

Trehalose Treatment Alleviates Salt Toxicity in Wheat Seedlings: A beneficial research attempt in agriculture sector of Pakistan

Bushra Fordil^{1*} and Nadia Khan¹

¹Department of Genetics, University of Karachi, Karachi, Pakistan.

Corresponding author: Bushra Fordil
Email address: bushrafordil848@gmail.com

Abstract

Osmoprotectants are small organic molecules that are synthesized and accumulate in plants which not only enhance growth parameters but also protect plants from damaging effects of abiotic stresses such as drought, heat, salt and metal toxicity. The most damaging being salinity stress, affecting every stage of wheat plant development. In the present study, the effect of an osmoprotectant compound commonly known as trehalose (Tre) on growth parameters of ten Pakistani wheat varieties (NIA Amber-10, Bhattoo, Bhattai, Borloug-16, DN-11, DN-84, Punjab-11, TJ-83, Zamindar-04 and Zincol-16) were assessed. Their 7 days old seedlings were subjected to Tre (10 and 50mM) with and without (150 mM) NaCl for five days were investigated. In the NaCl treated group, a reduction in all four growth parameters i.e. root length (cm), shoot length (cm), fresh weight (g) and dry weight (g) were evident. However, Tre alone at 10 and 50 mM enhanced the growth of wheat cultivars. Furthermore, a combination of NaCl and Tre (10

and 50 mM) alleviated the harmful effects of NaCl by enhancing growth of all aforementioned parameters. Results of the present study led us to conclude that Tre act as an osmoprotective agent in wheat seedlings by reducing the damage caused by salinity. Thus, practical implications of such molecules cannot be undermined which may facilitate the growth of such cash crops also that will promote a global cultivation map for medicinal plants under such challenging or hostile salt affected areas thereby positively affecting the economic revenues in agriculture sector.

Keywords:

Wheat, Osmoprotectant, Trehalose, Salt stress.

1. INTRODUCTION

Around the globe, bread wheat (*Triticum aestivum L.*) is the leading grown food cereal, owing a wider adaptability as well as quality of nutritive values than other cereals. Equally in te-

rms of production and land it also stands first. In the third world countries, it also serves as a strategic crop and has a substantial role on the national economy (Yadav *et al.*, 2018). While, to meet the food security of increasing population its demand is increasing (Jahan *et al.*, 2019). At the same time several abiotic stresses such as heat, drought and salinity influence the productivity of wheat across the globe and among them the most important is soil salinity, particularly in arid and semi-arid regions (Out *et al.*, 2018). The primary environmental difficulties that negatively influence the development and growth of plants by altering the physio-biochemical process is soil salinity (Allakhverdiev *et al.*, 2000). Due to which 60% crop productivity is globally lost (Xie *et al.*, 2016). Nearly 20% of the total cultivated land across the world is affected by salt stress Oproi and Madosa (2014).

It is well-established that under salt stress, owing to higher osmotic stress uptake of high concentrations of soluble salts limits the water uptake through the roots system. Consequently, membrane stability changes because of limited water uptake in plant cells that influences the turgor pressure (Sairam *et al.*, 2002), as the uptake of essential nutrients is limited, high concentration of ions is absorbed by plant cells causing nutrient deficiency (Goudarzi and Pakniyat, 2008). Exposure of 2, 4, 6 and 8 ds m⁻¹ salinity decreased the number of foliages, root growth and dry matter in *Aloe vera*. The reduced levels of total soluble solids (TSS) is the main limiting factor for these damages. When compared to the control this resulted in lower sprout production (30%) under salt stressed plants (Moghbeli *et al.*, 2012).

In terms of cash crops, an attractive and e-

conomical approach is development of salt tolerant wheat varieties to enhance wheat production under saline areas. However, modern genomics technologies are required for discovery of salt tolerant genes and their precise mobilization into salt sensitive wheat varieties that are agronomically superior (Moose and Mumm, 2008). During osmotic and ionic stress osmoprotectant compounds play a vital role as membrane stabilizer with capabilities of protecting cell membrane, organelles and enzymes that are sensitive to dehydration and ionic damage (Sairam and Tyagi, 2004). They are a group of organic molecules also known as compatible osmolytes or compatible solutes, that have no charge, polar and have soluble nature (Fang and Xiang, 2015). These organic osmolytes are synthesized and accumulates in plants that protects them from salinity-induced damages and osmotic stress. Many efforts were made to use osmoprotectant compounds as a pre-sowing seed treatment or exogenous application as foliar treatment on plants at different growth stages to improve salinity tolerance of different crops. These compounds include proline, glycine betaine, or trehalose (Dawood and Sadak, 2014). One of these compounds trehalose, is a non-reducing disaccharide of glucose (Fig. 1) which plays an important role as a stress protectant in aquatic plants. Additionally, an energy source, protect biological structures from damage during desiccation, the unique physicochemical properties efficiently stabilize dehydrated enzymes, proteins and lipid membranes (Fernandez *et al.*, 2010).

Trehalose being a signaling and antioxidant molecule has the added advantage. During detoxification and stress response it also acts as stress responsive element of genes (Bae *et al.*, 2005). However, to ameliorate stress in-

duced adverse effects in most plants Tre production is not sufficient. On the other hand, external Tre application has been suggested as an alternative approach to induce stress tolerance that increases the internal level of this osmolyte (Chen and Murata, 2002). Different abiotic stresses including heat, water deficit, and salinity in wheat and drought in maize were alleviated by its exogenously application of Tre (Ali and Ashraf, 2011). It is an osmoprotectant that can enhance salinity stress tolerance of wheat plant through increasing some osmoprotectant compounds such as glucose, trehalose, proline, and free amino acids (Sadak, 2019). Therefore, the aim of the present study was to investigate the effects of exogenous application of Tre on different growth parameters such as root and shoot lengths and fr-

esh and dry weights of different wheat cultivars with particular focus on their use for future wheat breeding programs.

2. MATERIALS AND METHODS

2.1. Screening of Wheat Genotypes at Seedling Stage

The experiment was carried out *in vitro* (petri dishes) at Department of Genetics, University of Karachi, Pakistan.

