seeds can be recommended to obtain resistant as well as uniform seedlings under salt stress conditions.

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