

Effects of exogenous glucose and sucrose on photosynthesis in triticale seedlings under salt stress

L.H. WANG, G.L. LI, S. WEI, L. J. LI, S.Y. ZUO, X. LIU, W.R. GU, and J. LI[†]

College of Agriculture, Northeast Agricultural University, Harbin 150030, Heilongjiang Province, China

Abstract

Salinization disturbs metabolic processes of plants and results in reduced growth and productivity. In our study, the Dongnong 8809 variety of triticale (*×Triticosecale* Wittmack) was evaluated for its physiological responses to salt stress during the seedling stage. We evaluated biomass production, relative chlorophyll content, chlorophyll fluorescence, and leaf gas-exchange parameters. Our results indicated that salt stress greatly reduced matter accumulation, SPAD value, photosynthetic capacity. However, seedlings treated with exogenous sugars showed significantly enhanced matter accumulation, SPAD value, quantum yield of PSII, electron transport rate, net photosynthetic rate, decreased stomatal limitation value, and improved photosynthetic capacity compared to seedlings treated with salt stress alone. Exogenous glucose and sucrose can alleviate the inhibitory effect of salt stress on the growth of triticale, and of the two exogenous sugars, sucrose had a stronger alleviating effect than that of glucose under salt stress.

Additional key words: exogenous sugar; growth; photosynthesis; tolerance.

Introduction

Triticale (*×Triticosecale* Wittmack) is a cereal crop cultivar obtained by cross-fertilization of wheat (*Triticum* spp.) and rye (*Secale* spp.) (Rasouli and Kiani-Pouya 2015) that offers high forage production and is an important potential protein source for animal feed (Samad and Karmoker 2013). Climate-resilient crops such as triticale are important for food production.

Salinization has become a major concern throughout the world (Liu *et al.* 2015) and especially in China (Sharathchandra *et al.* 2016). More than 7% of the world land is salt-affected (Liang *et al.* 2005, Setia *et al.* 2013), especially, land in arid and semiarid regions (Bray *et al.* 2000). Salt stress affects plant growth and productivity during all developmental stages (Akram *et al.* 2011). The introduction and utilization of salt-tolerant crop plants is one of the most efficient and useful ways to cope with salt stress in these regions. Seed germination represents the primary period of plant growth, and at this stage, salt stress affects triticale most seriously. Since the photosynthetic activity of plants is inefficient during this phase, the ionic effect of salt stress is the dominant factor during the germination. Later, in young seedlings, the salt stress can lead to serious stomatal closure in leaves and a reduction of plant water potential; as a result, growth rate

and production decline (Teng *et al.* 2012, Hu *et al.* 2013).

Sugar is indispensable in the life cycle of plants; it provides plants with energy and participates in the synthesis of the cell wall but also acts as a primary messenger. Sugars, such as glucose, sucrose, trehalose, and fructose, participate in many signaling, regulatory, and metabolic pathways in plants (Smeekens *et al.* 2009, Trouvelot *et al.* 2014). These pathways include biotic stress resistance; for instance, the exogenous application of trehalose has been shown to enhance the resistance of wheat (*Triticum aestivum* L.) against powdery mildew caused by *Blumeria graminis* (Reignault *et al.* 2001, Muchembled *et al.* 2006, Tayeh *et al.* 2014). Glucose acts as a direct signaling molecule in plant metabolism and is closely related to phytohormones. If excessive glucose is supplied to the external environment of the seedlings, the growth of the seedlings is stopped and the differentiation is interrupted, the cotyledons cannot expand and turn yellow. This phenomenon may be related to the increase of abscisic acid (ABA). Garg *et al.* (2002) found that glucose functions as a compatible solute in stabilization of biological structures under abiotic stress in bacteria, fungi, and invertebrates. Sucrose can reduce the water potential of the cells to resist adverse environments. Baque *et al.* (2011) also found that 5% sucrose supply was ideal for the production of root dry mass in *Periploca sepium* Bunge, but higher sucrose

Received 18 October 2017, accepted 16 August 2018.

[†]Corresponding author; phone: +86 13204513712; fax: +86-451-55190447; e-mail: jingli1027@163.com

Abbreviations: ABA—abscisic acid; Chl—chlorophyll; C_i —intracellular CO_2 concentration; E —transpiration rate; ETR—electron transport rate; F_0 —minimal fluorescence yield of the dark-adapted state; F_0' —minimal fluorescence yield of the light-adapted state; F_m —maximal fluorescence yield of the dark-adapted state; F_m' —maximal fluorescence yield of the light-adapted state; F_s —steady-state fluorescence yield; F_v/F_m —maximum quantum yield of PSII photochemistry; F_v/F_0 —potential photochemical efficiency; g_s —stomatal conductance; L_s —stomatal limitation value; NPQ—nonphotochemical quenching; P_N —net photosynthetic rate; q_p —photochemical quenching coefficient; WUE—water-use efficiency; WUE_i —intrinsic water-use efficiency; Y —quantum yield of PSII.

