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ROLE OF CYCLOHEXIMIDE IN ALLEVIATED SALT STRESS IN TERMS OF SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN CUCUMBER (*CUCUMIS SATIVUS* L.)

Maha Hussein and Evan Ibrahim Merhij*

Department of Biology, College of Science, University of Babylon, Babel, Iraq

Abstract

This research was carried out in order to study the effect of cycloheximide in alleviation salt stress. *Cucumis sativus* L. exposed to salt stress and treated with cycloheximide in terms of some physiological and biochemical indicators. Cucumber seeds were germinated with different concentrations of NaCl (0, 25, 75, 125, 150) mM for 7 days and concentration 150 mM was the most stressful concentration. NaCl caused decrease in germination indices as germination percentage (55.56%). Also, seeds germinated in different cycloheximide concentrations (0, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5) µg/L and the concentration of 0.2 µg/L was the optimum concentration for germination of cucumber seeds, the germination percentage (87%), this was not significantly different from the control treatment (92.5%). After determination the stressful NaCl concentration as well as the effective cycloheximide concentration. An experiment was conducted to determine the appropriate priming time at a concentration of CHX 0.2µg/L cycloheximide for three time durations (2, 4 and 6 hours) and the effective priming time is 2 hours in terms of germination indices, as well biochemical parameters, since α-amylase activity increased as well as chlorophyll content and carbohydrates content as compared to salt treatment.

Key words*:* Salt stress, cycloheximide, carbohydrates, α-amylase activity, *Cucumis sativus* L.

Introduction

Due to the climate change environmental stresses are frequently associated with plant production significantly. Worldwide, abiotic stresses have been estimated to reduce average crop yields by 50% (Hasanuzzaman *et al.,* 2012). Salinity is one of the harsh environmental conditions and one of the factors limiting plant crop productivity, since most plants are sensitive to salinity caused by high salt concentration in the soil (Shrivastava and Kumar, 2015). *Cucumis sativus* L. is a salty medium-sensitive plant, so its exposure to salinity causes many harmful morphological, physiological and biochemical effects. Organic and inorganic compounds have been used by many researchers to minimize the negative impact of salt stress (Tatlioglu, 1997). Mahajan and Tuteja (2005) noted that salinity negatively affected germination and plant growth as well as physiological processes (photosynthesis, respiration and transpiration), nutritional balance, membrane properties, cellular balance, metabolic activities and metabolism. Also, salinity affects the reduction of vegetative and root length and dry weight in all species (Ologundudu *et al.,* 2014). Saline stress also breaks down protein, reduces chlorophyll and inhibits electron transport (Tuna and Higgs, 2008). During germination, the embryo triggers starch degradation by releasing gibberellins, which stimulate cells of the aleurone layer to secrete degradative enzymes into the endosperm (Beck and Ziegler, 1989). A number of enzymes vital to starch degradation have been identified from this system. One of the most important enzymes is α-amylase, an endohydrolase that is able to rapidly degrade the starch into soluble substrates for other enzymes to attack (Beck and Ziegler, 1989). However, the circumstances of starch degradation in endosperm are unlike those in other plant tissues, as the majority of enzymes involved in breakdown of endosperm starch are secreted enzymes, acting in an acellular matrix rather than within a living organelle.

Material and Methods

Cucumber (*Cucumis sativus* var. Ghazeer) seeds provided from Chile. NaCl solutions were prepared in

***Author for correspondence :** E-mail: evan.ebraheem@yahoo.com

Table 1: Effect of NaCl on some germination indices.

Parameters / NaCl Conc. (mM)	Germination percentage %	Fresh Weight (g)	Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
	Mean±S.D				
0	1.6±99.3	0.05±1.08	0.02±0.13	0.30±4.08	0.19±1.93
25	11.2±81.7	0.04±1.06	0.01±0.11	0.50±1.80	0.34±0.65
75	8.4±67.0	0.01±1.05	0.19±0.002	0.22±0.73	0.02±0.40
125	7.7±50.0	0.13±0.62	0.02±0.09	0.25±0.63	0.22±0.50
150	6.7±44.0	0.06±0.75	0.01±0.10	0.17±0.68	0.17±0.38
Value LSD (0.05)	8.099	0.142	0.029	0.288	0.505
Sig.	0.00012	0.0025	0.00019	0.00088	0.001

Table 2: Effect of different cycloheximide concentrations on germination indices.