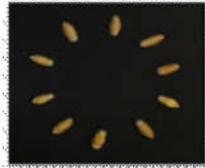
2.2. Germplasm Collection

Ten wheat varieties namely NIA Amber-10, Bhatoor, Bhattai, Borloug-16, DN-11, DN-84, Punjab-11, TJ-83, Zamindar-04 and Zincol-16 were collected from different provinces of Pakistan (Table 1).

Table 1. Sources of wheat germplasm from different provinces of Pakistan

S.No	Genotypes	Sources of Germplasm
1	NIA Amber-10	 Sindh
2	Bhatoor	 Sindh

3	Bhittai	 <p>Sindh</p>
4	Borloug-16	 <p>NARC Islamabad</p>
5	DN-11	 <p>KPK</p>
6	DN-84	 <p>KPK</p>
7	Punjab-11	 <p>Punjab</p>

8	TJ-83	 <p style="text-align: center;">Sindh</p>
9	Zamindar-04	 <p style="text-align: center;">Balochistan</p>
10	Zincol-16	 <p style="text-align: center;">NARC Islamabad</p>

2.3. In vitro Experiment

An experiment was conducted with completely randomized design (Three replications of each treatment) Control (dH₂O), Saline conditions (150mM NaCl) treated group (10 and 50mM) with Tre. Seeds from different wheat varieties were treated with sodium hypochlorite solution (1%) for 1h followed by repeat washing with distilled water. Seeds were germinated under normal condition for 7days at room temperature (15-20°C). Seven days old seedlings were subjected to Tretreatment (10 and 50mM) without and with NaCl (150mM) for consecutive five days. After twelve days old seedlings were harvested after stress period, root and shoot lengths and fresh weight were recorded. After

drying all the seedlings using incubator (55°C) for 48 hours, dry weight was also noted.

2.4. Statistical Analysis

The data was analyzed by using the analysis of variance (ANOVA);genotypes and treatments were considered as fixed factors and replications as random factor. ANOVA was performed by Duncan’s multiple range test (DMRT) for mean comparison using the statistical software SPSS version 16.

3. RESULTS AND DISCUSSION

Control (dH₂O), Saline conditions (150 mM NaCl) and treated group (10 and 50mM) with

Tre data was collected for root and shoot length of wheat seedlings also for fresh and dry weight from ten genotypes performed to statistical analysis i.e.(ANOVA). Mean squares revealed that main effect (genotypes and treatments) and

their interaction was significant for root length, shoot length, fresh weight and dry weight (Table 2). Data was subjected to Duncan's multiple range test (DMRT) to evaluate significance of mean comparisons (Table 3).

Table 2. Mean squares for root, shoot length (cm), fresh and dry weight (g) of wheat genotypes under Control, NaCl and Tre treatments.

Sources of Variance	d.f	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
Genotypes (G)	9	28.059***	42.156***	0.834***	0.011***
Treatments (T)	5	112.357***	77.365***	5.474***	0.296***
G×T	45	5.057***	1.735***	0.221***	0.004***

Note: d.f: degrees of freedom; * $P < 0.05$ level; ** $P < 0.01$ level; *** $P < 0.001$ level

3.1 a) Root and shoot length of wheat seedlings

Salt stress caused a significant amount of reduction in shoot with a range of 5-39% and root length 6-52% of all wheat cultivars. Application of Tre as a foliar spray caused a significant increase in shoot (91%) and root length (13%) under non-saline conditions when compared to control group with means 14.3 and 12.8 cm respectively. Exogenous application

of Tre appeared to be effective in increasing the shoot and root length of all cultivars with a range 8-34% and 6-33% under saline conditions. However, the maximum promotion was observed at 50 mM Tre compared to 10 mM (Fig. 2). Under saline condition exogenous Tre alleviated the adverse effect of NaCl by enhancing growth of shoot and root of wheat seedlings when compared to salt alone (Fig.3).

Table 3a: Mean comparisons for root length (cm) in wheat genotypes under Control, NaCl and Tre treatments

Genotypes	Control	T1	T2	T3	T4	T5
NIA Amber-10	10 ^{cd} ±0.45	9.3 ^a ±0.17	11.1 ^{bc} ±0.35	11.7 ^g ±0.17	10.5 ^a ±0.32	11.5 ^{bc} ±0.26
Bhatoor	12.1 ^b ±0.03	9 ^d ±0.09	11.2 ^{bc} ±0.12	13.2 ^{de} ±0.45	10 ^a ±0.03	12 ^a ±0.03
Bhittai	10.4 ^c ±0.46	9.6 ^a ±0.30	11.8 ^b ±0.15	12.2 ^{fg} ±0.31	10.6 ^a ±0.31	11.2 ^{bc} ±0.34
Borloug-16	13.4 ^a ±0.30	8.5 ^b ±0.29	11.1 ^{bc} ±0.20	14.6 ^c ±0.28	8.6 ^b ±0.31	9.1 ^c ±0.18
DN-11	10.6 ^c ±0.30	9.5 ^a ±0.29	11.6 ^b ±0.22	16.8 ^b ±0.15	10.2 ^a ±0.44	11.6 ^{bc} ±0.35
DN-84	8.8 ^{de} ±0.18	7.1 ^c ±0.23	9.3 ^d ±0.15	12.6 ^{ef} ±0.21	7.3 ^c ±0.15	7.8 ^d ±0.22
Punjab-11	13.3 ^a ±0.44	9.1 ^{bc} ±0.06	10.5 ^c ±0.29	14.5 ^c ±0.29	10.1 ^a ±0.24	10.8 ^b ±0.12
TJ-83	14.2 ^a ±0.67	9.5 ^a ±0.29	16.6 ^a ±0.30	18.6 ^a ±0.21	10.6 ^a ±0.30	11.6 ^{bc} ±0.30
Zamindar-04	11.0 ^{bc} ±0.59	9.6 ^a ±0.22	11.1 ^{bc} ±0.06	15.0 ^c ±0.50	10.4 ^a ±0.32	11.2 ^b ±0.27
Zincol-16	8.1 ^e ±0.30	8.5 ^b ±0.29	11.2 ^{bc} ±0.44	13.6 ^d ±0.24	8.0 ^{bc} ±0.03	8.2 ^d ±0.03
Average	11.19	8.97	11.54	14.26	9.62	10.49

