Turkish Journal of Agriculture - Food Science and Technology, 12(4): 608-618, 2024
DOI: https://doi.org/10.24925/turjaf.v12i4.608-618.6668

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Morphological and Physiological Responses of Different Cotton Genotypes Primed with Salicylic Acid Under Salinity Conditions

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ARTICLE INFO

Research Article

Received : 11.01.2024
Accepted : 29.02.2024

Keywords:
Germination
Gossypium hirsutum
Salinity
Seed priming
SPAD

ABSTRACT

This study was conducted as both petri dishes and pot experiments on four different salt-sensitive cotton genotypes (Laser, May 505, May 455 and Selin) in order to investigate the role of exogenous salicylic acid applications in reducing the effects of salt stress. Six saline treatments; 0, 30, 60, 90, 120 mM NaCl were used. Each group divided into three sub-groups (hydo-primed control, 0.5 mM and 1.0 mM SA) on the basis of seed priming treatments. They were applied in three replications according to the randomized block design. In all genotypes, 90 mM and 120 mM salt stress negatively affected germination and seedling development. In salt stress up to 60 mM, it was recommended to May 505 and Selin genotypes with 0.5 mM salicylic acid pre-application to the seeds.

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Introduction

Cotton is considered a salt-tolerant crop after barley, with a salt stress threshold level of 7.7 dS m^{-1}, with moderate tolerance to salt stress (Alizade & Mammodova, 2023; Muhammad et al., 2023). The roots of the cotton are the first organs to be affected by salt and root development (Öz & Karasu, 2007), growth, yield and fiber quality are adversely affected.

Salt stress is the second most common abiotic stress after drought, negatively affecting plant growth and significantly limiting plant yield (Anwar et al., 2023). Salty and alkaline soils constitute more than 6% of the world's soils. Seed germination is affected by adverse environmental conditions, including salinity (Abdi et al., 2022). Salt stress causes a decrease in biomass, stem thickness, leaf area, root and shoot weight and seed yield in cotton (Sharif et al., 2019) and stimulates antioxidant enzymes such as superoxide dismutase, peroxidase, catalase and glutathione reductase (Alizade & Mammodova, 2023; Muhammad et al., 2023), on the other hand, it tends to decrease as a result of increasing salicylic acid (SA) level (Abdi et al., 2022).

In conditions of intense salt stress, seeds cannot absorb sufficient water due to higher osmotic potential, which reduces or delays the germination process in seeds (Azeem et al., 2019). Salt stress causes a significant decrease in leaf chlorophyll index (Demming & Adams, 1996) and photosynthesis rate, which is one of the main regions in photosynthesis and is directly related to photosynthesis performance (Harizanova & Koleva-Valkova, 2019).

Germination is the most sensitive stage in the life cycle of a plant and salt-induced stress inhibits the germination and development of seeds (Biswas et al., 2023; Radwan et al., 2023; Taşan, 2023). Salinity affects germination negatively in cotton (Malik et al., 1994; Amjad et al., 2002; Munawar et al., 2021) and as the salt dose increases biomass, root length and root surface area decrease. High salt concentration prevents germination and seedling growth in cotton (Yan et al., 2019). Salt stress reduces plant height in cotton (Shahzad et al., 2020), reduces germination rate (Ergin et al., 2021), biomass (Guo et al., 2019), negatively affects growth (Long et al., 2019; Ergin et al., 2021; Hamani et al., 2021).

One of the methods used to alleviate the effects of salt stress is seed priming (Fujikura et al., 1993; Radwan et al., 2023). This technique provides rapid and uniform seedling emergence, plays a role in breaking seed dormancy, increases protein synthesis, plant growth and development by providing resistance to environmental stress factors (Moreno et al., 2018; Moghaddam et al., 2020; Anwar et al., 2023).

Salicylic acid a phenolic phytohormone, causes many metabolic and biochemical changes in germinating seeds (Farooq et al., 2013), regulates growth with various physiological responses and also plays an important role in reducing the effects of temperature, salt, osmotic and oxidative stress (Khan et al., 2012; Riaz et al., 2019; Sofy et al., 2020; Abdi et al., 2022; Biswas et al., 2023; Maqsood et al., 2023; Ogunsiji et al., 2023). It significantly increases salinity tolerance based on glycine betaine to saline conditions, followed by an increase in water content. While salinity stimulates antioxidant enzymes such as superoxide dismutase, peroxidase, catalase and glutathione reductase, it tends to decrease as a result of increasing salicylic acid level (Abdi et al., 2022).

Salicylic acid has a positive effect on growth, yield and quality characteristics of cotton (Al-Rawi et al., 2014), strawberry (Lolaei et al., 2012) and tomato (Yıldırım & Dursun, 2009). Salicylic acid increases plant tolerance to stress conditions by balancing the decrease in dry weight in plants. This is due to the stimulating effect of salicylic acid on shoot growth and the accumulation of more assimilates in the shoots (Pirasteh-Anosheh et al., 2014). With the application of SA to wheat and maize, abscisic acid and indole acetic acid accumulate (Fahad et al., 2015). Salinity reduces fresh weight (40%) and chlorophyll (39%) in wheat, whereas root fresh weight and chlorophyll b increase after 20 mM SA priming to seeds (Maqsood et al., 2023). Salicylic acid increases cell membrane damage caused by salt stress, thus reducing the transpiration rate, facilitating the adjustment of the optimum amount of water in plant tissues and minimizing water loss (Fairoj et al., 2022).

