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Effects of sodium chloride on germination and seedling growth of Sunflower (*Helianthus annuus* L.)

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Abstract
The response of BARI Sunflower-2 (*Helianthus annuus* L.) to sodium chloride (NaCl) at germination and early seedling growth was investigated. The seeds and seedlings of sunflower were treated with a series of eight different concentrations of NaCl viz 25, 50, 75, 100, 125, 150, 175 and 200 mM and distilled water (control) and were allowed to grow under plate culture condition. The results showed that the highest salinity concentration (200 mM NaCl) remarkably decreased the germination percentage, germination index and speed of germination of BARI Sunflower-2. Fresh and dry masses of both shoot and root were significantly reduced at a 25-200 mM NaCl solution. Significant reductions of shoot and root length were also noted with the increase of NaCl concentration. The results also illustrate that up to 50 mM NaCl concentration, all of the germination indices and fresh and dry biomass were withstand more than 70%. Thus the present study concluded that the BARI Sunflower-2 could be cultivated in moderately saline soil.

Key words: Salinity, Germination percentage, Germination index, Germination speed.

INTRODUCTION

Sunflower is the most important oil seed crop of the world next to groundnut, rapeseed and mustard (Talia *et al.*, 2011). Sunflower is a newly introduced oil seed crop in Bangladesh. Sunflower as a photo and thermo-neutral crop can be grown both in Rabi and Kharip seasons. It can fill up the gap between production and consumption of edible oil by expanding the area of sunflower production. A large portion of coastal area or southern parts of Bangladesh is now affected in sodium chloride salinity (Khatun *et al.*, 2016). As a result the saline tolerant cultivar can be grown in this area at Rabi season when paddy land remain fallow. If any cultivar of sunflower would release as saline tolerant, it would be beneficial for our farmers as well as our country. Though its cultivation is lower due to increase of the rice producing area, it has bright future because of cropping intensity which is increasing gradually (Jahangir *et al*., 2006). In order to mitigate the problem of oil seed crisis, it is necessary to increase the oil seed production in our country.

Salinity is considered as a major abiotic stress and significant factor affecting crop production all over the world and especially in arid and semi-arid region (Davidson and Chevalier, 1987; Khajeh-Hosseni *et al.*, 2003). The common salts normally present in saline soils are the chlorides, sulfates, bicarbonates, carbonates and borates of Na, K, Ca and Mg. Soil salinity is characterized by high amounts of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-

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which inhibits growth and productivity of the plant due to accumulation of these salts in the root zone and affected the absorption of water and nutrient elements (Szabolcs, 1979). Salinity impairs seed germination, retards plant development and reduces crop yield. At the later stage salinity decreases crop yield by affecting crop growth and yield parameters (Munns & Tester, 2008; Wu *et al*., 2015). Salinity affects plant growth by distributing water relation, creating water imbalance in plant nutrition and affecting plant physiological and biochemical processes (Yeo *et al*., 1985; Campos *et al*., 1989; Bethke *et al*., 1992 and Karim *et al*., 1993).

Seed germination is one of the most fundamental and vital phases in the growth cycle of plants that determine plant establishment and the yield of the crops. Salinity have many-fold effects on the germination process: it alters the imbibition of water by seeds due to lower osmotic potential of germination media (Khan & Weber, 2008), causes toxicity which changes the activity of enzymes of nucleic acid metabolism (Gomes-Filho *et al*., 2008), alters protein metabolism (Yupsanis *et al*.,1994 ; Dantas *et al*., 2007), disturbs hormonal balance (Khan and Rizvi, 1994), and reduces the utilization of seed reserves (Promila & Kumar, 2000 ; Othman *et al*., 2006).

The current study was undertaken to investigate the effect of sodium chloride on germination percentage, germination index, germination speed, shoot and root length along with biomass of BARI Sunflower-2.

MATERIALS AND METHODS

Seeds of Sunflower, cultivar BARI Sunflower-2 (*Helianthus annuus* L.) were collected from Bangladesh Agricultural Research Institute (BARI) and were used as plant material. On the basis of previous research information (Carbonell-Barrachina and Burlo-Carbonell, 1997) eight different concentrations of (NaCl) solution viz 25, 50, 75, 100, 125, 150, 175 and 200 mM and distilled water as control were used as treatment. In each treatment, 10 ml of NaCl solution was applied per petridish. No Nutrient solution was applied in petridish. The germination experiment was carried out in the Plant Physiology and Plant Biochemistry laboratory of Botany Department, Jahangirnagar University, Savar, Dhaka.