3.1 b) Fresh and dry weight of wheat seedlings

Wheat seedlings were supplemented with Tre increased fresh and dry weight (13% and 85%) when compared to control seedlings with values of mean 2.35 and 0.49g, respectively. Supplementation with Tre (50 mM) markedly increased fresh and dry weight as compared to 10 mM which caused a slight increase in fresh weight. Salt stress caused a significant reduction

in shoot, fresh and dry weights with a range of 4-46% and 13-26% of all ten cultivars. Under salt stress, exogenous Tre promoted growth of wheat varieties with a range of 2-27% and 12-14% as compared to stress alone. Fresh and dry weight of seedlings supplied with 50 mM Tre increased significantly, as compared to salt-stressed seedlings without osmoprotectant. Tre 10 (mM) demonstrated a slight increase in fresh and dry weight presented in Fig.4.

Table 3b. Mean comparisons for shoot length (cm) in wheat genotypes under Control, NaCl and Tre treatments.

Genotypes	Control	T1	T2	T3	T4	T5
NIA Amber-10	10 ^e ±0.25	9.5 ^c ±0.29	11.1 ^e ±0.43	11.5 ^e ±0.25	9.8 ^c ±0.06	9.5 ^d ±0.09
Bhatoor	12.5 ^c ±0.09	8.5 ^d ±0.23	12.6 ^c ±0.07	13 ^d ±0.06	8.8 ^d ±0.25	9.5 ^d ±0.38
Bhittai	14.3 ^a ±0.12	10.7 ^a ±0.15	15.1 ^a ±0.66	15.2 ^b ±0.28	11.4 ^a ±0.36	12.2 ^a ±0.23
Borloug-16	10.1 ^e ±0.06	7.0 ^d ±0.20	11.6 ^{de} ±0.09	12.5 ^d ±0.29	7.5 ^e ±0.50	9.4 ^d ±0.47
DN-11	10 ^e ±0.12	8.6 ^c ±0.20	10 ^f ±0.15	10.6 ^e ±0.09	8.9 ^{cd} ±0.32	9.9 ^{cd} ±0.46
DN-84	10 ^e ±0.12	9 ^{bc} ±0.06	12.2 ^{cd} ±0.15	12.9 ^d ±0.39	10.3 ^b ±0.25	10.7 ^{bc} ±0.15
Punjab-11	12.1 ^c ±0.19	8.7 ^c ±0.15	13.7 ^c ±0.18	14 ^c ±0.09	9.4 ^c ±0.38	9.5 ^d ±0.12
TJ-83	11.5 ^d ±0.18	9.6 ^c ±0.23	12.5 ^c ±0.21	13 ^d ±0.30	10.4 ^b ±0.40	11 ^b ±0.06
Zamindar-04	14.1 ^b ±0.05	10.5 ^b ±0.29	14.6 ^b ±0.09	16.1 ^a ±0.58	11.4 ^a ±0.32	12.5 ^a ±0.17
Zincol-16	8.2 ^f ±0.24	7.3 ^d ±0.14	9.0 ^g ±0.10	9.7 ^f ±0.09	7.6 ^e ±0.55	8.4 ^{±e} 0.20
Average	11.58	8.96	12.27	12.86	9.52	10.28

Table 3c. Mean comparison for fresh weight (g) in wheat genotypes under Control, NaCl and Tre treatments

Genotypes	Control	T1	T2	T3	T4	T5
NIA Amber-10	1.33 ^d ±0.05	1.27 ^d ±0.01	1.40 ^d ±0.03	2.24 ^d ±0.09	1.30 ^d ±0.01	1.34 ^b ±0.03
Bhatoor	1.40 ^d ±0.01	0.82 ^f ±0.01	1.29 ^e ±0.03	1.61 ^f ±0.06	0.90 ^d ±0.05	1.05 ^d ±0.03
Bhittai	1.40 ^b ±0.03	1.24 ^d ±0.02	2.37 ^a ±0.03	2.84 ^b ±0.08	1.34 ^d ±0.04	1.41 ^b ±0.03
Borloug-16	1.57 ^c ±0.00	1.34 ^{bc} ±0.01	1.62 ^c ±0.03	2.36 ^d ±0.08	1.36 ^d ±0.02	1.41 ^b ±0.03

DN-11	1.60 ^{bc} ±0.03	0.86 ^f ±0.02	1.22 ^c ±0.05	1.33 ^g ±0.05	0.91 ^d ±0.02	1.02 ^d ±0.04
DN-84	1.66 ^{abc} ±0.03	1.29 ^{cd} ±0.01	1.57 ^c ±0.04	2.80 ^b ±0.04	1.34 ^d ±0.01	1.37 ^b ±0.03
Punjab-11	1.72 ^{ab} ±0.04	1.36 ^b ±0.02	1.41 ^d ±0.04	2.29 ^d ±0.03	1.42 ^d ±0.07	1.39 ^b ±0.01
TJ-83	1.64 ^{abc} ±0.03	1.13 ^e ±0.03	1.76 ^b ±0.03	2.66 ^c ±0.10	1.14 ^c ±0.03	1.24 ^c ±0.03
Zamindar-04	1.75 ^a ±0.07	1.11 ^e ±0.00	1.80 ^b ±0.03	3.34 ^a ±0.02	1.15 ^c ±0.03	1.25 ^c ±0.03
Zincol-16	1.58 ^c ±0.00	1.41 ^a ±0.03	1.49 ^d ±0.01	2.05 ^e ±0.01	1.70 ^a ±0.04	1.84 ^a ±0.04
Average	1.56	1.19	1.59	2.35	1.26	1.33

Table 3d: Mean comparison for dry weight (g) of wheat genotypes under Control, NaCl and Tre treatments