The application of phytohormones such as salicylic acid will be effective in mitigating or minimizing the negative impact of salinity on plant growth and productivity (Moles et al., 2019).

Therefore, it was aimed to determine the effect of salicylic acid priming on seed germination, seedling growth, morphological and physiological parameters in four cotton genotypes grown under salinity stress in this study. The hypothesis is that salicylic acid application may increase the salinity tolerance of cotton genotypes during germination and seedling periods.

Materials and Methods

Location of the Experiment

The study was carried out at Ege University, Faculty of Agriculture, Department of Field Crops and Aydın Nazilli Cotton Research Institute.

Plant Material

The characteristics of four different cotton genotypes used (Laser, May 505, May 455 and Selin) in the experiment are shown in Table 1.

Soil Properties

The properties of the soil used in the experiment are given in Table 2. This soil used was mixed with peat at a ratio of 1:1 and added to each pot equally. A total of 216 pots were used.

According to the soil analyzes, it was determined that the soil texture is sandy-loamy, moderately alkaline, poor in $CaCO_3$ ($CaCO_3$ %<2.5) and there is no salinity problem (total salt %< 0.150).

Treatments and Experimental Design

A completely randomized design was adopted with the levels of different salinity doses (0; 30; 60; 90; 120 mM) and salicylic acid concentrations (0; 0.5; 1.0 mM).

Germination tests were carried out in the growth incubating chamber (28 ± 1°C) in Aydın Nazilli Cotton Research Institute Laboratory, with a diameter of 9 cm and a depth of 2 cm (0.18 lt) petri dishes placed with double filter paper and 15 seeds on them. Pot experiments were carried out in growth incubating chamber, 15 cm diameter and 20 cm deep pots (3 L) in Aydın Nazilli Cotton Research Institute Laboratory.

Table 1. Characteristics of cotton genotypes used in the experiment

Properties	Genotypes			
	Lazer	Selin	May455	May505
100 grain weight (g)	7.78	8.43	11.01	9.59
Fiber fineness (mikroinere)	4.6 - 4.8	4.7	4.4 - 4.8	4 - 4.6
Fiber strength (g/tex)	34 - 36	31.5	32 - 35	32 - 36
Fiber length (mm)	31 - 32	28.7	30 - 31	30
SCI	160 - 180	-	135	135
Boll opening rate	Medium	Late	Early	Early
Average seed cotton yield ($kg.ha^{-1}$)	58.4	54.8	56.2	41.6
Ginning outturn (%)	45 - 47	42.8	44 - 46	41- 43
Fiber yield ($kg.ha^{-1}$)	26.52	23.46	24.15	20.13

Table 2. Properties of the soil used in the experiment

pH	NaCl (%)	$CaCO_3$ (%)	Sand (%)	Silt (%)	Clay (%)	P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)
7.9	0.15	2.04	65.7	26.9	7.4	2.65	145
	Na ($mg.kg^{-1}$)	Fe ($mg.kg^{-1}$)	Ca ($mg.kg^{-1}$)	Mg ($mg.kg^{-1}$)	Zn ($mg.kg^{-1}$)	Cu ($mg.kg^{-1}$)	S ($mg.kg^{-1}$)
	118	7.09	2230	457	0.38	1.12	120.4

Seed Priming

Cotton seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 2 minutes and then thoroughly washed with sterilized water (Azeem et al., 2019). Seeds were soaked either in water (hydro-priming) or 0.5 and 1.0 mM SA solutions (SA priming), for 12 h.

Seed Germination in Laboratory

Healthy and uniform seeds from primed treatments were transferred into petri plates (15 seeds per plate), lined with double layer of filter paper. Petri plates were divided into five groups, moistened with 10 ml of 1) distilled water (non-saline control), 2) 30 mM NaCl solution, 3) 60 mM NaCl solution, 4) 90 mM NaCl solution and 5) 120 mM NaCl solution. Each group was further divided into three treatments, 1) hydro-priming 2) primed with 0.5 mM SA and 3) primed with 1.0 mM SA. Each treatment had 3 replicates. Petri-plates were placed in growth incubating chamber at 28 ± 1ºC, (12 h light and 12 h dark) for 14 days. A total of 216 petri plates were used. The protuberance of radical (2 mm) was considered as a mark of germination (Mohammadi, 2009). After 14 days, seedlings (3 of each replicates) were kept in oven (70ºC for 48 h) for dry weight measurements. The plumule and radicle lengths, fresh weight, dry weight and chlorophyl content index (SPAD) were measured.

Pot experiment

Fifteen seeds of three priming treatments (0, 0.5 and 1 mM SA) were sown in 3 L plastic pots containing (15-cm diameter at the top and 20-cm depth) filled with 2.8 kg of soil and peat at 1:1 ratio. By using 15.10.10 compound fertilizer, 15 kg of pure nitrogen, 10 kg of pure phosphorus and 10 kg of pure potassium were fertilized per decare. Pots were placed in climate laboratory with 6 saline treatments, 1) 0 mM (non-saline), 2) 30 mM NaCl, 3) 60 mM NaCl, 4) 90 mM NaCl and 5) 120 mM NaCl were used. Each group divided into three sub-groups on the basis of seed priming treatments 1) hydo-primed control, 2) 0.5 mM SA and 3) 1.0 mM SA. All pots were placed according to completely block design. Pots were observed every day from the start of the study and thinned 28 days after the emergence of seeds.

At the end of four weeks, the plants were harvested and observations were taken. Root length (cm), shoot length (cm), root, shoot fresh and dry weights (g) and chlorophyl content index (SPAD) were evaluated in the seedlings.

