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EFFECT OF SALINITY STRESS ON THE GERMINATION, GROWTH AND DEVELOPMENT OF BEET ROOTS



S M Noor-Ullah, P K Dash and M A Mannan

Agrotechnology Discipline, Life Science School Khulna University, Khulna, Bangladesh.

Abstract

The southern zone of Bangladesh has faced trouble in the production of beets due to increasing soil salinization. Tolerant varieties are of utmost importance to compensate for the salinity of the soil. The goal of this research study was to assess the effect of salinity stress on the growth and development of beets. Seeds were germinated in Petri dishes and plants were grown in pots. Salinity (ECe) was adjusted to 3, 6, 9, 12, and 15 dSm⁻¹ via the application of NaCl in both the Petridis and the pots. Untreated seeds and plants were included as a control. Germination speed and other germination and growth parameters were detected that the seedling did not exhibit any salt tolerance. The seedling did exhibit a negative linear relationship between increasing salinity and germination parameters. As for the potted plants, rates of NaCl induced salinity higher than 9 dSm⁻¹ had marked adverse effect on vegetative parameters and decreased beets yield. Thus, salt imposition progressively impairs seed germination, vegetative growth and development parameters of the beets cultivar (SB001) and SB001 beet cultivar is considered as moderately salinity tolerant and we can cultivate it on lower salinity condition.

Key words: Beet, Salinity, Vegetables, Germination, Growth, Development.

Introduction

Salinity is one of the oldest and most serious environmental stresses in crop production (McWilliam 1985). Extreme soil salinity has detrimental effects on crop production, especially in arid and semi-arid regions (Moud and Maghsoudi 2008; Keshavarzi 2011). High soil salinity is deleterious to most plants, and the nature and composition of soluble salts, salt dynamics, and water regimes during the growth season strongly influence crop growth (Clarke et al. 2015). Due to osmotic gradient, salinity drastically reduces the soil water availability for germinating seeds ultimately hindering seed germination. In addition, same process impedes cell division and elongation and shoot growth (Kaymakanova 2009). Na⁺ and Cl⁻ are typically the acting ions in this process (Kaya et al. 2006; Kandil 1993). Among crop species, only cotton

and barley are more tolerant than beet to salinity (Maas 1990; Allen *et al.* 1998). Two mechanisms employed by sugar beet for tolerance to salinity are the sequestration of Na in vacuoles and osmotic adjustment (Katerji *et al.* 1997). Salinity has a negative effect on the yield and quality of sugar beet, especially from the excessive absorption of sodium (Mekki and El Gazaar 1999; Cheggour and Fares 2002).

Soil salinization has ruined approximately 20% of the total cultivable land (>0.9 billion hectares of land) worldwide and 6% of that active agricultural land. The bulk of that salinity is derived from sodium chloride (NaCl) (Munns and Tester 2008). In Bangladesh, the total cultivable area in coastal belt is more than 30% (Haque 2006). About 0.83 million hectares out of the 2.85 million hectares of the coastal and offshore areas are affected by different degrees of salinity (Batenet *et al.* 2015).





Table 1 Units for measuring salinity and conversion factors

Further, the salt-affected area is significantly increasing day by day. The presence of excess soluble salt in the soil is one of the major factors that reduce the growth and development of cultivated crop plant in coastal areas of Bangladesh. The salinity problem is most severe in the dry period during the winter growing season due to upward or lateral movement of saline water (Clarke *et al.* 2015; Haque 2006).

In Bangladesh, vegetables are produced on about 9.3 acres of land area, with a total production of about 3 M tons in 2013 (BBS 2013). Khulna is one of the most important vegetables growing zones in the southern part of Bangladesh. In recent years vegetable production in the southern region has suffered due to pronounced increase of salinity levels in the soil. Determination of salt-tolerant vegetable varieties has been advised to compensate for the increasing salinity of the soil (Jahan and Islam 2010; Sivritepe *et al.* 2003).

The beet (*Beta vulgaris* L.) is belonging to the Chenopodiaceae family, subfamily Betoideae (Lange *et al.* 1999). Beets can withstand in adverse climate that they grow in tropical, subtropical and even in temperate countries (Katyal and Chandha 1985). These crops are annuals as cultivated or biennials if grown from seed. The best quality and root color are obtained when the air temperature ranges between 10 and 18°C. These crops prefer deep, friable, well-drained, sandy loams to silt loam soils for proper growth (Rashid 1999). Recently beet consumption has expanded due to the increased popularity of betalain phytonutrients within beets that provide antioxidant, anti-inflammatory, and detoxification support (Julian 2013).

It is hypothesized that beet production can aid in meeting the demand and need for vegetables in the southern region. Therefore, the present research work was undertaken keeping in mind the following objectives:

• To assess the effect of salinity on germination of beet.