Parameters / CHX Conc. (µg/L)	Germination percentage %	Fresh Weight (g)	Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
	Mean±S.D				
0	9.6±92.5	0.01±0.097	0.003±0.0122	0.7±7.2	0.6±6.1
0.2	0.87±2.3	0.009±0.094	0.001±0117.0	1.3±5.7	0.9±5.4
0.4	6.0±77.0	0.01±0.083	0.001±0.0107	1.6±4.2	0.8±3.4
0.6	6.8±81.0	0.02±0.080	0.001±0090.0	1.4±3.3	0.9±4.6
0.8	6.0±77.0	0.008±0.089	0.002±0107.0	2.0±5.0	0.7±4.3
1	3.8±79.0	0.01±0.085	0.004±0083.0	1.9±3.7	1.1±3.3
2	4.0±82.0	0.03±0.091	0.001±0093.0	0.4±1.1	0.1±0.5
3	5.1±82.0	0.02±0.069	0.002±0153.0	1.3±2.5	0.2±1.2
4	5.7±80.0	0.01±0.052	0.001±0143.0	0.7±2.2	0.6±2.1
5	6.0±79.0	0.007±0.063	0.001±0157.0	1.6±3.9	0.8±2.2
LSD (0.05)	7.007	0.016	0.002	0.843	0.742
Sig.	0.025	0.0038	0.0095	0.0023	0.0017

concentrations (25, 75, 125 and 150) mM and distilled water applied as control treatment. Seven replicates of 25 seeds were germinated in petri dishes contained filter papers with 10 ml of solutions for 7 days in plant growth chamber at 25±1°C, RH 60-70%. Germination indices (germination percentage, radical and shoot lengths and fresh and dry seedlings weight) were measured at the end of experiment. The experimental design was in a CRD with 7 replicates and 25 seeds per replicate. α-amylase activity was measured according to method of William and Peterson (1973). Carbohydrates content was determined according to Yemm and Wills (1954), chlorophyll while gibberelline was determined to method (Ergun *et al*., 2002).

Results and Discussion

Cucumber is moderately sensitive to stresses, including salt stress (Tatilioglu, 1997). The physiological growth indicators decreased with increasing salt concentration and the concentration was 150 mM as the most stressful (Table 1), which significantly reduced the percentage of germination, which was consistent with the results of Chartzoulakis (2015), this decrease by germination due to the negative effect of NaCl. It is attributed to low α-amylase activity (Add *et al*., 2014, Liu *et al*., 2018). Also, the effect of salinity on α-amylase activity depends on the concentration of NaCl, it decreased when salt concentration reached 150 mM (Adda *et al*., 2014).

Turan *et al*., (2009) have indicated that NaCl salt reduces the carbohydrate content that is necessary for growth by reducing the efficiency of photosynthesis. Also, total carbohydrates in seeds were reduced by increased seawater salinity levels (Sadak *et al*., 2015). Reduced fresh and dry weights of cucumber seedlings are prone to saline stress (Trajkova and Papadantonakis, 2006). The

Table 3: Effect of cycloheximide in alleviation of salt stress in terms of germination indices.

Priming Time (h)	Subsequent treatment for 7 days	Germination percentage %	Fresh Weight(g)	Dry Weight (g) Mean±S.D.	Shoot Length (cm)	Root Length (cm)
Distilled water	Distilled water	2.31±98.67	0.04±1.06	0.013±0.125	0.36±4.10	0.21±1.97
NaCl (150mM)	NaCl (150mM)	5.64±36.67	0.03 ±0.86	0.190±0.025	0.17±0.50	0.10±0.30
2	Distilled water	4.01±96.00	0.04±1.07	0.005±103.0	0.50±5.00	0.73±2.33
2	NaCl (150mM)	4.00±76.00	0.06±0.78	0.028±0.139	0.12±0.93	0.40±0.93
4	Distilled water	1.20±96.00	0.01±1.10	0.016±129.0	0.50±3.50	0.29±1.83
4	NaCl (150mM)	2.31±72.67	0.19±0.70	0.041±170.0	0.17±1.40	0.15±0.83
6	Distilled water	1.00±92.00	0.05±1.16	0.013±119.0	0.76±3.83	0.64±2.23
6	NaCl (150mM)	2.36±78.67	0.03±0.56	0.012±137.0	0.36±1.10	0.10±0.50
LSD (0.05)		5.222	0.109	0.031	0.599	0.800
Sig.		0.0012	0.0025	0.0019	0.0088	0.001

Table 4: Effect of cycloheximide in alleviation of salt stress in terms of chlorophyll content.