Results and Discussions

Labaratory Petri Plates Analyses

The results of the analysis of variance for the observations measured 14 days after sowing of cotton genotypes in which different salt doses were applied under laboratory conditions are summarized in Table 3. The interaction of genotype x salicylic acid x salinity was found to be statistically significant in other properties except plumula and radicle length. Besides, salicylic acid x salinity interaction was found to be significant for plumula length and genotype x salicylic acid interaction for radicle length.

Germination Percentage (%) and Chlorophyll Content Index (SPAD)

The germination percentage of Selin genotype was the highest (100%) under control conditions, followed by Lazer genotype (80%) (Table 4). However, in both genotype, it was observed that the germination percentage was negatively affected by salicylic acid application under salt stress conditions. In general, for all genotypes, the germination (%) decreased at 90 mM salt stress. Although the Selin genotype had the highest germination rate, salicylic acid application could not prevent the decrease in germination as the salt stress increased.

Azeem et al. (2019) emphasized that as salt stress increases in wheat, germination rate is inhibited, and seed priming process significantly reduces seed dormancy. Salicylic acid application to broad bean seeds against salt stress improved germination (Anaya et al., 2018). A positive effect of 20 mM dose of salicylic acid on seed germination rate was observed (Heidarian & Roshandel, 2021). In another study, salicylic acid (10 mM) increased germination percentage in black bean under salt stress (50 mM and 100 mM) conditions. To reduce the adverse effects of salinity on seed germination and plant performance of *Citrullus lanatus*, priming with the pre-sowing seaweed *Ulva lactuca* improved germination and seedling growth (Radwan et al., 2023). In a study conducted on wheat, it was observed that salicylic acid application against salinity increased germination and seedling growth (Shakirova, 2007).

Under salt stress conditions, the effect of salicylic acid applications on SPAD values of genotypes was found to be significant (Table 4). The genotype with the highest SPAD value was Laser.

Table 3. Analysis of the variance of the data of petri plates observations of cotton genotypes

Sources of Variation	DF	Mean Square Values					
		GP	PL	RL	FW	DW	CCI
Genotype (A)	3	33.79 **	177.73 **	416.72ns	12.559 **	0.369 **	289.46 **
Salicylic acid (B)	2	26.39 **	88.29 ns	89.38ns	11.059 **	0.273 **	207.67 **
Salinity (C)	5	66.06 **	95.55 *	226.57 **	2.595 **	0.074 **	84.41 **
A×C	15	25.54 **	41.84ns	86.79ns	0.764ns	0.066 **	88.86 **
A×B	6	4.48ns	26.72 ns	253.43 **	0.519ns	0.021ns	121.07 **
B×C	10	32.99 **	97.71 *	64.81ns	1.529 *	0.078 **	90.91 **
A×B×C	30	24.89 **	33.52ns	57.71ns	1.180 **	0.063 **	73.86 **
Error	142	2.67	40.49	65.56	0.678	0.019	5.63

GP: Germination percentage; PL: Plumule length; RL: Radicle length; FW: Fresh weight; DW: Dry weight; CCI: Chlorohyll content index (SPAD); * and ** indicate significance at 0.05 and 0.01 levels of probability, respectively. ns; not significant;

Table 4. Effect of seed priming of salicylic acid on germination percentage and SPAD value of cotton genotypes

SA (mM)	Salinity (mM)	Germination percentage (%)				Chlorophyll content index (SPAD)			
		Lazer	Selin	May 455	May 505	Lazer	Selin	May 455	May 505
0	0	80.0b	82.2a	66.6d	71.1c	33.53a	23.53b	32.38a	24.93b
	30	64.4a	24.4d	60.0b	62.2a	32.30a	22.94b	33.84a	19.71b
	50	37.7b	28.8d	33.3c	62.2a	30.53a	20.06bc	16.38c	23.62b
	60	28.8d	62.2b	73.3a	53.3c	30.18a	30.65a	26.94ab	23.47b
	90	24.4d	53.3c	60.0a	55.5b	30.13ab	26.36b	33.10a	18.92c
	120	77.7b	100.0a	48.8d	75.5c	30.33a	23.63b	25.98b	27.35ab
0.5	0	68.8a	53.3c	53.3c	64.4b	39.37a	23.39c	23.35c	27.69b
	30	73.3b	84.4a	20.0d	66.6c	19.30b	22.57ab	12.73c	24.66a
	50	53.3d	75.5a	62.2c	68.8b	33.54a	21.33c	19.51c	25.31b
	60	51.1c	37.7d	60.0a	57.7b	33.87a	16.85c	29.35b	30.89ab
	90	22.2d	75.5a	46.6b	33.3c	17.99b	24.13a	17.81b	20.61ab
	120	17.7c	28.8b	26.6b	75.5a	18.94bc	22.43b	17.75c	32.35a
1.0	0	46.6b	26.6d	42.2c	68.8a	26.94a	17.97b	21.50b	20.69b
	30	62.2b	62.2b	64.4a	28.8c	29.86a	20.63bc	23.29b	16.89c
	50	73.3a	28.8d	57.7b	35.5c	33.63a	27.22b	23.27c	21.23d
	60	26.6d	55.5a	33.3c	48.8b	29.24a	25.80a	20.27b	21.16b
	90	24.4c	68.8a	11.1d	55.5b	19.73b	22.57b	14.07c	28.37a
	120	15.5d	53.3a	40.0c	42.2b	17.77c	24.06b	34.19a	25.38b
		$LSD_{(A\times B\times C)}$ = 2.652				$LSD_{(A\times B\times C)}$= 3.850			