Genotypes	Control	T1	T2	T3	T4	T5
NIA Amber-10	0.29 ^{bc} ±0.01	0.24 ^a ±0.01	0.30 ^c ±0.02	0.39 ^d ±0.02	0.35 ^{bc} ±0.00	0.41 ^{bc} ±0.02
Bhatoor	0.23 ^f ±0.01	0.20 ^{de} ±0.01	0.34 ^{bc} ±0.01	0.48 ^{bc} ±0.02	0.32 ^c ±0.00	0.40 ^{bc} ±0.01
Bhittai	0.25 ^{de} ±0.01	0.22 ^{cd} ±0.01	0.43 ^{ab} ±0.01	0.57 ^a ±0.02	0.41 ^{ab} ±0.01	0.49 ^a ±0.02
Borloug-16	0.33 ^a ±0.01	0.24 ^a ±0.01	0.35 ^{bc} ±0.02	0.47 ^{bc} ±0.02	0.36 ^{ab} ±0.00	0.41 ^{bc} ±0.03
DN-11	0.24 ^{cf} ±0.01	0.18 ^e ±0.00	0.39 ^{abc} ±0.01	0.52 ^{ab} ±0.02	0.41 ^a ±0.01	0.45 ^{abc} ±0.02
DN-84	0.26 ^{de} ±0.01	0.18 ^e ±0.00	0.34 ^{bc} ±0.03	0.44 ^{cd} ±0.02	0.34 ^{bc} ±0.01	0.41 ^{bc} ±0.02
Punjab-11	0.25 ^{de} ±0.01	0.20 ^{cd} ±0.01	0.31 ^c ±0.01	0.56 ^a ±0.02	0.36 ^{bc} ±0.00	0.45 ^{abc} ±0.02
TJ-83	0.24 ^f ±0.01	0.21 ^{cd} ±0.00	0.37 ^{abc} ±0.02	0.43 ^{cd} ±0.02	0.33 ^c ±0.01	0.39 ^{bc} ±0.02

Zamindar-04	0.30 ^{ab} ±0.01	0.24 ^{ab} ±0.01	0.36 ^{abc} ±0.05	0.49 ^{bc} ±0.02	0.33 ^c ±0.00	0.38 ^c ±0.04
Zincol-16	0.27 ^{cd} ±0.01	0.22 ^{bc} ±0.01	0.46 ^a ±0.02	0.51 ^{ab} ±0.02	0.42 ^a ±0.01	0.46 ^{ab} ±0.03
Average	0.27	0.21	0.38	0.49	0.36	0.42

Wheat genetically consists of $2n = 6x = 42$ chromosomes composed of three distinctive genomes A, B and D namely allohexaploid (AABBDD). Each genome has 7 pairs of chromosomes (1-7A, 1-7B and 1-7D) (Shaista *et al.*, 2010). For global food security it constitutes one of the most pivotal cereal crops (Chairi *et al.*, 2020). It is one of the principal sources of calories and protein. The world's 82-85 percent population mainly rely wheat crop (Caverzan *et al.*, 2016). It also serves as a main food component for the population of Pakistan (Zafar *et al.*, 2015) and ranked 2nd after maize (Zeb *et al.*, 2009). According to a Global Agricultural Information Network report from the US Department of Agriculture (USDA) the current year's wheat production forecast in Pakistan is 25.5 million tonnes, a 1.2-million-tonne increase over the previous year. Pakistan procured 6.5 million tonnes of wheat from this year's harvest, about 80% of its goal. Salinity or sodicity affects more than 6 percent of the world's land (Priyanka and Arun, 2015). An estimated value affected by salinity in irrigated areas of Pakistan is 20% (Asian Water Development Outlook, 2013). As a result, plant growth and development are adversely affected by salt stress (Li *et al.*, 2011; Mahmood *et al.*, 2012).

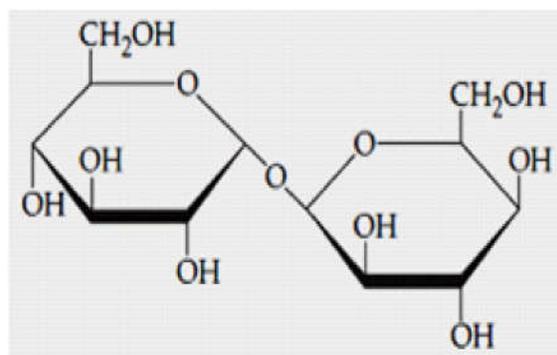


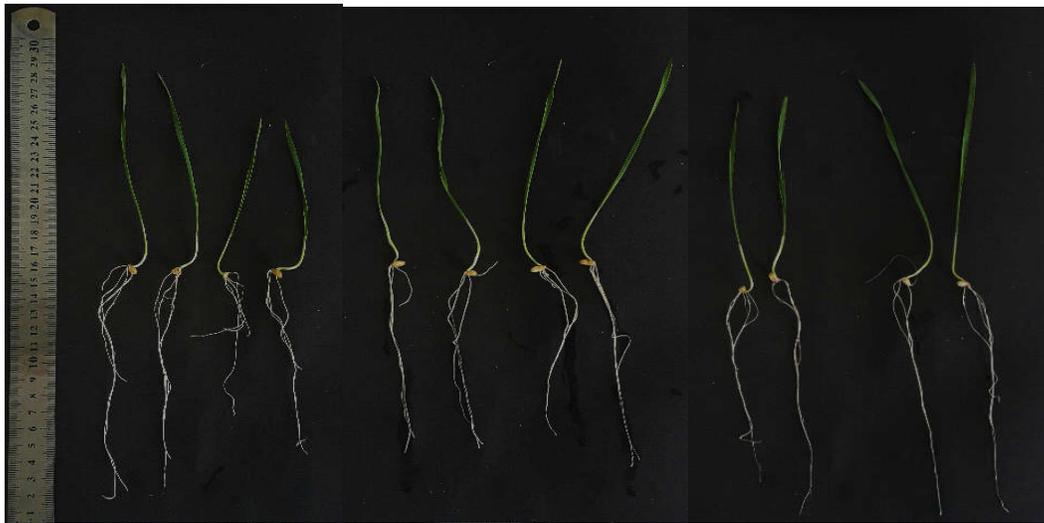
Fig.1: Trehalose, a disaccharide consisting of two glucose units linked by α -(1'!1) glycosidic bond.

