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EVALUATION OF THE EFFECT OF EXOGENOUS APPLICATION OF INDOLE-3-ACETIC ACID ON MORPHOPHYSIOLOGICAL PARAMETERS UNDER SALT (NaCl) STRESS IN COTTON (*G. hirsutum* L.)

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Abstract. Soil salinity is caused by a high concentration of soluble salts that retain water in the soil and accentuate the problem of desertification. In this research, the effect of Indole-3-Acetic Acid (IAA) on germination parameters, total chlorophyll content and morphometric parameters of cotton seedlings under stress conditions of 100 mM and 200 mM NaCl was evaluated in 5 cultivars. An increase in final germination percentage (FGP) was determined in all cultivars affected by IAA in the control variant and in 3 cultivars under both stress conditions. Among the genotypes treated with IAA under 250 mM concentration of NaCl, the highest value of germination index was observed in Bless (176) and Livzara (181) varieties. Treatment with IAA at a concentration of 250 mM salt caused an increase in total chlorophyll content (TCC) in all genotypes. The application of IAA in Ayzek variety caused an increase in the height and mass of the root and shoot only under the condition of plant growth regulator (PGR) + without NaCl.

Keywords: *Gossypium hirsutum, salt stress, chlorophyll content, germination index, indole-3-acetic acid.*

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1. Introduction

Salinity is an edaphic stress that has affected 45 million hectares of irrigated land out of 230 million hectares, causing annual losses of approximately US$ 12 billion globally and is a major threat to global agricultural productivity (FAO, 2020; Saleem *et*

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al., 2021). Excessive concentration of soluble salts in soils negatively affects agricultural lands and crops and subsequently the livelihood of people all around the world (Alizada *et al*., 2020; Alizade, 2022a; Alizade & Mammadova 2023). Agriculture is one of the mainincome sector of the economy of developing countries (Mammadova *et al*., 2023; Mammadova *et al*., 2024a; Salimov *et al*., 2024) and more than 100 countries are facing the problem of soil salinity along with salinization of groundwater (Alizade *et al*., 2023, Alizada & Aliyeva, 2024). Excessive salts primarily disrupt the cellular osmotic balance by lowering the water potential inside cells. The salts like chlorides and sulphates of sodium, calcium and magnesium along with sodium carbonate and sodium bicarbonate prevailed in saline soils negatively affect plant growth and productivity as they change the osmotic balance between plant roots and soil and interfere with physiological and metabolic processes of plant (Srivastava *et al*., 2019). The reduction in plant growth exposed to saline environments could be due to either adverse water relations or the effects of ions on metabolism.

Phytohormoness are active members of the signal compounds involved in the induction of plant stress responses (Pedranzani *et al*., 2018; Shahzad *et al*., 2022; Amrahov *et al*., 2024). IAA is the most common phytohormone of the auxin class and it regulates various aspects of plant growth and development (Fu *et al*., 2015; Alizade, 2022b). IAA can control cell elongation, vascular tissue development and apical dominance (Wang *et al*., 2001; Mammadova *et al*., 2024b).

The IAA signal transduction pathway in plants has been described in detail (Zhang *et al*., 2022). Also, it has been reported that IAA responds -to salinity in plants (Latef *et al*., 2021; Ma *et al*., 2022; Chiangmai *et al*., 2022; Alam *et al*., 2020). GA3 application during the salinisation period partly overcome the effect of salinity on reducing IAA levels and this shows that salinity may influence hormone balances by affecting plant growth and development. Significant reduction in IAA levels in rice five days after NaCl treatment (Nilsen & Orcutt, 1996) and also salinity caused 75% reduction in IAA levels of tomato (Dunlap & Binzel, 1996).

However, little information seems to be available on the relationship between salinity stress and auxin levels in plants. The variations in indol asetic acid (IAA) content under stress conditions appeared to be similar to those of abscisic acid (Ribaut & Pilet, 1991) and increased levels of IAA have also been correlated with reduced growth (Ribaut & Pilet, 1994). Therefore, reduction in plant growth under stress conditions could be an outcome of altered hormonal balance and hence, their exogenous application provides an attractive approach to counter the stress conditions.