Table 5. Effect of seed priming of salicylic acid on plumule length of cotton

SA (mM)	Salinity (mM)	Plumule length (mm)			
		Lazer	Selin	May 455	May 505
0	0	23.70a	27.30a	23.70a	23.30a
	30	26.00a	26.87a	28.88a	27.18a
	50	26.14a	25.00a	18.33a	24.14a
	60	25.39a	27.79a	21.98a	23.37a
	90	17.79a	21.90a	16.91a	24.26a
	120	23.67a	27.28a	27.37a	23.28a
0.5	0	21.83a	21.83a	21.04a	24.83a
	30	16.10b	25.20a	14.40b	21.40a
	50	31.87a	21.95a	29.58a	25.98a
	60	20.33b	27.67ab	26.49b	37.60a
	90	17.77a	22.83a	20.85a	25.07a
	120	11.36b	20.83ab	22.92a	22.60a
1.0	0	17.15a	17.67a	23.46a	21.67a
	30	18.33a	18.51a	27.10a	18.10a
	50	19.40a	21.87a	22.32a	24.33a
	60	20.33a	25.95a	20.83a	25.05a
	90	19.37a	20.70a	14.83a	24.83a
	120	20.0a	25.75a	26.04a	27.71a
		$LSD_{(B\times C)}$= 10.322			

Application of 0.5 mM salicylic acid under control conditions increased all genotypes except May 455 genotype. However, the SPAD value decreased with increasing salicylic acid dose. In a study conducted under salt stress conditions in wheat, it was found that chlorophyll content, tillering number and K^+/Na^+ ratio decreased, but there was a significant improvement after salicylic acid application (Suhaib et al., 2018).

Plumule Length (cm)

The most important parameters in the sensitivity of seeds to salinity are root and plumule length (Jamil et al., 2006). Plumula length was significantly affected by salicylic acid and salt applications (Table 5). However, difference was not significant between genotypes in terms of plumule lengths. *Lathyrus sativus* L. seeds primed with salicylic acid gave longer radicles and plumules length, regardless of the salicylic acid dose. Salicylic acid application under salt stress increased the fresh and dry weight of *Lathyrus sativus* L. seedlings. The effect on seedling dry weight was observed only at a dose of 0.2 mM salicylic acid (Moghaddam et al., 2020). In another study, salt stress adversely affected germination, root length, plumule length, root fresh weight, plumule fresh weight and mean germination time in canola. However, in canola primed with ascorbic acid, it was observed that ascorbic acid alleviated the negative effect of salt on these properties (Taşan, 2023).

Radicle length (cm)

The Lazer genotype under control condition gave the highest radicle length with 0.5 mM and 1.0 mM salicylic acid doses (Table 6). Delavari et al. (2014) reported that due to salicylic acid application to *Ocimum basilicum* under salt stress, osmotic stress decreased and better water uptake was achieved, germination, root and shoot length, fresh and dry weight increased. In saline conditions, soaking bell pepper with salicylic acid improved relative moisture content, radicle and seedling length, dry weight, and vigor, mitigating the toxic effects of salt on the plant (Júnior et al., 2020). Similarly, as the salt dose increased, the decrease in *Lathyrus sativus* plumula and seedling length was greater. Salinity and priming with salicylic acid affected the radicle, plumule and seedling length of *Lathyrus sativus*, but it turned out that the observed effect was not dependent on the dose of salicylic acid (Moghaddam et al., 2020).

Fresh and Dry Weight (g)

The highest fresh weight value of seeds germinated in petri dishes was obtained in the Selin genotype with 3.78 g under control conditions (Table 7). After salicylic acid application (0.5 mM) under salt stress conditions (50 mM), May 505 variety ranked second with 3.60 g. As salt stress increased, a decrease was observed in the Selin genotype fresh weight value, while a high decrease was not detected in the May 505 genotype. In addition, at the highest salt dose (120 mM), fresh weights increased as the salicylic acid dose increased in all cultivars. Priming the seeds with salicylic acid against salt stress causes the plants to accumulate abscisic acid for adaptation.

Abscisic acid promotes various anti-stress proteins that protect plants against stress conditions (Pirasteh-Anosheh et al., 2014; Heidarian & Roshandel, 2021). The cultivar with the highest dry weight was Selin genotype with 0.79 g under the conditions of 120 mM salt application and no salicylic acid (Table 7). Following this, under 120 mM NaCl conditions, May 505 genotype (0.70 g) without salicylic acid gave the highest value, followed by Selin genotype. May 505 genotype gave generally higher values compared to other genotypes when salicylic acid and NaCl doses were taken into account. A study conducted revealed that salt stress significantly reduced seedling dry weight and priming with 0.2 mM salicylic acid increased (Moghaddam et al., 2020). External salicylic acid application to the plant improved fresh weight, dry weight, leaf number (Hayat et al., 2005) and leaf area (Khan et al., 2003a). Treatment of wheat seeds with salicylic acid increased the seedlings' tolerance to salt (Hamada & Al-Hakimi, 2001; Shakirova et al., 2003). There was a significant decrease in growth parameters in cumin under severe salt stress (50 mM NaCl), but application of Amla extract to cumin seeds before sowing improved plant height, number of branches, fresh weight, number of seeds and seed weight, and photosynthetic pigments (Said & Mohammed, 2023).

Pot Experiment Results

Root and shoot related values and chlorophyll content index (SPAD) were significantly affected by the genotype × salicylic acid × salinity interaction (Table 8, Table 9).