In plant's life cycle, seedling has been considered as the most vulnerable stage. In *Thymus maroccanus* (an antibacterial plant) salinity caused a significant reduction in the seedling growth by suspending cell division, inhibiting reserve food mobilization, enlarging and injuring hypocotyls. Similar results were noticeable for *basil*, *chamomile* and *marjoram* (Said-Al Ahl and Omer, 2011). Our study demonstrated that seedling growth under stress condition with 150 mM NaCl was drastically inhibited. During the experimental period salt stress induced a significant variation in plant growth conditions. All wheat varieties showed considerable reductions in all studied growth parameters. Statistical analysis stated that var-

ieties, treatments and their interaction were significant at $P < 0.001$ level for all the growth parameters such as Root, shoot, fresh and dry weight. Under stress condition, Zamindar-04 showed maximum root length (9.6 cm) (Fig. 2a) where as DN-84 had minimum root length (7.1 cm). In Bhattai maximum shoot length (10.7 cm) (Fig. 2b) while Zincol-16 showed minimum shoot length (7.3 cm). The overall reduction in root length was 18.0% and shoot length was 21% confirmed the negative effect of NaCl treatments on plant growth at early seedling stage. Similar results were obtained for fresh (24%) and dry weight (20%). When compared with control group these parameters were highly affected by salt stress with greater amount of reduction. Decrease in root and shoot develop-

ment is due to the higher level of NaCl toxic effects as well as unbalanced nutrient uptake by seedlings. Under high salt stress condition initially inhibition of the root and shoot elongation occurs which further slowing down the water uptake for overall osmotic adjustments of the plant structure making it difficult for further plant to grow and develop. The reduction of these growth parameters was reported previously (Singh *et al.*, 1994) that salt stress reduces dry matter accumulation, leaf area and photosynthetic ability. Salt effect mainly makes plant growth processes sensitive, thus biomass production and growth rates provide reliable criteria for assessing the degree of salt stress and the ability of a plant to survive (Amor *et al.*, 2005).

a) Bhattai

**C****T1****T2****T3****T4****T5**

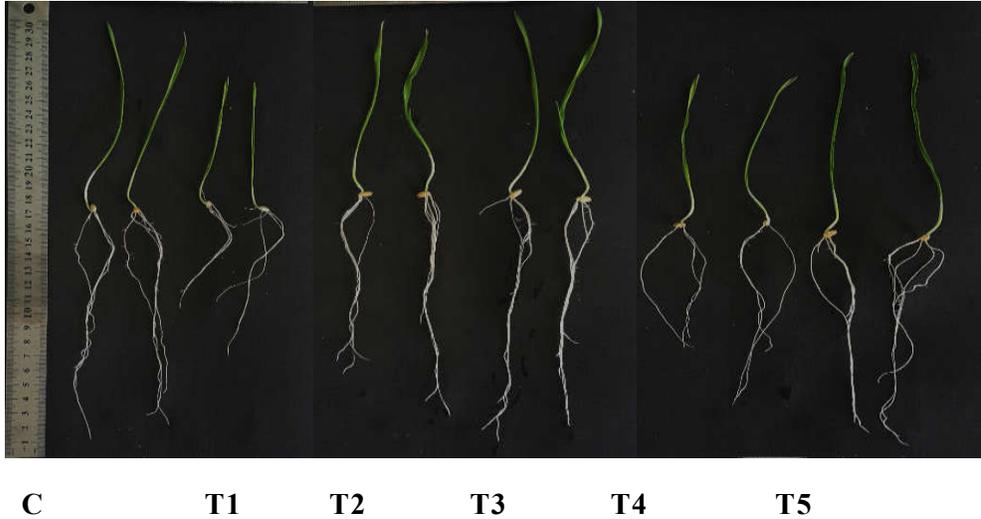
b) Zamindar-04

Fig.2: The effect of sodium chloride and trehalose on wheat varieties at seedling stage.

Control (C) and treatments: Sodium chloride (T1, 150 mM), Trehalose (T2, 10mM and T3, 50mM), T1 + T2 (T4) and T1 + T3 (T5).

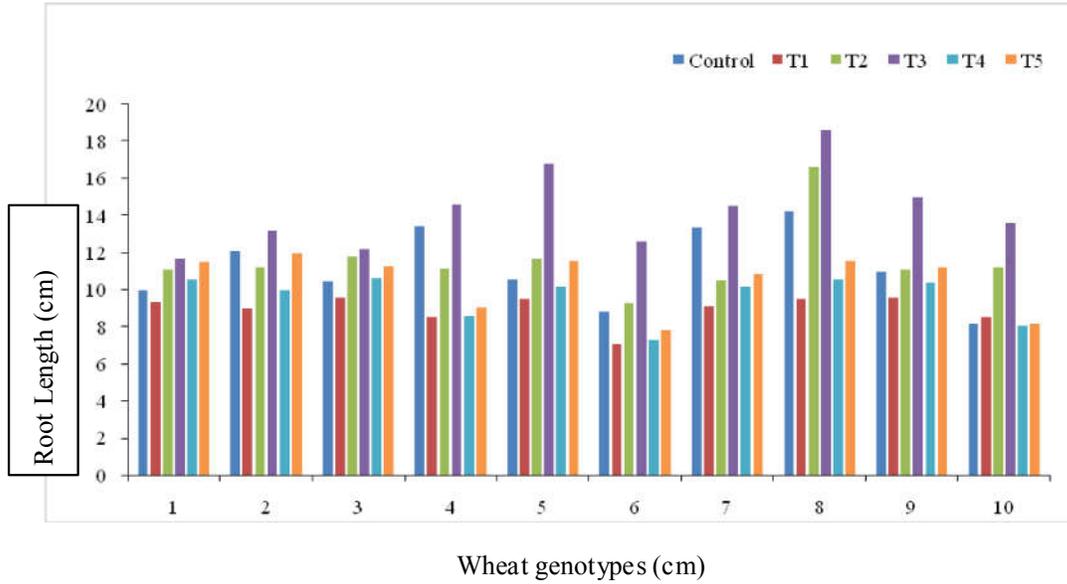
The scale on the right hand was used to measure the shoot and root lengths (cm).