Among industrial crops, cotton is one of the most valuable as it is used as raw material for textile industruy, to make biofuel and edible oil (Jenkins *et al*., 2014; Mammadova *et al*., 2021; Khalid *et al*., 2023; Akparov *et al*., 2021). Throughout its lifespan, cotton is subjected to a variety of biotic and abiotic stresses, with salinity being one of the most serious threats to global cotton production. Salinization of the cultivated land is the major cause of less yield production of cotton that is a great economical loss worldwide (Alizada *et al*., 2024). This current research was conducted to show the effect of exogenous application of indole-3-acetic acid on germination parameters, total chlorophyll content and vegetative growth parameters in cotton under salt stress (NaCl) conditions.

2. Materials and methods

The research was carried out in the Department of Industrial and Forage Crops of the Institute of Genetic Resources of the Ministry of Science and Education of Azerbaijan. Five cotton varieties (Bless, Livzara, Lodos, Turkan, Ayzek) belonging to the *G. hirsutum* L. species were used as the research material. The seeds of cotton genotypes were pre-treated with 0.2% potassium permanganate for 8 minutes. For evaluation germination and morphophysiological parameters experimental design was based on a combination of two factors: stress (0, 100 and 250 mM NaCl) and treatment of IAA (1 μM). There were six treatment conditions as follows: control (C), 0 mM NaCl; 100 mM NaCl; 250 mM NaCl; 0 mM NaCl + IAA; 100 mM NaCl + IAA; 250 mM NaCl + IAA.

For the study of germination parameters, 25 seeds of each genotype were planted in 6 variants in Petri dishes in 4 replications and germinated seeds were counted every day for 10 days.

Final Germination Percentage (1) calculated as follows:

$$\mathrm{FGP} = \frac{Na}{Nt}\ x\ 100, \tag{1}$$

where, *Na* is the number of germinated seeds and *Nt* is the total number of planted seeds.

Coefficient of velocity of germination (2) (CVG or Kotowski`s) calculated by the formula proposed Bewley and Black (1994) (Kotowski, 1926; Scott *et al*., 1984):

$$\mathrm{CVG} = \frac{\sum_{i=1}^{k} N_i}{\sum_{i=1}^{k} N_i T_i}\ x\ 100\ , \tag{2}$$

where, *Ti* is the time from the start of the experiment to the *i*th interval, *Ni* is the number of seeds germinated in the *i*th time interval and *k* is the total number of time intervals.

Mean germination (3) time (MGT or *T*) calculated by the formula proposed Ranal and Santana (2006):

$$\mathrm{MGT} = \frac{\sum_{i=1}^{k} N_i T_i}{\sum_{i=1}^{k} N_i}\ x\ 100\ , \tag{3}$$

where, *Ti* is the time from the start of the experiment to the *i*th interval, *Ni* is the number of seeds germinated in the *i*th time interval (not the accumulated number, but the number corresponding to the *i*th interval) and *k* is the total number of time intervals.

Germination Index (*GI*) (4) estimated as follows (Melville *et al*., 1980):

$$GI = \sum_{\mathrm{i}=0}^{\mathrm{k}} \frac{|(T_k - T_i)\, N_i|}{N_t}\ , \tag{4}$$

where, *Ti* is the time from the start of the experiment to the *i*th interval (day for the example), *Ni* is the number of seeds germinated in the *i*th time interval (not the accumulated number, but the number corresponding to the *i*th interval), *Nt* is the total number of seeds used in the test and *k* is the total number of time intervals.

Germination Rate Index (Speed of germination or index of velocity of germination or Emergence rate index) (5) calculated as follows (Wardle *et al.*, 1991):

$$GRI = \sum_{i=0}^{k} \frac{N_i}{T_i}, \quad (5)$$

where, *Ti* is the time from the start of the experiment to the *i*th interval, *Ni* is the number of seeds germinated in the *i*th time interval (not the accumulated number, but the number corresponding to the *i*th interval) and *k* is the total number of time intervals.