Table 6. Seed priming of salicylic acid regulates the radicle length of cotton

SA (mM)	Radicle length (mm)			
	Lazer	Selin	May 455	May 505
0	34.95 a	20.49 b	23.04 b	23.84 b
0.5	25.77 ab	20.99 b	23.35 ab	27.14 a
1.0	24.59 a	24.75 a	23.14 a	20.93 a
	LSD $_{(A×B)}$= 5.362			

Table 7. Effect of seed priming of salicylic acid on fresh and dry weight of cotton

SA (mM)	Salinity (mM)	Fresh weight (g)				Dry weight (g)			
		Lazer	Selin	May 455	May 505	Lazer	Selin	May 455	May 505
0	0	1.52b	3.78a	2.95a	2.57a	0.43a	0.63a	0.56a	0.58a
	30	1.76ab	0.98a	2.40a	2.26ab	0.41ab	0.21b	0.52a	0.50a
	50	2.18ab	1.78b	0.94b	2.52a	0.48a	0.25b	0.26ab	0.41ab
	60	0.70b	2.04a	2.91a	2.66a	0.20b	0.53a	0.68a	0.62a
	90	0.67b	1.22ab	1.63ab	2.11a	0.22b	0.37ab	0.53a	0.46a
	120	1.38b	3.14a	1.95ab	2.78a	0.42c	0.79a	0.54bc	0.70ab
0.5	0	1.23a	1.90a	2.07a	2.43a	0.40a	0.45a	0.49a	0.49a
	30	1.19ab	2.29a	0.43b	1.93a	0.38a	0.54a	0.12b	0.54a
	50	1.19c	2.07bc	2.84ab	3.60a	0.36b	0.50ab	0.55ab	0.63a
	60	0.99b	1.11b	1.77ab	3.07a	0.22b	0.31ab	0.46a	0.52a
	90	0.59a	1.50a	1.32a	1.29a	0.14b	0.48a	0.30ab	0.26ab
	120	0.30b	0.86b	0.94b	2.57a	0.06b	0.17b	0.24b	0.67a
1.0	0	0.98ab	0.68b	0.41ab	2.07a	0.27b	0.20b	0.51a	0.57a
	30	1.03a	1.24a	1.13a	0.87a	0.35ab	0.37ab	0.43a	0.17b
	50	1.22a	0.85a	1.65a	1.71a	0.47a	0.22b	0.54a	0.34ab
	60	0.60a	1.70a	0.96a	1.38a	0.16b	0.46a	0.32ab	0.43a
	90	0.57ab	1.51ab	0.31b	1.77a	0.14b	0.50a	0.08b	0.46a
	120	0.43b	1.43ab	1.73ab	2.20a	0.10b	0.47a	0.43a	0.43a
		LSD$_{(A×B×C)}$= 1.335				LSD $_{(A×B×C)}$= 0.225			

Table 8. Analysis of the variance of the root data of pot experiments of cotton genotypes

Sources of Variation	DF	Mean Square Values		
		Root length	Root fresh weight	Root dry weight
Genotype (A)	3	13.39 **	0.16 **	0.018 **
Salicylic acid (B)	2	73.55 **	0.22 **	0.014 **
Salinity (C)	5	422.39 **	0.83 **	0.007 **
A×C	15	92.00 **	0.17 **	0.033 **
A×B	6	55.34 **	0.21 **	0.010 **
B×C	10	153.80 **	0.26 **	0.011 **
A×B×C	30	102.66 **	0.19 **	0.011 **
Error	142	0.09	0.00	0.00

* and ** indicate significance at 0.05 and 0.01 levels of probability, respectively. ns; not significant

Table 9. Analysis of the variance of the shoot data of pot experiments of cotton genotypes

Sources of Variation	DF	Mean Square Values			
		Shoot length	Shoot fresh weight	Shoot dry weight	Chloroyll content index (SPAD)
Genotype (A)	3	285.48 **	2.37 **	0.023 **	774.81 **
Salicylic acid (B)	2	69.77 **	0.19 **	0.094 **	781.81 **
Salinity (C)	5	1010.90 **	37.55 **	0.433 **	702.99 **
A×C	15	56.27 **	5.68 **	0.047 **	138.98 **
A×B	6	36.30 **	9.69 **	0.093 **	171.59 **
B×C	10	56.93 **	5.09 **	0.043 **	309.78 **
A×B×C	30	34.25 **	6.21 **	0.048 **	128.19 **
Error	142	0.08	0.00	0.00	5.96

Table 10 Effect of seed priming of salicylic acid on root and shoot length of cotton genotypes