For germination experiment 25 seeds based on seed uniformity were placed on moist blotting paper in 9 cm diameter petridishes covered with a lid. The petridishes were kept under laboratory conditions where temperature fluctuated between 23-25^{o}C. The treatment solutions were applied separately for each petridish regularly. The number of seeds that were germinated was recorded at 24 hours interval for 7 days. After seven days of growth germination percentage, germination index, germination speed, shoot and root length, and fresh weight and dry weight of root and shoot were recorded.
The germination percentage, germination index and speed of germination was calculated by the following formula:

$$\textbf{Germination Percentage (GP)} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seed}} \times 100$$

The germination index was calculated after final germination by the following equation (Karim *et al*., 1992)

$$\textbf{Germination Index (GI)} = \frac{\text{Germination \% in each treatment}}{\text{Germination \% in the control}} \times 100$$

Speed of germination was calculated according to Mohammed *et al*., 1989 by the following equation

$$\textbf{Speed of Germination (SG)} = \frac{\text{No. of Seedlings}}{\text{First count on day 1}} + \text{----} + \frac{\text{No. of Seedlings}}{\text{Final count on day 7}}$$

Seedling height and root lengths were recorded on the 7^{th} day after the seeds were placed for germination. At harvest on 7^{th} day, the seedlings were kept in oven and dried at 72ºC until they reached a constant weight. Data on germination behavior and seedlings characteristics for each NaCl treatment were compared with those in the control treatment. The experiment was arranged out in randomized block design with three replications. The data recorded on different parameters were statistically analyzed with the help of Microsoft Excel 2013 and Duncan multiple range test (DMRT) was done by using SPSS Program 16.00. The difference (LSD) level of significance was also found out after performing ANOVA.

RESULTS AND DISCUSSION

Germination Percentage (GP) and Germination index (GI): In the present study, the highest germination percentage was recorded 90% in the control and the lowest value was 53.33% in 200 mM NaCl (Table 1). The moderate germination percentages were recorded 76.67%, 73.34%, 70.00% and 66.67% in 25, 50, 75, 100 and 125 mM NaCl levels respectively. The germination index (GI) was significantly decreased as the NaCl levels were increased. The highest germination index was 100.00 in the control and lowest 59.26 in 200 mM NaCl (Table 1). The moderate germination indices were 85.20, 81.49, 77.78 and 74.08 in 25, 50, 75, 100 and 125 mM of NaCl levels. The result agrees with the work of Karim *et al*. (1992) and Mondal *et al*. (1988) that salinity delays germination. This result was in similar with Francois *et al*. (1986) noticed that in wheat germination was significantly delayed and the final germination percentage was markedly reduced by salinity.

The mean values of germination percentages and germination indices of BARI Sunflower-2 are presented in Table 1 which show a decreasing tendency of the germination percentage and germination index with the increase of NaCl concentration.

Germination percentage of a crop is considered as the most important criteria for a successful crop establishment under stress conditions because under saline conditions germination is the first stage of a crop exposed to salinity. Usually a crop with high germination percentage and GI performs well under saline conditions than with low GI. Presumably the osmotic effect due to salinity on inhibition of seeds was the main cause for the reduction of germination percentage and varieties differences for GI as indicated by Akbar and Ponnamperuma (1982) and Uhvits (1946). The negative effect of salinity on seed germination might be happened due to salinity-induced ionic imbalance or toxicity (Panuccio *et al*., 2014).

Speed of Germination (SG): The highest germination speed was obtained 3.77 (100%) at the control and the lowest was 0.76 (20.16%) at 200 mM NaCl (Table 1). In the present study at 125 mM of NaCl salinity, the germination speed was 71.09% which is an indication of salt tolerance (Halim *et al*., 2004). The speed of germination was decreased with the increase of NaCl concentrations (Table 1). The decreasing tendency of the speed of germination due to salinity was in conformity with the reports of others (Muhammad *et al*., 1986; Khan *et al*., 1997). The SG is an indicative of crop performance under saline conditions. The speed of germination may have decreased due to inactivation of hydrolase enzymes at the germination stage of seeds at high concentrations of NaCl salinity (Maas & Hoffman, 1977; Blum, 1988).