Acknowledgements: This work was funded and supported by Key Research and Development Program of 13th Five-Year No. 2017YFD0300405.

concentrations inhibited the accumulation of root dry mass. The sugar-mediated repression of photosynthesis is among the general mechanisms that maintain photosynthetic homeostasis over a wide range of growing conditions (Mortain-Bertrand *et al.* 2008).

Since the area of saline soils in the world continues to increase (Munns 2002, Arzani 2008), studies on the effects of combined stress factors seem to be of great importance. In contrast to the large body of work that has focused on the effect of trehalose on photosynthesis (Pego *et al.* 2000, Gibson 2005, Araya *et al.* 2006), few studies have been performed on the effects of glucose and sucrose on photosynthesis in triticale seedlings under salt stress. Thus, the role of glucose and sucrose in salt tolerance must be examined further. In this study, we investigated the response of triticale seedlings to salt stress and sugar pretreatment in terms of growth and photosynthetic characteristics. We hope to reveal roles of glucose and sucrose in increasing triticale tolerance to salt stress and show that osmoregulators, such as glucose and sucrose, can be used to protect plants against hostile environmental conditions.

Materials and methods

Plant material and experimental design: Dongnong 8809 was chosen as the experimental materials, and triticale seeds were supplied by the Physical and Culture Research Center of Wheat at Northeast Agricultural University, China. Triticale seeds were chosen for their similar size and no damage, and then they were surface-sterilized according to Samad and Karmoker (2012). After sterilization, the seeds were placed in a computer-controlled incubator and kept in the dark at a temperature of 25°C to facilitate germination. Germinated triticale seeds with the same shoot length were moved to a HPG-280HX phytotron (East Union Hall, China) with day/night temperatures of 25 ± 2°C/20°C ± 2°C, day/night length of 12/12 h, and relative humidity of 60–80%. After germination, the seedlings were grown in half-strength Hoagland nutrient solution for 8 d (the seedlings had two leaves). Then they were treated with 0.5 mmol L⁻¹ sucrose, or 0.5 mmol L⁻¹ glucose or 0.5 mmol L⁻¹ mannitol dissolved in half-strength Hoagland nutrient for 3 d. After pretreatment, the solution was changed to normal growth (in half-strength Hoagland nutrient) solution with or without 100 mM NaCl, after which the seedlings were left to grow for 6 d. At the end of the experiments, seven types of treatment were evaluated: the control (CK) without treatments, glucose-pretreated (G), sucrose-pretreated (T) seedlings, salt-treated (S), glucose-pretreated, and salt-treated (G+S), sucrose-pretreated and salt-treated (T+S), and mannitol-pretreated and salt-treated (M+S) seedlings. Three replicates were used in each treatment, one hundred seeds were prepared in each replicate. After germination, we had to eliminate seeds, which did not germinate and showed shoot length inconsistency, and select the better growing seeds to grow in the computer-controlled incubator. The remaining seeds included three replicates per treatment, sixty seeds per replicate.

Biomass production: For each of the seven treatments, ten seedlings treated for 6 d were taken. The aboveground and underground parts were separated, and each was washed with distilled water and dried with filter paper. Then, the fresh samples were weighed, and the ratios of shoots to roots were calculated. The fresh samples were de-enzymed at 105°C for 15 min in a drying oven and then dried at 80°C for 48 h. Then, the dry matter mass was determined.

Photosynthetic characteristics: All photosynthetic parameters were determined using the middle part of the second fully expanded leaf, avoiding the leaf vein on the sixth day of the treatment with or without salt between 9:00 and 12:00 h in sunny weather. Ten replicates were used in each treatment.

SPAD values were determined with a CCM-200plus SPAD chlorophyll (Chl) content meter (Opti-Sciences, USA). The result of these measurements was displayed digitally as the so-called SPAD units. The value of these units is proportional to the Chl content of the analyzed leaf surface (6 mm²) (Monje and Bugbee 1992).