For evaluation morphophysiological indicators 4 seeds for each genotype were planted in 7 cm plastic cups containing perlite in five replications. The seedlings were grown in a phytotron (Taisite, GZX-300 E) at relative humidity of 65-75% and average night and day temperature ranging from 22 to 24 °C. The light length was 14/10 hours day/night. After germination, plants were thinned out and each pot contained 2 cotton plants.

As a nutrient medium was used Steiner's solution (https://s3-eu-west-1.amazonaws.com/pstorage-acs-6854636/5112742/es0501971_si_001.pdf) during the entire growth period, starting from the first days of planting. IAA priming variants were treated with 1 μM solution of IAA. The experiment was designed as a completely randomized model with three replications for each treatment and each replication contained 10 cotton plants. The plants were grown under these treatment conditions for 28 days.

After 28 days of salinization, measurement of the root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW) and shoot dry weight (SDW), root fresh height (RFH), shoot fresh height (SFH), root dry height (RDH) and shoot dry height (SDH) were recorded from 10 plants. The dry weights were measured after drying in an aerated oven at 80 °C for 96 h to reach a constant weight. Total chlorophyll content determinations was carried out by using the method proposed by Rosyara (2010).

The mean ± SD values were calculated using statistical functions of Microsoft Excel 2010 (Microsoft Corporation, USA). One-way analysis of variance (ANOVA) was performed using SPSS software (IBM SPSS v.25).

3. Results

The salts in the soil prevent the water from entering the seed by either constituting osmotic pressure which creates a barrier to the seed or toxic effects caused by Na^+ and Cl^- ions. Germination and growth of seedlings under stress conditions, an indicator routinely employed to assess the degree of damage to plant cells (Tarchoun *et al.*, 2022). Pre-soaking treatment may be used to mitigate the impact of many stresses on seed germination. Seed treatment with plant growth regulators (PGR) is sometimes an easy and low-cost approach to alleviate the detrimental effects of stresses (Salih *et al.*, 2022). The main effects of IAA and salinity on cotton variety germination parameters were shown in Table 1.

Increasing of salinity level caused a significant decrease in final germination percentage, coefficient of velocity of germination, germination rate index and germination index in all studied genotypes while mean germination time delayed. The CVG gives an indication of the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases (Kader, 2005).

Table 1. Germination parameters of cotton genotypes

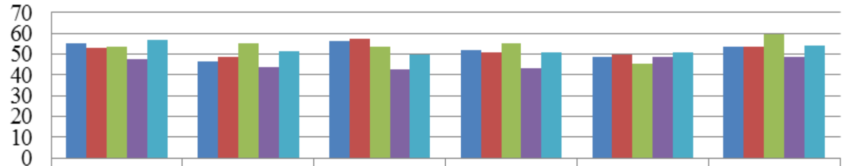
Genotype	Treatment	FGP%	CVG	MGT	GRI	GI
Lodos	Control	91	28.17	3.55	27.67	678
	Control + IAA	94	28.39	3.52	28.83	703
	100 mM NaCl	69	26.74	3.74	20.45	501
	100 mM NaCl + IAA	72	25.99	3.84	20.91	515
	250 mM NaCl	18	18.37	5.44	3.59	100
	250 mM NaCl + IAA	20	16.28	6.14	3.71	102
Bless	Control	95	30.45	3.28	30.93	733
	Control + IAA	98	30.25	3.31	31.68	754
	100 mM NaCl	70	29.54	3.39	22.83	533
	100 mM NaCl + IAA	75	27.57	3.63	23.55	553
	250 mM NaCl	27	19.85	5.04	6.21	161
	250 mM NaCl + IAA	32	18.18	5.51	6.85	176
Livzara	Control	94	27.17	3.68	27.87	688
	Control + IAA	97	26.94	3.71	28.53	707
	100 mM NaCl	68	24.19	4.13	18.08	467
	100 mM NaCl + IAA	73	19.16	5.22	14.83	422
	250 mM NaCl	31	17.22	5.81	5.71	161
	250 mM NaCl + IAA	35	17.16	5.83	6.43	181
Turkan	Control	94	25.89	3.86	26.5	671
	Control + IAA	95	26.46	3.78	27.37	686
	100 mM NaCl	70	22.74	4.39	18.69	482
	100 mM NaCl + IAA	70	22.58	4.43	17.48	460
	250 mM NaCl	29	18.23	5.48	5.58	160
	250 mM NaCl + IAA	30	17.44	5.73	5.48	158
Ayzek	Control	92	28.53	3.51	27.82	682
	Control + IAA	93	29.81	3.35	29.77	711
	100 mM NaCl	67	24.36	4.11	18.04	462
	100 mM NaCl + IAA	60	23.91	4.18	15.87	409
	250 mM NaCl	28	16.57	6.04	4.95	139
	250 mM NaCl + IAA	25	16.44	6.08	4.38	123