SA (mM)	Salinity (mM)	Root length (cm)				Shoot length (cm)			
		Lazer	Selin	May 455	May 505	Lazer	Selin	May 455	May 505
0	0	9.53d	10.3c	23.53a	14.50b	18.38d	28.50a	19.43c	23.87b
	30	20.60a	15.47c	17.83b	21.10a	18.33d	27.93a	20.00c	21.93b
	50	16.60b	3.33c	19.33a	16.17b	17.70b	4.33c	17.53b	23.27a
	60	11.33d	19.73c	23.47b	30.93a	17.20b	17.43b	19.40a	15.50c
	90	21.93b	25.77a	15.37d	17.93c	10.60c	18.53a	18.00b	18.27ab
	120	4.30b	3.67b	3.67b	3.33b	5.27a	4.67b	4.67b	4.33b
0.5	0	13.93b	14.77a	14.27a	12.20c	16.93d	22.80a	22.13b	21.20c
	30	11.33c	27.30a	19.20b	11.50c	21.83a	20.83b	20.40b	20.57b
	50	29.30a	19.50b	19.33b	14.20c	18.07c	16.90d	21.23a	19.83b
	60	27.30a	15.27c	27.30a	16.83b	13.27c	16.77a	16.23b	17.17a
	90	9.30d	22.87a	11.43c	20.93b	7.83d	13.03b	12.23c	18.03a
	120	17.27a	3.33d	5.77c	14.20b	5.73b	4.33c	5.83b	11.20a
1.0	0	18.00b	14.37d	16.00c	18.93a	16.67d	26.20b	25.73c	32.17a
	30	14.57d	15.90c	17.40b	24.80a	15.50c	19.33b	17.67c	20.17a
	50	9.87d	21.83b	23.20a	13.37c	13.87c	21.77b	23.97a	21.83b
	60	22.43a	13.50d	14.87c	16.43b	7.87d	19.83b	20.40a	17.63c
	90	16.27c	22.50a	19.80b	15.80c	12.17bc	12.43b	11.87c	24.43a
	120	21.80a	14.90c	10.80d	20.67b	10.33b	5.77c	10.40b	19.23a
		$LSD_{(A×B×C)}$=0.50				$LSD_{(A×B×C)}$=0.444			

Root and Shoot Length (cm)

In Table 10, it was revealed that the root length decreased as the salt dose increased. The highest root length was obtained from May 505 genotype (30.93 cm) in pots with 60 mM NaCl dose and no salicylic acid application. In the experiment, the highest value Lazer genotype was observed after 0.5 mM salicylic acid application against salt stress. However, as the Laser genotype and salt dose increased, it was determined that salicylic acid applications were not effective on root length. By increasing the salicylic acid dose to 1.0 mM, the Lazer genotype with a root length of 22.43 cm at 60 mM NaCl stress and the May 505 genotype with a root length of 24.80 cm at 30 mM NaCl stress came to the fore. It was observed that the highest shoot length value was not salt stress, but May 505 genotype was obtained with 1.0 mM dose of salicylic acid. In terms of shoot length, May 505 genotype also gave high values under salt stress conditions. Salicylic acid doses (1.0 mM) under high salt stress to Lazer, Selin and May 455 genotypes did not have a positive effect on shoot length values. In pots without salt stress and salicylic acid applied, Selin genotype gave the highest shoot length of 28.50 cm. Increasing salt doses caused a significant decrease in shoot length in all cultivars with salicylic acid. The Laser genotype gave a value of 21.83 cm with 0.5 mM SA at 30 mM salinity and the May 455 genotype gave a value of 21.23 cm. In black bean, salinity caused a decrease in seedling length, but priming with 10 mM salicylic acid gave the most effective result compared to 2 and 20 mM doses and the seedling length increased under salt stress (Heidarian & Roshandel, 2021). Hussein et al. (2007) emphasized that salicylic acid increased growth in maize against saline conditions. Salicylic acid increased growth and yield in chickpea under salt stress conditions (Riaz et al., 2019). Priming with salicylic acid suppressed the phytotoxic effects caused by salinity in cotton, affected plant growth, improved the phenotypic appearance of the plant and increased salt tolerance in cotton (Keya et al., 2023).

Root Fresh Weight and Shoot Fresh Weight (g)

In Table 11, the highest root fresh weight was observed in the Selin genotype (1.54 g), which was not treated with salicylic acid at 30 mM salt stress. It was revealed that root growth decreased in all cultivars in general at 120 mM NaCl dose, where salt stress was the highest. However, increasing the salicylic acid had a positive effect on root growth. The highest root fresh weight was obtained in the Selin genotype under control conditions. However, the root fresh weight of Selin genotype decreased significantly when salt doses were increased, except for 30 mM salt stress. In the applied 50 mM NaCl and 0.5 mM salicylic acid, the Laser genotype had the same value at 1.0 mM salicylic acid, and May 455 was in the front row with 0.88 g. The highest shoot fresh weight (90 mM salt + 0.5 mM salicylic acid) was obtained from May 455 genotype with 10.22 g (Table 11). In all other genotypes, shoot fresh weight decreased significantly as salt stress increased, regardless of salicylic acid dose. In the experiment where salicylic acid was not applied and the salt stress was 30 mM, the highest shoot fresh weight was obtained as 7.03 g from Selin genotype. As the salicylic acid dose increased in Selin genotype, shoot fresh weight decreased. Salt dosage caused a decrease in shoot fresh weight. When the salicylic acid dose was increased to 0.5 mM, the May 505 genotype ranked higher in high salt conditions. Further increasing the salicylic acid dose resulted in less decrease in fresh weight values. While high values were obtained at the 1.0 mM SA without salinity, the salicylic acid effect was less at the 120 mM salt dose. The highest value in shoot fresh weight values was obtained from applications without the use of salicylic acid. Salicylic acid-induced abiotic stress tolerance is due to the fact that osmolyte accumulation via salicylic acid helps maintain osmotic homeostasis and improves the regulation of mineral substance intake (Abdi et al., 2022). Salicylic acid to maize exposed to salt stress improved plant height, root and shoot fresh and dry weight (Khodary, 2004). Priming against salt stress caused an increase in seedling weight (Heidarian & Roshandel, 2021). Foliar salicylic acid application to cotton against salinity reduced the negative effect of salt on cotton seedlings (Hamani et al., 2021).