Table 1. Effect of different levels of salinity on germination percentage, germination index and germination speed of BARI Sunflower-2

Treatments NaCl (mM)	Germination percentage	Germination index	Germination speed
Control	90.00a	100.00a	3.77a
25	76.67b	85.20b	3.30b (87.53)
50	73.34bc	81.49c	3.17b (84.08)
75	70.00bc	77.78d	1.27c (33.69)
100	66.67bc	74.08e	1.17d (31.03)
125	66.67bc	74.08e	1.09e (28.91)
150	63.34bc	70.38f	1.05e (27.85)
175	60.00cd	66.67g	1.03e (27.32)
200	53.33d	59.26h	0.76f (20.16)
Cultivar Mean	68.89	76.55	1.85
LSD (5%)	0.64	0.47	0.26
CV%	17.23	11.88	36.46

- Data expressed as mean values of 3 replicates.
- In a column followed by same letters do not differ significantly at 5% level of significance.
- Values within parentheses indicate percent change over control.

Shoot and root length: At the highest level of salinity (200 mM) the shoot length decreased up to 6.5 cm whereas in the control the shoot length was found 14 cm. Shoot length was found 75% compared to the control at 50 mM NaCl salinity whereas the lowest shoot length 46.42% was exhibited compared to the control at the highest level of NaCl treatment (Table 2). The decreasing tendency of shoot growth from 50 mM to 150 mM was slow. It indicated that at least 65% growth rate remained at 150 mM NaCl salinity treatment but drastic reduction was observed at 200 mM. Reduction of shoot length is a common phenomenon of many crop plants under saline conditions as noticed earlier by several workers (Javed & Khan, 1975; Amin *et al*., 1996). Under saline condition plant suffers from osmotic stress. To adjust osmotically the plant has to synthesize compatible metabolites and thus need extra energy. For this reason the growth processes suffer considerably (Taiz and Zaiger, 1991). Karim *et al*. (1992) pointed out that shoot growth was more sensitive to salinity than germination and root growth.

Table 2. Effect of different levels of salinity on shoot and root length (cm) of BARI Sunflower-2

Treatments NaCl (mM)	Shoot length	Root length
Control	14.00a	13.00a
25	11.84b	12.67a
	(84.57)	(97.46)
50	10.50bc	12.33a
	(75.00)	(94.85)
75	10.33bc	11.67ab
	(73.79)	(89.76)
100	9.33c	10.50ab
	(66.64)	(80.76)
125	9.17c	9.10bc
	(65.50)	(70.00)
150	9.13c	8.84bc
	(65.22)	(68.00)
175	6.50d	7.17c
	(46.42)	(55.16)
200	6.50d	7.17c
	(46.42)	(55.16)
Cultivar Mean	9.70	10.84
LSD (5%)	0.76	1.46
CV%	29.64	22.38

- Data expressed as mean values of 3 replicates.
- In a column followed by same letters do not differ significantly at 5% level of significance.
- Values within parentheses indicate percent change over control.

The mean value of root length decreased significantly with the increase in salinity levels (Table 2). At the highest level of salinity (200 mM) the root length was 7.17 cm and in the control the root length was 13 cm. Root length was observed 80% compared to the control at 100 mM NaCl salinity. At 200 mM NaCl salinity root growth was decreased by 55.16% compared to the control while maximum root growth was found at control treatment. Root growth was suppressed by salinity. The results of this experiment are in similar with the findings of Cramer *et al.* (1988) and Ashraf *et al.* (2005). Under saline conditions roots play an important role by excluding Na salt or by controlling easy pass of the Na to the shoot (Karim *et al.*, 1992; Amin *et al.*, 1996). Moreover the longer roots have an advantage for absorbing higher amount of water than shorter roots.

Table 3. Effect of different levels of salinity on fresh weight of shoot and root (gm/ plant) of seedling of BARI Sunflower-2

Treatments NaCl (mM)	Fresh weight of shoot	Fresh weight of root
Control	1.25a	0.69a
25	1.16a (92.8)	0.46b (66.67)
50	0.92ab (73.60)	0.41bc (59.42)
75	0.73b (58.40)	0.38c (55.07)
100	0.69b (55.20)	0.31d (44.90)
125	0.65b (52.00)	0.31d (44.90)
150	0.64b (51.20)	0.27d (39.13)
175	0.19c (15.20)	0.06e (8.69)
200	0.18c (14.40)	0.06e (8.69)
Cultivar Mean	0.71	0.33
LSD (5%)	0.60	0.28
CV%	55.80	57.78

- Data expressed as mean values of 3 replicates.
- In a column followed by same letters do not differ significantly at 5% level of significance.
- Values within parentheses indicate percent change over control.