After the application of IAA, no significant change in the FGP parameter was recorded in 100 mM concentration of NaCl in the Turkan variety and a decrease was observed in the Ayzek variety under both salt concentration conditions. After the application of IAA, the GI index increased in all samples, except for the 100 mM salt concentration of NaCl salt in the Livzara variety and both salt variations in the Ayzek variety.

As a result of the comparative analysis of stress and stress + IAA variants, a decrease in the MGT indicator and an increase in the CVG indicator were observed in all genotypes. In general, with the application of IAA, the increasing of the FGP parameter under 100 mM concentration of NaCl was observed in Bless and Livzara varieties and at 250 mM salt concentration it was observed in Bless variety. The results obtained on the change of germination indicators with the increase of the severity of stress were similar to the results of the experiments conducted by Hmissi et al. (2003) on wheat (*Triticum durum* Desf.), Ergin et al. (2021) on the cotton plant. In cotton the application of 20 mg L^{-1} IAA in the medium without NaCl caused an increase in Germination index, Germination rate and Mean germination time (Zhao *et al*., 2020). Furthermore, the interaction between application of IAA and salinity levels significantly affected final germination percentage in wheat (Saeidi *et al*., 2014).

Chl content is considered to be one of the parameters of salt tolerance in crop plant (Heidari *et al.*, 2014) and Chl *a* and *b* and chlorophyll SPAD can be used to discriminate between salt-tolerant and salt-sensitive varieties (Saleh, 2012).

Among the samples, an increase in the total chlorophyll content was determined in the control + IAA only in Ayzek variety. The increase of total chlorophyll content in 100 mM NaCl + IAA variant compared to 100 mM NaCl was determined in Ayzek, Turkan and Livzara varieties. In variant 250 mM NaCl + IAA, the TCC was higher than the 250 mM NaCl in all varieties. After application of IAA, the highest chlorophyll contents were recorded in Ayzek variety for all variants (Figure 1).



	Control	Control+IAA	100 mM NaCl	100 mM NaCl + IAA	250 mM NaCl	250 mM NaCl + IAA
Lodos	55,2	46,2	56,2	52	48,6	53,7
Bless	52,9	48,9	57,3	51	49,7	53,4
Ayzek	53,6	55,1	53,8	55,3	45,4	59,5
Turkan	47,4	43,8	42,9	43,4	48,6	48,8
Livzara	57	51,2	49,6	51	50,6	53,9

Figure 1. Total chlorophyll content in different variants by varieties

In all samples, except the Turkan variety, it was determined that the total chlorophyll content was lower than the control at high salt concentration (250 mM). Under 100 mM concentration of NaCl, the increasing of the total content of chlorophyll compared to control was determined in Lodos, Bless and Ayzek varieties. In cotton it was determined that compared to control plants, under 150 mM concentration of NaCl stressed plant had significantly greater chlorophyll content (SPAD value). The plants grown under salt stress environment had 11.64% higher SPAD, than those grown under control (Ma *et al.*, 2021). In maize foliar application of IAA improved the chlorophyll level in salinity-stressed plants. Foliar application of IAA, especially at 2 mM, counteracted some of the salt induced adverse effects by enhancing essential inorganic nutrients as well as by maintaining membrane permeability (Kaya *et al.*, 2010). During the research conducted on two okra genotypes, Esan et al. (2020) observed increasing of chlorophyll b content under 100 mM NaCl treated than the control group.