Root Dry Weight and Shoot Dry Weight (g)

The highest root dry weight was obtained from Selin genotype with 0.36 g (90 mM salt + 0 mM salicylic acid) (Table 12). However, in terms of root fresh weight, Selin genotype had the highest value at 30 mM salt stress. This shows that as the salt stress increases, the organic and inorganic substances in the roots decrease. Increasing the dose of salicylic acid also had no effect on root dry weight. Root dry weight values gave high values in cultivars that were not treated with salicylic acid under salt stress conditions.

Table 11. Effect of seed priming of salicylic acid on root and shoot fresh weight of cotton genotypes

SA (mM)	Salinity (mM)	Root fresh weight (g)				Shoot fresh weight (g)			
		Lazer	Selin	May 455	May 505	Lazer	Selin	May 455	May 505
0	0	0.26d	0.74a	0.61b	0.37c	3.10b	6.81a	2.36d	2.53c
	30	0.75b	1.54a	0.43c	0.37d	3.91b	7.03a	2.90c	2.76d
	50	0.68a	0.07d	0.36c	0.41b	3.20a	0.35d	1.91c	2.61b
	60	0.36c	0.29d	0.67a	0.54b	2.61a	1.60c	2.23b	1.46d
	90	0.89b	1.50a	0.42d	0.50c	2.92a	2.83a	1.70c	2.09b
	120	0.09a	0.06b	0.06b	0.07b	0.35a	0.35a	0.35a	0.35a
0.5	0	0.35c	0.44b	0.50a	0.33d	2.11d	2.76c	3.48a	2.92b
	30	0.51b	0.74a	0.43c	0.28c	4.37a	2.85b	1.65d	2.08c
	50	0.88a	0.74b	0.53c	0.38d	3.32a	2.81c	2.98b	2.01d
	60	0.31b	0.31b	0.29c	0.46a	1.52c	1.68b	1.26d	1.87a
	90	0.09d	0.36b	0.21c	0.86a	0.61d	1.37c	10.22a	1.85b
	120	0.22a	0.06c	0.07b	0.23a	0.51b	0.35c	0.53b	0.96a
1.0	0	0.50c	0.46d	0.67a	0.57b	2.18d	4.98a	3.73c	4.77b
	30	0.35d	0.50c	0.64b	0.86a	2.34c	2.79b	2.25c	3.49a
	50	0.20d	0.84b	0.88a	0.36c	1.55c	2.90b	3.18a	3.09a
	60	0.23d	0.59a	0.53c	0.55b	0.63d	2.56b	2.76a	2.06c
	90	0.41b	0.32d	0.35c	0.57a	1.70b	1.33c	1.06d	2.96a
	120	0.40b	0.18d	0.20c	0.52a	0.82b	0.46c	0.89b	1.82a
		$LSD_{(A×B×C)}$=0.009				$LSD_{(A×B×C)}$=0.100			

Table 12 Effect of seed priming of salicylic acid on root and shoot dry weight of cotton genotypes

SA (mM)	Salinity (mM)	Root dry weight (g)				Shoot dry weight (g)			
		Lazer	Selin	May 455	May 505	Lazer	Selin	May 455	May 505
0	0	0.04c	0.09a	0.09a	0.05b	0.28b	0.74a	0.28b	0.28b
	30	0.10b	0.26a	0.08c	0.04d	0.44b	0.86a	0.33c	0.30d
	50	0.18a	0.01c	0.08b	0.03c	0.47a	0.08d	0.26c	0.37b
	60	0.06c	0.04c	0.28a	0.09b	0.30c	0.19d	0.44a	0.24b
	90	0.13b	0.36a	0.09c	0.08c	0.41b	0.48a	0.28d	0.32c
	120	0.02a	0.01a	0.01a	0.02a	0.05b	0.09a	0.08a	0.08a
0.5	0	0.04b	0.06b	0.09a	0.05b	0.27d	0.29c	0.48a	0.31b
	30	0.07b	0.16a	0.09b	0.04c	0.57a	0.34c	0.42b	0.23d
	50	0.15a	0.14a	0.11b	0.05c	0.52a	0.40b	0.39b	0.29c
	60	0.04a	0.05a	0.05a	0.05a	0.20b	0.21b	0.18c	0.27a
	90	0.02d	0.08b	0.04c	0.11a	0.09d	0.22c	0.23b	0.33a
	120	0.05a	0.01b	0.03ab	0.033a	0.09b	0.09b	0.09b	0.14a
1.0	0	0.07ab	0.05b	0.11a	0.09a	0.24d	0.59b	0.50c	0.63a
	30	0.09b	0.07b	0.09b	0.11a	0.36b	0.35b	0.36b	0.50a
	50	0.03d	0.17b	0.26a	0.06c	0.19d	0.43b	0.50a	0.42c
	60	0.08b	0.11a	0.10a	0.07b	0.17d	0.36b	0.42a	0.31c
	90	0.07b	0.06b	0.13a	0.07b	0.28b	0.20d	0.24c	0.49a
	120	0.04b	0.02c	0.02c	0.07a	0.21b	0.09d	0.19c	0.33a
		LSD $_{(A\times B\times C)}$=0.023				LSD $_{(A\times B\times C)}$=0.010			

Table 13 Effect of seed priming of salicylic acid on chlorophyll content index (SPAD) of cotton genotypes