Biomass (Seedling Fresh weight and Dry weight of BARI Sunflower-2): In the present study, plant biomass decreased markedly with the increasing level of sodium chloride concentration, which contradict the result of Carbonell-Barrachina *et al.* (1997). The shoot fresh weight decreased significantly with the increasing level of salinity (Table 3).

At the highest level of salinity (200 mM) the shoot fresh weight was 0.18 gm and in the control the shoot fresh weight was 1.25 gm after seven days. Fresh weight of shoot was reduced 26.40% at 50 mM NaCl salinity whereas up to 200 mM it was reduced to 85.60%. This may be due to diversion of energy in the process of osmotic adjustment. Results obtained from this study are in confirmation with the findings of Ashraf & Leary (1997).

The root fresh weight decreased significantly with the increasing level of salinity (Table 3). At the highest level of salinity (200 mM) the root fresh weight was 0.06 gm and in the control the root fresh weight was 0.69 gm. The highest salinity level (200 mM NaCl) was drastically reduced root fresh weight by 91.31%, while at slightly salinity level (25 mM NaCl), it was 66.67%. Results obtained from the experiment clearly indicates that root fresh weight also decreased with the increasing NaCl levels. This may be due to diversion of energy in the process of osmotic adjustment. The results are in confirmation with the findings of Ashraf & Leary (1997).

The shoot dry weight decreased significantly with the increasing level of salinity (Table 4). At the highest level of salinity (200 mM) the shoot dry weight was 0.08 gm and in the control the shoot dry weight was 0.39 gm. High concentration (200 mM NaCl) of NaCl led to significant reduction of shoot dry mass by 79.49%, while at 50 mM NaCl it was 71.20%. The results are in similar with the findings of Cramer *et al*. (1994), Halim *et al*. (2004) and Mansour *et al*. (2005). Shoot dry weight was decreased by salinity. Shoot dry weight at seedling stage is an indication of seedling vigour. Under saline conditions the variety with higher shoot mass performs better than a variety with lower shoot mass (Blum, 1988; Karim *et al*., 1992).

Table 4. Effect of different levels of salinity on dry weight of shoot and root (gm/plant) of seedling of BARI Sunflower-2

Treatments NaCl (mM)	Dry weight of shoot	Dry weight of root
Control	0.39a	0.57a
25	0.34a	0.52b
	(87.18)	(91.22)
50	0.28b	0.38c
	(71.20)	(66.67)
75	0.25bc	0.34c
	(64.10)	(59.64)
100	0.22c	0.29d
	(56.41)	(50.87)
125	0.16d	0.27d
	(41.02)	(47.36)
150	0.15d	0.17e
	(38.46)	(29.82)
175	0.08e	0.11f
	(20.51)	(19.29)
200	0.08e	0.10f
	(20.51)	(17.54)
Cultivar Mean	0.22	0.31
LSD (5%)	0.18	0.27
CV%	49.91	53.38

- Data expressed as mean values of 3 replicates.
- In a column followed by same letters do not differ significantly at 5% level of significance.
- Values within parentheses indicate percent change over control.

The root dry weight decreased significantly with the increasing level of salinity (Table 4). At the highest level of salinity (200 mM) the root dry weight was 0.10 gm and in the control the root dry weight was 0.57 gm. Dry weight of root was observed 50.87% at 100 mM NaCl salinity and up to 200 mM NaCl salinity dry weight of root reduced to 82.46%. The results are in similar with the findings of Akram *et al*. (2007) reported that root dry weight of all corn hybrids showed a decline towards increase in salinity level. Root dry weight decreased with the increase in the salinity levels. Usually higher fresh weight of root is an indication of higher root dry weight. Usually tolerant variety possess higher root mass compared to salt susceptible variety.

This study showed that at least 65% shoot growth rate was sustained up to 150 mM NaCl salinity treatment but drastic reduction (46.42%) was found at 200 mM. Moreover, more than 50% dry weight of shoot and root at 100 mM NaCl salinity treatment was found. These results of this study indicate that the seedlings of BARI Sunflower-2 could be withstand up to moderate salinity level. This findings will be helpful for further experiments to determine the physiological consequences on salt tolerant and salt susceptible cultivars at seedling stages.

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