Under normal condition addition of Tre to promote seedling growth of wheat cultivars as compared to control group. Tre treatment alone played a significant role in improved growth condition. All ten wheat cultivars performed adequately at both concentrations of Tre (10 and 50mM) which predominantly enhanced the overall growth conditions. In foliar application of Tre Zamindar-04 and Bhittai showed maximum shoot length (15.1 cm and 14.6 cm) (Fig. 4a and b). Maximum root length was observed in TJ-83 (18.6 cm) and minimum NIA Amber-10 (11.7 cm). The Tre at 50 mM was more beneficial to improve the length and weight of root and shoot with overall promotion 92%, 13%, 51% and 85% respectively.

These results were similar with earlier report that Tre applied exogenously mainly protects plants from desiccation by water recovery, maintains enzymes level, proteins and lipid membranes from aggregation also causes the changes in the metabolism of sugar. Zeid (2009) mentioned the effect of Tre in maize. According to his observation before exposure to salt treatment exogenous accumulation of Tre was able to improve growth of salt-stressed and unstressed maize plants. Furthermore, under stress condition exogenous supply of Tre dramatically supply of Tre dramatically reversed the negative effects of salt and significantly improved the overall growth of the wheat seedlings. Combined effect of Tre and NaCl, Zamindar-04 demonstrated

maximum shoot length (12.5 cm) whereas Zincol-16 showed minimum length (8.4 cm). Maximum root length was noticed in Bhatoor (12 cm) and minimum in DN-84 (7.8 cm).

a) Root length (cm)



a) Shoot length (cm)

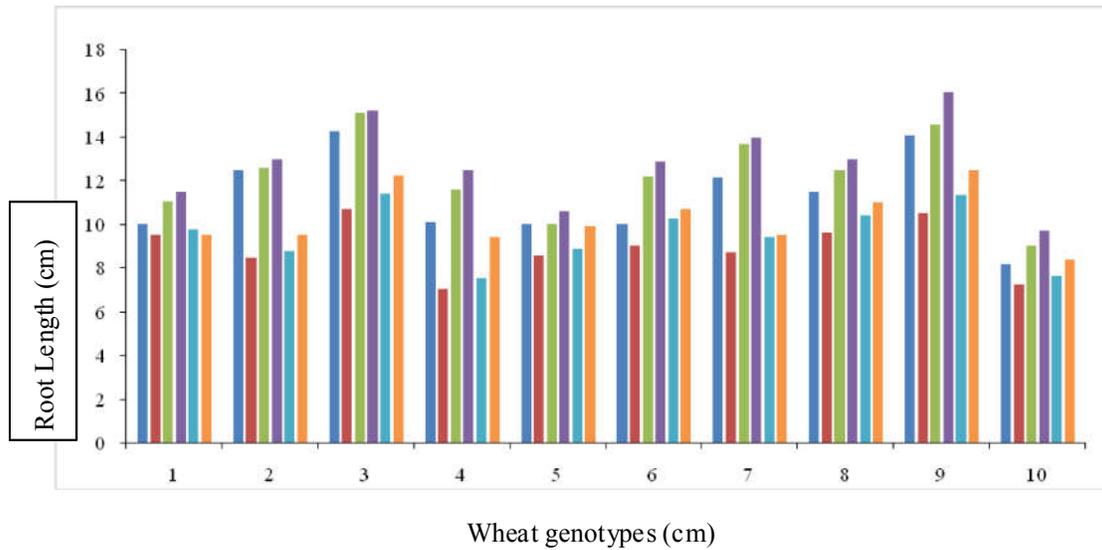
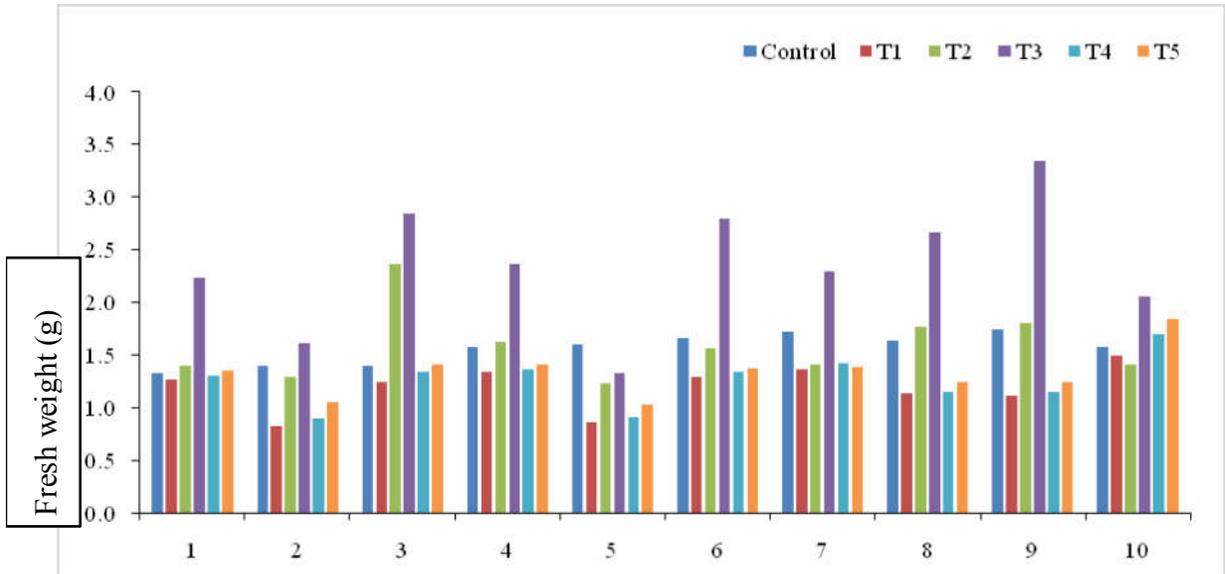


Fig. 3: Root and shoot length of wheat seedling of various wheat genotypes.