Priming Time (h)	Subsequent treatment for 48 days	A	B	Total
		Mean±S.D.		
Distilled water	Distilled water	0.53±1.66	1.26±5.66	1.94±7.32
NaCl (150mM)	NaCl (150mM)	0.48±1.08	1.09±3.41	1.31±4.89
2	Distilled water	0.28±1.27	0.46±3.81	0.21±5.08
2	NaCl (150mM)	0.02±1.44	0.37±3.92	0.36±5.36
4	Distilled water	0.11±1.29	0.61±4.61	0.20±5.46
4	NaCl (150mM)	0.14±1.45	0.10±4.33	0.24±5.78
6	Distilled water	0.33±1.10	0.41±3.49	0.48±4.58
6	NaCl (150mM)	0.31±1.18	1.17±3.23	1.42±4.41
LSD (0.05)		0.420	1.668	1.427
Sig.		0.540	0.120	0.026

decrease in chlorophyll is one of the indicators of salt stress (Table 4). This is consistent with (Khan, 2013) as the chlorophyll content in NaCl treated cucumber decreased.

The effect of CHX was studied in two ways: seeds were planted in different concentrations of CHX (0, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5) µg/L for 7 days and observed its effect on the studied parameters, as well as priming the seeds for different time periods 2, 4 and 6 hours before cultivated with 150 mM NaCl.

As a way to alleviate the intensity of salt stress, CHX priming seeds significantly increased growth indicators compared to salt treatment but did not reach growth indicators in control treatment. This may differ with some studies which have shown that CHX may cause inhibition of germination percentage (Narra *et al*., 2010), which is due to the different concentrations of CHX used and the duration of treatment. Some studies have indicated that the addition of CHX prevents the decrease in enzyme α-amylase activity (Nolan and Ho, 1988). The accumulation of mRNA α-amylase activity increases when rice (*Oryza sativa*) seeds germination (Shen *et al*., 1994). It is also found as a result of treatment with CHX which increased the α-amylase activity (Fig. 1). Thus increasing the percentage of germination. As well CHX maintained the carbohydrates content (Fig. 2), which coincided with the increased α-amylase activity (Fig. 1). It was in agreement with (Narra *et al*., 2010) which pointed out the role of CHX in preserving sugars content.

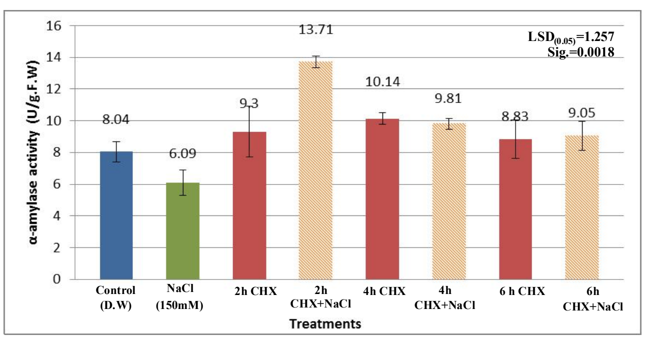

Fig. 1: Effect of cyclohexamide in alleviation of salt stress in terms of α-amylase activity.

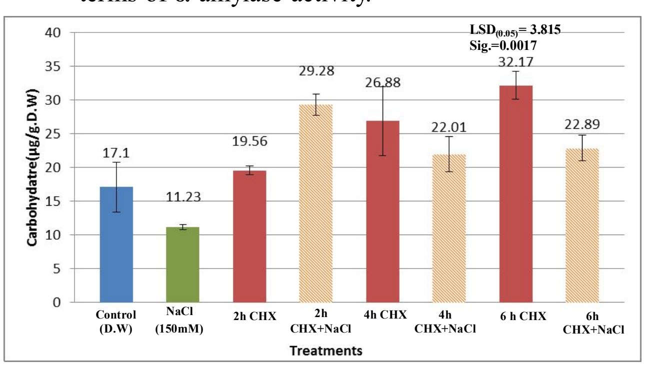

Fig. 2: Effect of cyclohexamide in alleviation of salt stress in terms of carbohydrates content.