• To determine the effect of salinity on yield and yield attributes of beet.

Materials and Methods Description of experimental sites

Two subsequent experiments were conducted in Petri dishes at the Molecular Horticultural Laboratory and in pots at the Germplasm Center of Agrotechnology Discipline greenhouse, Khulna University, Bangladesh in 2014. The pot experimental area was in the Agro Ecological Zone (AEZ) 13, or the Ganges Tidal Flood Plain. Geographical location of the experimental site was $22^{\circ} 47'$ North latitude, and $89^{\circ} 34'$ East longitude. The experimental site is a subtropical humid climate and is characterized by moderately high temperature and heavy rainfall during the kharif season (April-September) and moderately low temperature with low rainfall during the Rabi season (October-March). The soil used in pots was collected from the Germplasm Centre of Agrotechnology Discipline garden, Khulna University, which belongs to the sandy loam soil type. The texture of the collected soil was clay to clay loam. However, the initial soil condition was non-saline based on EC content (SRDI 2010).

Layout of experiment and treatments description

Germination and pot experiments were laid out in a Complete Randomized Design (CRD) with seven replications. Six salinity levels (ECe) (0, 3, 6, 9, 12 and 15 dSm⁻¹, respectively) were used as treatments in both experiments.

Planting materials

SB001 beets cultivar was used as planting material. It is popular cultivar of India. This popular variety is adaptable to wide range of climate. It matures earlier than any other cultivar. Bangladesh have no own beet cultivar. That's why Indian high yielding cultivar was used. Collected seeds were properly sun dried and then treated with Benomyl fungicide before being placed in Petri dishes.

Salinity levels preparation

NaCl compound have been used to simulate osmotic stress effects in petri dish (in vitro) for plants to maintain uniform water potential throughout the experimental period (Kulkarni and Deshpande 2007). Five different salinity levels (3 dSm⁻¹, 6 dSm⁻¹, 9 dSm⁻¹, 12 dSm⁻¹ and 15 dSm⁻¹) were used in this experiment.

Measurement and units	Application	1 dSm ⁻¹ is equal to:	Equivalent units	
Total dissolved salts (mgL ⁻¹)	Irrigation and river water	640 mgL ⁻¹ (approx.)	1 mgL ⁻¹ =1 ppm	
Molarity of NaCl (mM)	Laboratory	10 mM	1 mM=1 mmolL ⁻¹ =0.001molL ⁻¹	

The amount of salt (NaCl) required to make the desired salinity level was estimated using the following formula and then added to distilled water.

Percent of salt = $640 \text{ mg} \times \text{EC} (\text{dSm}^{-1})$

Treatments	Salinity Levels (dSm ⁻¹)	Required amount of salt (NaCl) in g to be added in 1L distilled water			
T ₁	0.66 (control)	0			
T ₂	3	1.92			
T ₃	6	3.84			
T_4	9	5.76			
T ₅	12	7.68			
T_6	15	9.6			

Each salt solution was separately prepared before application of solution in Petri dishes and pots.

Management of the experiment

Glass petri-dishes (9 cm in diameter) with a tightfitting lid were used for the germination experiment. Three pieces of sterilized Whatman No.1 filter paper (9 cm in diameter) were used as a matrix for seed germination. Each filter paper was soaked with its respective salt solution, except the control, which was soaked with distilled water. They were then placed in their respective Petri dishes. A total of 1050 seeds was placed in 42 Petri dishes (25 seeds per Petri dish). Six treatments replicated seven times, or forty-two Petri dishes and pots were used in the germination and pot experiments, respectively. The plastic pots were 21 cm \times 19 cm \times 20 cm in length, width, and height. The pots were filled with proper sun-dried soil, inorganic fertilizers at 100 kg ha⁻¹ N, 85 kg ha⁻¹ P, 170 kg ha⁻¹ K, and organic manure. A full dose of cow dung, phosphorus, potassium and a half dose of nitrogen were initially mixed with the soil to fill the pots. The other half dose of N was then top dressed in two equal installments at 20 and 50 days after sowing (DAS). Healthy 30-day-old seedlings that grown on nursery were selected to be transferred into the pots. One

healthy seedling was transferred in an individual pot and all plants were survived properly. Before transferring the seedlings into the label pots, however, the soil medium was soaked with six different saline solutions prepared separately before application. To keep soil salinity (ECe content) uniform throughout the experiment, the soil of each individual pot, soil was analyzed several times in the Agrotechnology Discipline Soil Chemistry laboratory. Also, other cultural practices were performed whenever required.