SA (mM)	Salinity (mM)	Chlorophyll content index (SPAD)			
		Lazer	Selin	May 455	May 505
0	0	39.95c	46.92b	43.37bc	58.43a
	30	55.33b	44.67c	45.47c	61.13a
	50	53.87a	32.67c	46.80b	53.23a
	60	51.83bc	47.95c	60.98a	54.32b
	90	49.98b	57.00a	60.83a	58.48a
	120	33.66a	22.37b	31.73a	31.33a
0.5	0	42.39c	58.68a	48.18b	50.82b
	30	44.58b	40.85b	57.15a	59.32a
	50	55.67b	46.55c	40.75d	62.08a
	60	60.49a	41.93c	46.90b	60.67a
	90	48.60b	52.17b	52.16b	56.92a
	120	46.00b	32.37d	40.27c	60.10a
1.0	0	56.18b	60.20a	50.20c	45.20d
	30	49.80b	61.63a	58.72a	59.17a
	50	42.60c	47.12b	60.00a	43.12c
	60	61.28a	44.04b	61.27a	60.93a
	90	48.97b	50.83b	60.03a	59.32a
	120	61.55a	56.12b	49.23c	51.98c
	LSD $_{(A\times B\times C)}$= 3.959				

Selin was the genotype that gave the highest value in terms of shoot dry weight (Table 12). The highest value in terms of shoot dry weight was obtained from May 455 genotype. However, this value was found to be low in shoot dry weight. This situation can be interpreted as the high water intake capacity of May 455. In all cultivars, the increase in salt dose caused a decrease in shoot dry weight. However, May 505 genotype gave higher values at 1.0 mM salicylic acid dose compared to control conditions at 120 mM salt stress. This reveals that the May 505 genotype can be grown under high salt stress conditions with salicylic acid application. In the absence of salt stress, the Selin genotype may be preferred. Salicylic acid application to wheat increased root and shoot dry weight under salt stress conditions (Azeem et al., 2019; Abdi et al., 2022). Priming of baby corn seeds with SA in saline conditions (6 dS m^{-1}) significantly increased the root dry weight. At high salt concentrations (9-12 dS m^{-1}), treatment of seeds with salicylic acid had no significant effect. Priming baby corn seeds with 1 mM SA at moderate salinity has been suggested for production (Islam et al., 2022). Salicylic acid application under salt stress caused a significant increase in shoot dry weight in wheat (Arfan et al., 2005). Similar results were obtained in dry leaf weight of cotton (Hussein et al., 2012).

Chlorophyll Content Index (SPAD)

The highest SPAD value in plants was obtained from the May 505 genotype containing 0.5 mM salicylic acid under 50 mM salt stress (Table 13). In 0.5 mM salicylic acid application, SPAD value was higher than control at 120 mM salt stress in all cultivars. In applications where salt stress is high, the addition of salicylic acid caused an increase in the SPAD values of the plants. The lowest SPAD value was observed in Selin genotype with 0.5 mM salicylic acid application at 120 mM salt stress.

Pancheva et al. (1996) emphasized that the application of more than 1 mM SA reduces the rate of photosynthesis. Soaking wheat seeds with salicylic acid against salinity significantly reduced NaCl-induced phytotoxicity in terms of chlorophyll index (Alam et al., 2022). SPAD values obtained after salicylic acid applied to *Vigna radiata* grown under salinity are an important criterion for evaluating plant lines, and it can be used as a physiological measurement criterion in determining photosynthetic performance in plants under salt stress (Ogunsiji et al., 2023). High NaCl content caused a decrease in chlorophyll content due to inhibition of chlorophyll biosynthesis in wheat (Khan, 2003b). Salicylic acid application in salt conditions improved chlorophyll value in cotton (Souza et al., 2023). Photosynthesis and chlorophyll synthesis are inhibited in plants exposed to high salt (Cha-Um et al., 2010; Mahboob et al., 2016; Mahboob et al., 2017), but salicylic acid reduces the negative effects (El Tayeb, 2005; Afzal et al., 2006; Farooq et al., 2007; Hussain et al., 2011; Rehman et al., 2011; Pirasteh-Anosheh et al., 2012). In salt stress conditions, SA to wheat improved seedling length and total chlorophyll content (Azeem et al., 2019). Salicylic acid application under salt stress conditions increased the chlorophyll content index (SPAD) in barley (Pirasteh-Anosheh et al., 2014). Similar studies were also observed in different plants (Khodary, 2004; Parida et al., 2008; Nikolaeva et al., 2010; Pirasteh-Anosheh & Emam, 2012). Hamid et al. (2010) revealed that priming wheat seeds with salicylic acid under salinity stress conditions made the seedlings stronger and increased the plant chlorophyll content.

Conclusion

In the present study, we explored the exogenous SA-induced salt tolerance in cotton. High dose salt concentrations (90, 120 mM) negatively affected germination and development in seeds. Salicylic acid application was significantly effective in the germination and seedling growth of cotton seeds. Therefore, to obtain high yields in cotton under saline conditions, priming the seeds with 0.5 mM SA will be effective. Priming with SA reduced the degradation of photosynthetic pigments while increasing plant biomass. Exogenously applied SA increased the salinity tolerance of Selin and May 505, particularly by reducing the negative effects of salts. Salicylic acid application (0.5 mM) had positive effects on germination percentage, plant height, fresh and dry weight and SPAD parameters in cotton. Selin and May 505 genotypes can be recommended to be planted after priming the seeds with salicylic acid (0.5 mM) for soils with 50 mM-60 mM salt concentration.

Acknowledgements

We highly appreciate the Nazilli Cotton Research Institute for providing the cotton genotypes. We express the profound sense of reverence to the entire research team and any other person who contributed.

Author Contributions: Data curation, NO; Project administration, IY, EI. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest

Data availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

This Research was Conducted as a Master's Thesis

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