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ORIGINAL ARTICLE

Studying the effects of different levels of salinity which caused by NaCl on early growth and germination of *Lactuca Sativa* L. seedling

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Soil salinity is one of the most important constraints that limit crop production in arid and semi arid regions. Seed germination is a critical stage in the history of plants and salt tolerance during germination is crucial for the establishment of plants that grow in saline soils. This research was carried out in order to test the effects of different salinity levels on germination and early growth of lettuce (*Lactuca Sativa L.)*. The experiment was carried out using completely randomized design in four replication in 2011 Zabol University laboratory Iran. The results showed that by increasing salinity, percentage and race of germination decreased, So that, in the 150 mM of salinity level, germination reached to minimized (8.33%). Other measured parameters such as plumule length, radicle length, dry and wet weight decreased as well. All the results analyzed by SAS statistical software and comparison of average had done by Duncan test on 5% possible level.

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More than 900 million hectares of land world-wide, approx. 20 % of the total agricultural land, are affected by salt, accounting for more than 6% of the world's total land area. NaCl is the predominant salt causing salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation (Munns and Tester, 2008).

Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Despite the importance of seed germination under salt stress (Ungar, 1995), the mechanism (s) of salt tolerance in seeds is relatively poorly understood, especially when compared with the amount of information currently available about salt tolerance physiology and biochemistry in vegetative plants (Hester *et al*., 2001; Hu *et al*., 2005; Garthwaite *et al*., 2005; Kanai *et al*., 2007). In vegetative plants, salt stress causes reduced cell turgor and depressed rates of root and leaf elongation (Werner and Finkelstein, 1995; Fricke *et al*., 2006), suggesting that environmental salinity acts primarily on water uptake. Furthermore, high intracellular concentrations of both Na+ and Cl2 can inhibit the metabolism of dividing and expanding cells (Neumann, 1997), retarding germination and even leading to seed death.

The different results were dedicated from the effect of salinity stress on the quantitative and qualitative parameters. For instance, it was found that, increasing of salinity stress decreased almost all of growth parameters in *Nigella sativa*, some growth parameters and essential oil amount in chamomile (Razmjoo *et al*., 2008). Also Younis *et al*. (2008) reported that enhancing salinity treatments lead to growth reduction. It also reduces germination amounts and seedling weight. Ashraf and Orooj (2006) reported that salinity treatment lead to reduction of growth and plant developments.

Overall, salinity through enhancement of osmotic pressure leads reduction of water absorbance and metabolically and physiological processes will be under its effect. So it cause more delay in germination beginning following by enhancing seed germination duration (Kang and Saltveit, 2002).

MATERIALS AND METHODS

The experiment was carried out using completely randomized design in four replication and 4 salinity levels (0, 50, 100 and 150 mM) in 2011 at Zabol University laboratory in Iran. Each experimental unit includes 1 Petri dish with 100 Ч 150 mm dimension each contains 25 healthy and homogenous seeds which were on the No1 Watman filter paper. First of all, to disinfect seeds, we put them in 10% Hypochlorite Sodium solvent then we washed them 3 times by distilled water. Next, we added 6 ml NaCl solvent to each Petri dish in this way filter water was weltered by NaCl completely. Eventually, their lids were closed by parafilm and had been located in growth room. The temperature adjusted in 25º C. This experiment took 8 days.

The following characteristics were studied:

Germination Percentage (GP):

From second day, the germinated seeds were counted daily in specific time. At that time, those seeds were considered germinated which their radical length was more than 3 mm.

Counting continued till we could count more germinated seeds and the resulted final counting considered as final germination percentage.

GP: Ni / N x 100

Ni: number of germinated seed till i^{th} day)

N= total number of seeds.

Germination Race (GR):

In order that, from the second day to 7^{th} once a 24 hours we counted germinated seeds and its race was determined by Maguire equation (1962):

$$GR = \sum_{i=1}^{n} \frac{Si}{Di}$$

GR: Germination Race (number of germinated seed in each day)

Si: number of germination seeds in each numeration

Di: number of days till n^{th} numeration.

n: number of numeration times.

At the end of experiment we chose 10 plants from each Petri dish, separated their radicle and plumule and measure each plant's radicle and plumule length separately. Then we put each repetition on the filter separately. In order to make them dry and measure its dry weight, we put them in oven with 75ºC temperature for 24 hours, after we achieved pure numbers, we used SAS software for analyzing them and used Excel software to draw graphs.