Data collection procedure

Germination parameters: A seed was considered germinated at the emergence of the plumule and radicle. The data on germinated seeds was recorded daily, and germination expressed as a percentage. The germination data were calculated using the formulas of different researchers (Keshavarzi 2011; Scott *et al.* 1984; Bam *et al.* 2006).

Shoot and root length: The shoot and root length of randomly selected seedlings were recorded 7 days after germination using a measuring scale and expressed in centimeters.



Fresh and dry weight of seedlings: The fresh weight of seedlings was recorded 7 days after germination. The samples were oven dried at 70°C for 2 days (48 hours). After the samples were dry, dry weights were determined with an electronic balance.

Growth, yield and yield attributes parameters: Growth, yield, and yield attribute data were collected from each pot at seven-day intervals up to seven weeks. Data collection starting from two weeks after planting and the following data was recorded: number of leaves, plant height, length and width of the leaves, number of lateral roots, length of lateral roots, fresh and dry weight of lateral roots, fresh and dry weight of shoots, length and diameter of beets, fresh and dry weight of beets.

Statistical analysis

Data was analyzed with the help of SAS JMP 12.2.0 software and mean separation by the Tukey HSD test at $p \le 0.05$. Regression analysis was conducted via linear fit model to evaluate the relationship between different germination and growth parameters and salinity levels.

Results and Discussion

Germination percent, energy, capacity, and speed were significantly different ($p \le 0.01$) among the treatments (Fig. 1). The highest values of germination percent, energy, capacity, and speed There were no significant ($p \le 0.05$) differences between different salinity levels with respect to leaf number (Fig. 2). Salinity level had no significant ($p \le 0.05$) effect on plant height. Nevertheless, plant height did decrease with each increasing salinization (Fig. 2). Differences in leaf length and width were statistically non-significant $(p \le 0.05)$ among treatments (Fig. 2). At 21 days after planting, highest leaf length came from the control, which was statistically similar to the 3, 6, and 9 dSm⁻¹ salinity treatments. The lowest were from the 15 dSm⁻¹ and 12 dSm⁻¹treatments. The trend was similar at 49 and 56 days after planting (Fig. 2). The effect of salinity stress on the lateral root number and length was statistically non-significant ($p \le 0.05$) for beets (Table 3). There was no significant ($p \le 0.05$) variation among the treatments for fresh and dry weight of shoots (Table 3).

Beet diameter varied among the treatments ($p \le 0.01$) (Table 3). The highest beet diameter was found from control treatment which was statistically similar to the low salinity level 3 dSm⁻¹ while the lowest beet diameter was paired with the highest level of salinity 15 dSm⁻¹ which was statistically similar to the 12, 9, 6, and 3 dSm⁻¹ treatments. On the other hand, differences in beets length were non-significant among the treatments (Table 3). Significant ($p \le 0.01$ and $p \le 0.05$) influence was observed in fresh and dry weight of the beets (Table 1). Beets fresh and dry weights were highest in the control and lowest (15 dSm⁻¹) in highest saline treatment, which was statistically similar to the 12, 9 and 6 dSm^{-1} treatments (Table 3). Germination and growth parameters are adversely affected by salinity (Fig. 1). Salinity stress decreased the germination percent, energy, capacity, speed, shoot and root length, and dry weight of shoot and root. Hillel (1998) found that without salinity, germinating seed absorb solution and swell very fast, but the saline water stops this normal physiological process of the seeds. As a result, the radicle and plumule fail to or poorly emerge and are too weak for proper seedling growth. Na⁺ and Cl⁻ ions are one common medium by which this noxious high soil osmolarity is produced reported by Kaya et al. (2006) and Kandil (1993). Kaymakanova (2009) observed that in addition to lowering germination, cell division and elongation is limited due to Na⁺ and Cl⁻ ions. Similarly, salinity impaired germination in knol-khol seedlings reported by Biswas et al. (2016). In the present study, a strong negative linear relationship between salinity level and germination and growth parameters was observed (Fig. 1). Seed germination and root growth were significantly



(89.14%, 64.0%, 89.14%, 72.05%, respectively) were observed in the control condition, while the lowest were observed in the 15 dSm⁻¹ salinity treatment. Salt imposition progressively impairs seed germination parameters. All values of germination gradually decline with the increase of salinity (Fig. 1). The shoot and root lengths and fresh and dry weights of seedlings were significantly ($p \le 0.05$) decreased compared to the control (Fig. 1). The highest shoot and root lengths (6.02 cm, 3.01 cm) and fresh and dry weights (0.40 g, 0.06 g) of seedlings were found in the control treatment. Lowest shoot and root length and fresh and dry weight (2.02 cm, 1.11 cm, 0.07 g, 0.01 g, respectively) of seedlings were found at the highest level (15 dSm⁻¹) of salinity (Fig. 1).