References

Adda, A., Z. Rgagba, A. Latigui and O. Merah (2014). Effect of salt stress on (Alpha)-amylase activity, sugars mobilization and osmatic potential of *Phaseolus vulgaris L.* Seeds Var. 'Cocorose' and 'Djadida' during germination. *Journal of Biological Sciences.*, **14(5):** 370-375.

Beck, E. and P. Ziegler (1989). Biosynthesis and degradation of starch in higher plants. *Annual review of plant biology.,* **40(1):** 95-117.

Chartzoulakis, K. and M. Bertaki (2015). Sustainable water management in agriculture under climate change. *Agriculture and Agricultural Science Procedia.*, **4:** 88-98.

Ergun, N., S.F. Topcuoúlu and A. Yildiz (2002). Auxin (Indole-3-acetic acid), Gibberellic acid (GA3), Abscisic Acid (ABA) and Cytokinin (Zeatin) Production by Some Species of Mosses and Lichens., **26(1):**13-18.

Hasanuzzaman, M., M.A. Hossain, J.A.T. da Silva and M. Fujita (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In *Crop stress and its management: Perspectives and strategies.,* 261-315). Springer, Dordrecht.

Khan, A., I. Iqbal, A. Shah, A. Ahmad and M. Ibrahim (2010). Alleviation of adverse effects of salt stress in Brassica (*Brassica campestris*) by pre-sowing seed treatment with ascorbic acid. *Am. Euras. J. Agric. Environ. Sci.*, **7:** 557-560.

Liu, J. (2018). Syringe injectable electronics. In Biomimetics Through Nanoelectronics. 65-93. Springer, Cham.

Mahajan, S. and N. Tuteja (2005). Cold, salinity and drought stresses: an overview. Archives of biochemistry and biophysics, **444(2):** 139-158.

Narra, H., P. Mamidala and P.M. Mehta (2010). Gibberellic Acid and Cycloheximide Influenced the Growth and Biochemical Constituents of a Medicinally Important Plant-*Trachyspermum ammi* (L.) Sprague. *Current Trends in Biotechnology & Pharmacy.*, **4(1)**.

Nolan, R.C. and T.H.D. Ho (1988). Hormonal regulation of α-amylase expression in barley aleurone layers: The effects of gibberellic acid removal and abscisic acid and phaseic acid treatments. *Plant physiology.*, **88(3):** 588-593.

Ologundudu, A.F., A.A. Adelusi and R.O. Akinwale (2014). Effect of Salt Stress on Germination and Growth Parameters of Rice (*Oryza sativa* L.). *Notulae Scientia Biologicae.,* **6(2)**.

Sadak, M.S.H., M.T. Abdelhamid and U. Schmidhalter (2015). Effect of foliar application of amino acids on plant yield and some physiological parameters in bean plants irrigated with seawater. *Acta biol. Colomb.*, **20(1):** 141-152.

Shen, W.H., C.C. Moore, Y. Ikeda, K.L. Parker and H.A. Ingraham (1994). Nuclear receptor steroidogenic factor 1 regulates the Müllerian inhibiting substance gene: a link to the sex determination cascade. *Cell.,* **77(5):** 651-661.

Shrivastava, P., and R. Kumar (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi journal of biological sciences.*, **22(2):** 123-131.

Tatlioglu, T. (1997). Cucumber (*Cucumis sativus L.*) In: Kailov, G. and Bo Bergan, (eds). genetic improvement of vegetable Crops. Oxford Pergamon Press.197-227.

Trajkova, F., N. Papadantonakis and D. Savvas (2006). Comparative effects of NaCl and $CaCl_2$ salinity on cucumber grown in a closed hydroponic system. *Hort. Science.*, **41(2):** 437-441.

Tuna, A.L., C. Kaya, M. Dikilitas and D. Higgs (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany.*, **62(1):** 1-9.

Turan, M.A., A.H.A. Elkarim, N. Taban and S. Taban (2009). Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research.*, **4(9):** 893-897.

William, J.F. and M.L. Peterson (1973). Reaction between α-amylase activity and growth of rice seedling. *Crop. Sci.*, **13:** 612-615.

Yemm, E.W. and A. Willis (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical journal.*, **57(3):** 508.