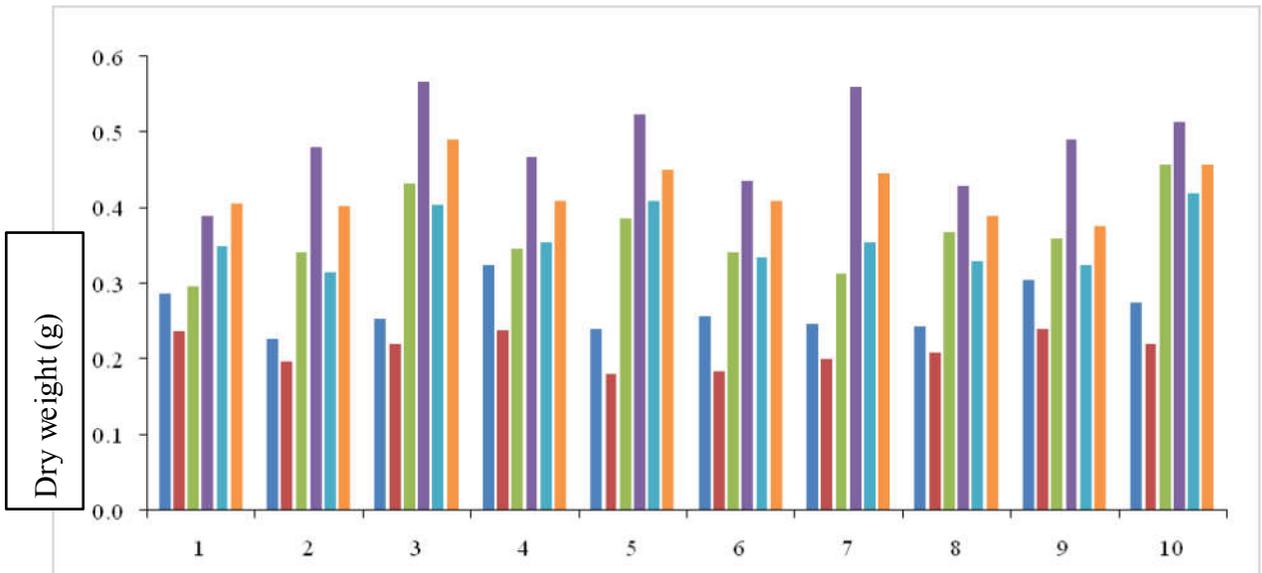
(1) NIA Amber-10,(2) Bhatoor, (3) Bhattai, (4) Borloug-16, (5) DN-11, (6)DN-84,(7) Punjab-11, (8) TJ-83, (9) Zamindar-04, (10) Zincol-16.Control (C) and treatments: Sodium chloride (T1,150 mM), Trehalose (T2, 10mM and T3 50mM), T1 + T2 (T4) and T1 + T3 (T5).

a) Fresh weight (g)



Wheat genotype

b) Dry weight (g)



Wheat genotype

Fig. 4: Fresh and dry weights of wheat seedling of various wheat genotypes

The adverse effect of salt stress was alleviated significantly by foliar-applied trehalose resulting increase in seedling root length up to 17% and shoot length 15% fresh weight and dry weight 12% and 13% at Tre 50 mM. These results were similar to the previously report (Shahbaz *et al.*, 2016) where shoot length and dry weight were enhanced by foliar-applied trehalose in rice. It was also confirmed that Tre is applied exogenously plants are better condition to recover from stress. (Djilianov *et al.*, 2005). It was also suggested that Tre accumulation influences the alteration of sugar metabolism leading to an osmoprotectant effect under stress condition. During high levels of salt accumulation and drought condition Tre could be used as a stress reducing approach as it promotes plant growth (Mosquera *et al.*, 2019).

4. CONCLUSION

Under saline medium, the growth rate of all wheat cultivars was significantly affected. However, foliar-applied Trehalose a protective role under saline conditions. Furthermore, Tre alone provided substantial attributes to growth parameters such as root length (cm), shoot length (cm), fresh weight (g) and dry weight (g). Moreover, this appears to be promising strategy for breeders to use this osmoprotectant as a beneficial source to reduce stress in plants to screen best yielded salt tolerant varieties at early stages with positive impact Tre. Positive aspect of this research will also impact on revenue generation in agriculture practice of this common food cereal. However further investigations on other stress parameters such as heat, drought and metal toxicity of different crops needs to be included in future breeding programs.

Conflict of interest

The is no conflict of interest

Acknowledgement

This study supported by the grant of Dean Faculty of Science, University of Karachi.

5. REFERENCES

1. Ali, Q., Ashraf, M. (2011). Exogenously applied glycinebetaine enhances seed and seed oil quality of maize (*Zea mays* L.) under water deficit conditions. *Environmental and Experimental Botany* 71: 249-259.
2. Allakhverdiev, S. I., Sakamoto, A., Nishiyama, Y., Inaba, M., Murata, N. (2000). Ionic and osmotic effects of NaCl - induced inactivation of photosystem I and II in *Synechococcus* sp. *Journal of Plant Physiology*. 123: 1047-1056.
3. Amor, N., Hamed K.B., Debez, A., Grignon, C., Crabbedly. (2005). Physiological and antioxidant responses of perennial halophyte *Crithmum maritimum* to salinity. *Plant Science*. 4: 889-899.
4. Lohani, B. N., Ait-Kadi, M. (2013). *Asian Water Development Outlook 2013: Measuring Water Security in Asia and the Pacific*.
5. Bae, H., Herman, E., Bailey, B., Bae, H.J., Sicher, R. (2005). Exogenous trehalose alters *Arabidopsis* transcripts involved in cell wall modification, abiotic stress nitrogen metabolism, and plant defense *Physiologia Plantarum*. 125:114-126.
6. Caverzan, A., Casassola, A., Brammer, S. P. (2016). Antioxidant responses of wheat plants under stress. *Genetics and Molecular Biology*. 39: 1-6.
7. Chairi, F., Aparicio, N., Serret, M. D., Araus, J. L. (2020). Breeding effects on the genotype×environment interaction for yield of durum wheat grown after the Green Revolution: The case of Spain. *The Crop Journal*. 8: 623-634.
8. Chen, T. H., and Murata, N. (2002). Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current Opinion in Plant Biology*. 5: 250-257.
9. Dawood, M G., Sadak, M.S. (2014). Physiological role of glycinebetaine in alleviating the deleterious effects of drought stress on canola plants (*Brassica napus* L.). *Middle East Journal of Agriculture Re-*