Regression analysis was conducted via linear fit model to evaluate the relationship between different germination and growth parameters and salinity levels. Negative linear relationship was observed among the studied parameters (Fig. 1).



Table 3. Effect of different levels of salinity on growth, yield, and yield attributes of beets.

Salinity levels (EC dSm ⁻¹)	No. of lateral roots	Lateral root length (cm)	Fresh wt. of beets (g)	Dry wt. of beets (g)	Diameter of beets (cm)	Length of beets (cm)	Fresh wt. of shoot (g)	Dry wt. of shoot (g)
0.66 (control)	41.71	48.80	167.28 a	23.11 a	7.22 a	6.70	122.14	15.57
3	36.74	42.64	145.42 ab	21.90 a	6.47 ab	6.28	106.85	15.42
6	33.74	42.41	130.26 ab	20.41 ab	6.09 b	5.79	104.85	13.57
9	31.85	38.47	127.57 ab	20.39ab	6.06 b	5.68	96.42	13.00
12	31.57	38.40	123.57 ab	18.02 ab	5.84 b	5.65	96.28	12.71
15	28.49	30.30	97.42 b	14.44 b	5.36 b	5.62	93.28	12.00
Level of significance	NS	NS	**	*	**	NS	NS	NS
P-value	0.07	0.08	0.01	0.05	0.01	0.08	0.09	0.06

Table values with the same letters in a column do not differ significantly whereas column having dissimilar letter differ significantly as per Tukey HSD test. Significance level **, * and NS means significant at 1% and 5%, or and insignificant, respectively.

affected by salt solution with EC up to 8 dSm⁻¹ and 4 dSm⁻¹, respectively in four tested cultivars of beet noticed by Asghar et al. (2007). However, the effect of salinity on onion seed germination is up to 10 dSm⁻¹ whereas 12 dSm⁻¹ for sugar beet (Wannamaker and Pike 1987; Jafarzadeh and Aliasgharzad 2001; Mohammadian 1995). These findings give a new avenue of sensitivity of salinity on germination of beet. Moreover, NaCl have deleterious effects on plant growth noticed by Volkmaret et al. (1998). In this research, most of the growth, yield, and yield attributes parameters such as leaf number, plant height, leaf length and width, lateral root number and length, fresh and dry weight of shoots, and length of beets were statistically insignificant (Fig. 2 and Table 3). It is because salinity stress had little effect on the growth and development of already-established beet plants. Similar results were found in Knol-khol, this vegetable tolerant up to 9 dS/m salinity levels without hampering growth and development of crops (Biswas et al. 2016). After physiological changes in the salt soil solution, the salt ions in the soil migrated to the upper vegetative parts of the plant without any adverse effects on the growth and development of the plant. On the other hand, diameter and fresh and dry weight of the beets were badly affected by salinity stress. Katerji et al. (2003) observed that salinity affected different parameters of beet cultivars such as leaf water potential, stomatal conductance, leaf area, evapotranspiration, and yield. In the same way, West et al. (1986) and Yeo et al. (1985) reported that salinity decreased osmotic potential, which reduced water availability for the plant, resulting water stress and finally affects the stomatal conductance, leaf growth and photosynthesis. Volkmar et al. (1998) detected that salt accretion in the root zone is cause

severe problems, which affected by dynamics of water and ion movements into the plants. High saline content solution on the soil impaired water uptake in these tissues, in particular, ultimately reducing the number of nutrients available in the soil as well. So, the growth and development of the taproots were stunted, which is concerning since those are the main yield parameters for beets. Dry and fresh weight of roots, leaf area were decreased with increasing salinity concentration reported by Jamil et al. (2007) and Jamil et al. (2005). Likewise, Jamil et al. (2007) found that the growth of sugar beet was significantly decreased with an increase in salt concentration up to 150 mM NaCl. Plant growth, reduced by salinity stress, although it is varies by species (Munns and Termaat 1986; Ashraf and Harris 2004). Kandil (1993) perceived that salt solution in the soil disturbed the nutrition and metabolism of the beet plants and altered the structure, permeability, and aeration of the soil. Thus, normal growth of the beets was disturbed.

Conclusion

Salt imposition progressively impairs seed germination, vegetative growth and development parameters of the beets cultivar (SB001). This indicates that salinity factors significantly interact with germination, growth and development factors of beet, which contribute to reduce yield. Indeed, significant correlation was found between salinity level and yield reduction of beet. This beet cultivar (SB001) is consider as moderately salinity tolerant and we can cultivate it on lower salinity condition. Further trials are needed with including more cultivars and salinity levels before final recommendation at farmer's level.



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