Application of IAA led to the increase of FSH, FSW, DSH and DSW parameters in Lodos cultivar under both stress conditions (Table 2). In Bless variety, treatment with IAA led to an increase in FRH index in both stress variants, DRH and DRW under concentration of 100 mM NaCl and FSW and DSW parameters under stress of 250 mM NaCl concentration.

Application of IAA increased FSH and DSH in control variant of Livzara variety, under 100 mM salt concentration of salt of DRW, under 250 mM concentration of salt of FSW and DSW and under both stress conditions FRH, FRW and DRH parameters. Treatment with IAA in the control variant of the Turkan cultivar caused an increase in all studied morphometric parameters, however, under concentration of 100 mM of NaCl, only the height of the shoot and root were increased. The application of

IAA in the Ayzek variety led to an increase in the values of all studied morphometric parameters only in the control variant.

Table 2. Morphometric parameters of cotton genotypes

	Variants	FRH	FSH	FSW	FRW	DRH	DSH	DSW	DRW
Lodos	Control	11.1±0.566	13.8±1.061	1.690±0.059	0.142±0.011	8.0±0.354	12.2±0.990	0.128±0.009	0.010±0.001
	Control + IAA	3.4±1.058	7.8±1.106	0.688±0.059	0.090±0.027	2.5±0.252	6.5±0.954	0.057±0.006	0.006±0.003
	100 mM NaCl	4.1±1.485	5.8±2.192	0.652±0.035	0.109±0.024	3.2±1.202	4.1±1.485	0.065±0.008	0.007±0.003
	100 mM NaCl + IAA	2.7±0.557	7.8±2.326	0.919±0.292	0.057±0.032	1.7±0.361	6.5±1.819	0.089±0.023	0.005±0.005
	250 mM NaCl	1.7±0.495	3.4±0.424	0.262±0.042	0.019±0.002	1.4±0.495	3.0±0.424	0.025±0.005	0.004±0.001
	250 mM NaCl + IAA	1.3±0.424	4.7±1.202	0.410±0.037	0.019±0.004	1.1±0.212	3.4±0.566	0.048±0.005	0.003±0.002
Bless	Control	3.7±1.404	8.2±2.895	0.894±0.395	0.105±0.025	3.0±1.252	6.2±2.959	0.073±0.026	0.009±0.003
	Control + IAA	3.2±1.445	5.5±1.882	0.486±0.158	0.073±0.030	2.8±1.839	5.4±2.030	0.046±0.011	0.005±0.004
	100 mM NaCl	2.7±0.600	8.5±2.626	0.963±0.400	0.075±0.014	1.9±0.239	6.6±2.616	0.072±0.027	0.007±0.001
	100 mM NaCl + IAA	3.6±1.606	7.7±1.086	0.670±0.208	0.032±0.013	2.7±1.273	6.1±0.760	0.065±0.014	0.008±0.003
	250 mM NaCl	4.3±0.636	5.5±1.697	0.297±0.121	0.056±0.008	2.7±1.980	4.9±1.273	0.030±0.002	0.009±0.005
	250 mM NaCl + IAA	5.3±2.116	3.9±1.178	0.381±0.111	0.050±0.024	2.4±1.064	3.4±1.721	0.046±0.007	0.006±0.003
Livzara	Control	4.5±2.227	7.2±1.751	0.557±0.147	0.067±0.020	3.2±1.681	5.2±1.598	0.048±0.009	0.007±0.002
	Control + IAA	2.0±0.700	7.9±1.652	0.473±0.221	0.060±0.007	1.8±0.656	6.3±1.562	0.042±0.011	0.005±0.004
	100 mM NaCl	2.1±0.071	9.3±1.556	0.928±0.132	0.075±0.026	1.9±0.566	8.0±1.697	0.067±0.008	0.006±0.001
	100 mM NaCl + IAA	3.4±0.905	6.2±2.461	0.575±0.178	0.077±0.019	2.3±1.349	5.1±2.367	0.055±0.012	0.009±0.003
	250 mM NaCl	2.8±1.344	5.3±0.212	0.391±0.018	0.032±0.005	2.1±1.202	4.4±0.354	0.038±0.006	0.010±0.001
	250 mM NaCl + IAA	3.6±2.033	4.3±0.987	0.429±0.063	0.039±0.013	2.3±1.389	3.4±1.084	0.054±0.007	0.008±0.001
Turkan	Control	3.9±0.354	10.0±0.919	0.732±0.023	0.067±0.006	3.2±0.424	8.5±0.495	0.059±0.005	0.010±0.002
	Control + IAA	4.1±0.141	11.7±0.283	0.801±0.026	0.069±0.004	3.3±0.141	10.0±0.212	0.061±0.011	0.012±0.002
	100 mM NaCl	2.4±0.849	8.9±0.566	0.714±0.091	0.074±0.030	1.7±1.202	7.3±1.061	0.062±0.003	0.007±0.003
	100 mM NaCl + IAA	2.5±0.283	9.8±0.495	0.668±0.016	0.050±0.004	2.0±0.283	7.9±0.283	0.059±0.004	0.005±0.003
	250 mM NaCl	2.4±0.283	5.2±0.141	0.397±0.035	0.047±0.008	1.8±0.354	4.2±0.212	0.052±0.005	0.009±0.004
	250 mM NaCl + IAA	1.9±0.764	3.6±1.411	0.313±0.169	0.028±0.019	1.1±0.513	2.7±1.457	0.047±0.008	0.003±0.001
Ayzek	Control	4.2±0.424	12.1±0.778	0.612±0.043	0.055±0.008	3.5±0.354	9.8±1.202	0.052±0.004	0.009±0.003
	Control + IAA	4.5±0.071	12.8±0.990	0.714±0.040	0.060±0.006	3.8±0.071	11.4±0.778	0.061±0.005	0.010±0.002
	100 mM NaCl	4.1±0.354	9.9±0.707	1.004±0.032	0.067±0.006	3.4±0.141	8.7±0.636	0.083±0.008	0.006±0.002
	100 mM NaCl + IAA	4.0±2.043	6.9±2.021	0.664±0.241	0.032±0.021	3.4±1.563	5.4±1.659	0.067±0.017	0.006±0.002
	250 mM NaCl	4.7±0.354	5.6±0.212	0.333±0.016	0.043±0.016	3.9±0.283	4.2±0.141	0.043±0.004	0.009±0.002
	250 mM NaCl + IAA	4.4±1.862	3.9±0.518	0.322±0.072	0.025±0.014	3.1±1.610	2.8±0.643	0.040±0.009	0.003±0.003