- search. 3:943-954.
10. Djilianov, D., Georgieva, T., Moyankova, D., Atanassov, A., Shinozaki, K., Smeeken, S. C. M., Verma, D.P.S., Murata, N. (2005). Improved abiotic stress tolerance in plants by accumulation of osmoprotectant genes: transfer approach. *Biotechnology and Biotechnological Equipment*. 19: 63-71.
 11. Fang, Y., Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences*. 72: 673-689.
 12. Fernandez, O., Béthencourt, L., Quero, A., Sangwan R.S., Clément, C. (2010). Trehalose and plant stress responses: friend or foe? *Trends in Plant Science*. 15: 409-417.
 13. Goudarzi, M., Pakniyat, H. (2008). Evaluation of wheat cultivars under salinity stress based on some agronomic and physiological traits. *Journal of Agriculture and Social Science*. 4: 35-38.
 14. Jahan, M. A. H. S., Hossain, A., Jaime, A., Da Silva, T., EL Sabagh, A., Rashid, M. H., Barutçular, C. (2019). Effect of naphthaleneacetic acid on root and plant growth and yield of ten irrigated wheat genotypes. *Pakistan Journal of Botany*. 51: 451-459.
 15. Li, J.T., Qiu, Z.B., Zhang, X.W., Wang, L.S. (2011) Exogenous hydrogen peroxide can enhance tolerance of wheat seedlings to salt stress. *Acta Physiologicae Plantarum*. 33:835-842.
 16. Mahmood, M., Bidabadi, S.S., Ghobadi, C., Gray, D.J (2012). Effect of methyl jasmonate treatments on alleviation of polyethylene glycol-mediated water stress in banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tip cultures. *Plant Growth Regulation*. 68:161-169.
 17. Moghbeli, E., Fathollahi, S., Salari, H., Ahmadi, G., Saliquehdar, F., Safari, A., Grouh, M.S.H. (2012) Effects of salinity stress on growth and yield of *Aloe vera* L. *Journal of Medicinal Plants Research*. 6:3272-3277.
 18. Mohamed, H., Akladios, S.A., El-Beltagi, H.S. (2018). Mitigation of the harmful effect of salt stress on physiological, biochemical and anatomical traits by foliar spray with trehalose on wheat cultivars. *Fresenius Environmental Bulletin*. 27:7054-7065.
 19. Moose, S.P., Mumm, R.H. (2008). Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiology*. 147: 969-977.
 20. Oproi, E., Madosa, M. (2014). Germination of Different Wheat Cultivars under Salinity Conditions. *Journal of Horticulture, Forestry and Biotechnology*. 18: 89-92.
 21. Out, H., Celiktas, V., Duzenli, S., Hossain, A., El Sabagh, A. (2018). Germination and early seedling growth of five durum wheat cultivars (*Triticum durum* desf.) is affected by different levels of salinity. *Fresenius Environmental Bulletin*. 27:7746-7757.
 22. Priyanka T. and G. Arun. 2015. An overview of impact of subsurface drainage project studies on salinity management in developing country. *Applied Water Science*. 29-4.
 23. Sadak, M.S. (2019). Physiological role of trehalose on enhancing salinity tolerance of wheat plant. *Bulletin of the National Research Centre*. 43:1-10.
 24. Said-Al Ahl, H.A.H., Omer, E.A. (2011). Medicinal and aromatic plants production under salt stress. A review. *Herba Polonica*. 57:72-87.
 25. Sairam, R.K., Rao, K.V., Srivastava, G.C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Journal of Plant Science*. 163: 1037-1046.
 26. Sairam, R.K., Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*. 86: 407-421.
 27. Shaista, B., Ahmad, H., Begum, K., Ghafoor, S., Khan, I.A. (2010). Identification of RAPD Marker for Chromosome 1D of Common Wheat. *Asian J. of Agricultural Sciences*. 2: 51-52.
 28. Singh, S.B., Singh, B.B., Singh, M. (1994). Effect of kinetin on chlorophyll, nitrogen and proline in mung bean under saline conditions. *Indian Journal of Plant Physiology*. 37: 37-39.
 29. Theerakulpisut, P., Phongngarm, S. (2013). Alleviation of adverse effects of salt stress on rice seedlings by exogenous trehalose. *Asian Journal of Crop Science*. 5: 405-415.
 30. USDA (2020). World agricultural supply & demand estimates.
 31. Xie, J., Dai, Y., Mu, H., De, Y., Chen, H., Wu, Z., Ren, W. (2016). Physiological and biochemical responses to NaCl salinity stress in three *Roegneria* (Poaceae) species. *Pakistan Journal of Botany*. 8: 2215-2222.
 32. Yadav, S. S., Redden, R. J., Hatfield, J.L., Ebert, A. W., Hunter, D. (2018). Food Security and Climate Change. John Wiley & Sons, Technology & Engineering. 568.

33. Zafar, S., Ashraf, M.Y., Niaz, M., Kausar, A., Hussain, J. (2015). Evaluation of wheat genotypes for salinity tolerance using physiological indices as screening tool. *Pakistan Journal of Botany*. 47: 397-405.
34. Zeb, B., Khan, I.A., Ali, S., Bacha, S., Mumtaz, S., Swati, Z.A. (2009). Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. *African Journal of Biotechnology*. 8: 4016-4019.
35. Zeid, I.M. (2009). Trehalose as osmoprotectant for maize under salinity-induced stress. *Research Journal of Agricultural and Biological Sciences*. 5: 613-622.