Table 1: Result of variance analysis on some germination and growth of seedling characteristics under NaCl concentration

		Mean Square					
S.O.V	**df**	**GP (%)**	**GR**	**RL (cm)**	**PL (cm)**	**WW (g)**	**DW (g)**
Treatment	3	4580.55**	44.91**	8.5**	3.45**	0.00093**	0.000012**
Error	12	18.75	0.125	0.06	0.092	0.0000025	0.00000011
C.V. (%)		10.02	11.40	19.19	7.46	13.31	17.32

Note: *and ** indicate significant difference at 5% and 1% probability level, respectively.

GR: Germination rate, GP: Germination percentage, PL: Plumule length, RL: Radicle length, WW: Wet weight, DW: Dry weight.

Table 2: Effect of different salinity concentration on some germination and growth of seedling characteristics

Salinity concentration (mM)	GP (%)	GR	RL (cm)	PL (cm)	WW (g)	DW (g)
0 (Control)	95a	8.77a	3.77a	2.53a	0.039	0.0059a
50	66.67b	5.59b	1.54b	2.01b	0.011b	0.003b
100	26.67c	1.43c	0.33c	0.74c	0.003c	0.00015c
150	8.33d	0.4d	0.093c	0.23d	0.0006c	0.000073d

Note: Similar letters in each column hadn't any significant statistical difference.

GR: Germination rate, GP: Germination percentage, PL: Plumule length, RL: Radicle length, WW: Wet weight, DW: Dry weight.

RESULTS AND DISCUSSION

Results of statistical analysis of experimental data have been written in table 1 and results of comparison between considered characteristics means have been written in table 2. As table 1 show, salinity made significant differences on all considered characteristics.

Germination percentage and race:

According to results of variance analysis, effect of salinity stress level on germination percentage and race were significant (Table 1). Comparison between means of different level of salinity's effects on germination race and percentage has been showed in table 2. As you see in salinity stress the most germination percentage was (95%) and the less germination percentage was related to 150 mM concentration (8.33%).

The most germination race was related to control also with (8.77) and the less related to 150 mM with (0.4). Its cause could be more than usual presence of anion, cation which in addition to toxication, decreased water potential that is because of its solubability in water. Ion's so plant can't absorb water and encounter to lake of water (Singah *et al.*, 1988). We also can say that this reduction in germination race relies on salinity bad effect on some physiological processes which are effective on seed germination (Khan *et al.*, 2002).

Radicle and plumule length:

The effects of salinity stress on radicle and plumule length have been showed in table 2 Results a significant difference in radicle and plumule length in 0.05% probable level (Table 1). Comparison of radicle and plumule length means in salinity different level showed that when salinity level increase, seedlings radicle and plumule length decrease.

The most reduction in radicle length and plumule related to 150 mM. In this relation Munns and Termaat (1986) suggested that salinity decrease radicle and plumule growth and if we increase salinity level, the amount of reduction will increase.

Also Salinity, declines plumule and radicle growth, and by increasing salinity these reduction increase. Salinity which is result of osmotic pressure leads reduction in water absorbance so cell division and differentiation reduce and reduction of plumule and radicle length will be explainable.

Salinity environment have shorter plumule and NaCl more than other salinity factors gas deterrent impact on embryo tissues appearance (Khan and Ungar, 1997).

Wet and dry weight:

Impact of salinity stress treatments, on dry and wet weight of lettuce seedling was significant (Table 1). Impact of salinity stress on dry and wet weight had been showed in Table 2. As you see by enhancing salinity levels, seedlings wet weight amounts decrease extremely. In this case in 150 mM we have 0.0006 gr also in other treatments (100, 50 and 0 mM), wet weights were 0.003, 0.011 and 0.039 in orderly. (Table 2). In addition, dry weight of seedling have similar results which when we increase salinity level till 150 mM dry weight decreases, means that it decrease from 0.0059 gr to 0.000073 gr. (Table 2).

Etesami and Galeshi (2008) reported that salinity is the cause of reduction in germination percentage, race and homogeneity of germination and dry weight of barley (*Hordeum vulgare*) seedling. Massai *et al.* (2004) say that salinity is delaying plant growth under reduction of photosynthesis effects, it is cause of closing stomata and reduction of water entrance into the plant and so that it cause duplicate reduction in plant weight.

Redman *et al.* (1994) showed that this reduction in dry weight of plumule and radicle which is results of enhancing the salinity concentration is a normal phenomenon and probably it is the result of low water absorbance by germinated seeds.

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