Latef et al. (2021) determined that FRW, FSW, DRW and DSW increased as a result of application of 1.15 mM IAA in faba plant (*Vicia faba* L.) under 150 mM concentration stress of NaCl salt. During the study of the combined effect of 0, 40, 60 or 80 mM NaCl with 0, 7 or 14 µM IAA on in vitro-grown *Solanum tuberosum* L. cultivars, it has been determined that IAA successfully alleviated the harmful effects of salt stress on all of the studied growth parameters (Khalid & Aftab, 2020). During the application of IAA to olive plantlets (*Olea europaea* L.) plants under 0 mM, 100 mM and 200 mM concentrations of NaCl, it was observed that DRW was decreased under 0 mM of NaCl, increased under 100 mM NaCl and remained unchanged under 100 mM NaCl (Cordovilla *et al*., 2023).

4. Conclusions

During the conducted research, it was determined that salt stress causes different changes in morphometric indicators in different cotton varieties. At the same time, the application of IAA under stress conditions was found to cause different changes in morphophysiological parameters in different cotton varieties.

The results obtained in this study can help in determining the effective dose of exogenous application of IAA to eliminate the harmful effect of salt stress. Therefore, more work needs to be done to determine the effect of exogenous application of IAA in open field conditions on biometric parameters under salt stress.

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