Chl fluorescence parameters were determined with a PAM-2500 Chl fluorometer (Walz, Germany). After a 20-min dark-adaptation period, the initial (F_0) and maximum fluorescence (F_m) were determined. Immediately, the leaves were exposed to an actinic irradiation of 447 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$ for 30 min to measure steady-state Chl *a* fluorescence (F_s), saturating pulses [$3,000\ \mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$] were applied to determine the maximum fluorescence (F_m'). Eventually, leaves were illuminated with far-red radiation to determine the minimal fluorescence (F_0'). Other parameters were calculated as follows: maximal photochemical efficiency of PSII, $F_v/F_m = (F_m - F_0)/F_m$, potential photochemical efficiency, $F_v/F_0 = (F_v/F_m)/(1 - F_v/F_m)$, actual photochemical efficiency of PSII in the light, $Y_{(II)} = (F_m' - F_s)/F_m'$ (Demmig-Adams 1996), photochemical quenching coefficient, $q_p = (F_m' - F_s)/(F_m' - F_0')$; nonphotochemical quenching coefficient, $NPQ = (F_m - F_m')/F_m'$, apparent photosynthetic electron transport rate, $ETR = \text{PPFD} \times 0.84 \times 0.5 \times \Phi_{\text{PSII}}$ (Maxwell and Johnson 2000, Baker 2008).

Different photosynthetic parameters, such as net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), leaf internal CO₂ concentration (C_i), and atmospheric CO₂ concentration (C_a), were determined with a CI-340 hand-held photosynthesis system (Walz, Germany). The instantaneous water-use efficiency (WUE) was calculated as P_N/E , and the intrinsic water-use efficiency (WUE_i) was determined as P_N/g_s . The stomatal limitation value (L_s) was defined as $1 - C_i/C_a$ (Yin *et al.* 2006). All photosynthetic measurements were taken at a saturating incident PPFD of 700 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$. The temperature was 25 ± 2°C and the concentration of CO₂ was 400 ± 5 $\mu\text{mol L}^{-1}$.

Statistical analysis: Experimental data are expressed as the mean with standard deviation. Statistical analysis was performed using SPSS 17.0 and Excel 2007, and all means were evaluated using Fisher's least significant difference

(LSD) test at a significance level of $p < 0.05$.

Results

Seedling growth: The growth of triticale seedlings showed significant differences between the seven treatments (Fig. 1). Without salt stress, compared to CK, T seedlings were in significantly better conditions, but G seedlings were more yellowish than that of CK, maybe the concentration of glucose was too high and stopped growth and differentiation. These results showed that sucrose can promote the growth of triticale seedlings, and glucose had an inhibiting effect at the high concentration.

Under salt stress, seedlings were weak, yellowish, and in a bad condition. Salt significantly affected the growth of the seedlings and caused serious lodging. However, seedlings pretreated with glucose and sucrose were not weak and maintained strong growth under saline conditions. Compared to S, seedlings pretreated with mannitol were stronger. But seedlings pretreated with mannitol were more yellowish than seedlings pretreated with glucose and sucrose under salt stress.

Biomass production: Compared with CK, the total dry mass of triticale in the G and T increased by 41.4 and 34.4%, and the total fresh mass increased by 59.7 and 65.7%, respectively, showing that glucose and sucrose pretreatments promoted the growth of triticale. The root/shoot ratios of G and T increased compared to CK.

Compared with those in CK, the total dry mass and total fresh mass of triticale in S decreased by 60.9 and

55.5%, respectively. The total dry mass of G+S and T+S were 3.91 and 3.34 times as high as those of S, while the total fresh mass G+S and T+S were 3.04 and 3.03 times as high as those of S. All growth parameters of the seedlings increased in G+S and T+S compared to S. Compared to S, the dry mass and fresh mass of the shoot and the root of G+S and T+S increased, while the differences in dry mass and fresh mass of the shoot and the root between M+S and S were not significant. The root/shoot ratios of G+S and T+S greatly increased compared to S. These results showed that salt stress significantly inhibited the growth of plants. Exogenous glucose and sucrose can enhance the salt resistance of plants and promote growth and dry matter accumulation. The same concentration of mannitol did not cause this effect, showing that the ameliorating effect of exogenous sugar on salt stress is not because of osmosis.

SPAD value: Chl contents are highly correlated with SPAD, thus, the changes in SPAD represent changes in the Chl content. Compared with CK, SPAD value increased in G by 41% at the sixth day, and SPAD value increased in T by 31% at the sixth day. It showed that glucose and sucrose can increase the Chl content in triticale (Fig. 2). SPAD values decreased with the application of salt. Compared with CK, SPAD values were lower in S by 13%. The pretreatment of glucose and sucrose could change the decline caused by salt. Compared to S, SPAD values were higher in G+S and T+S by 52 and 53%, respectively, and SPAD values in M+S were similar to S. These results showed that glucose and sucrose pretreatment could alleviate the Chl decline caused by salt stress and help

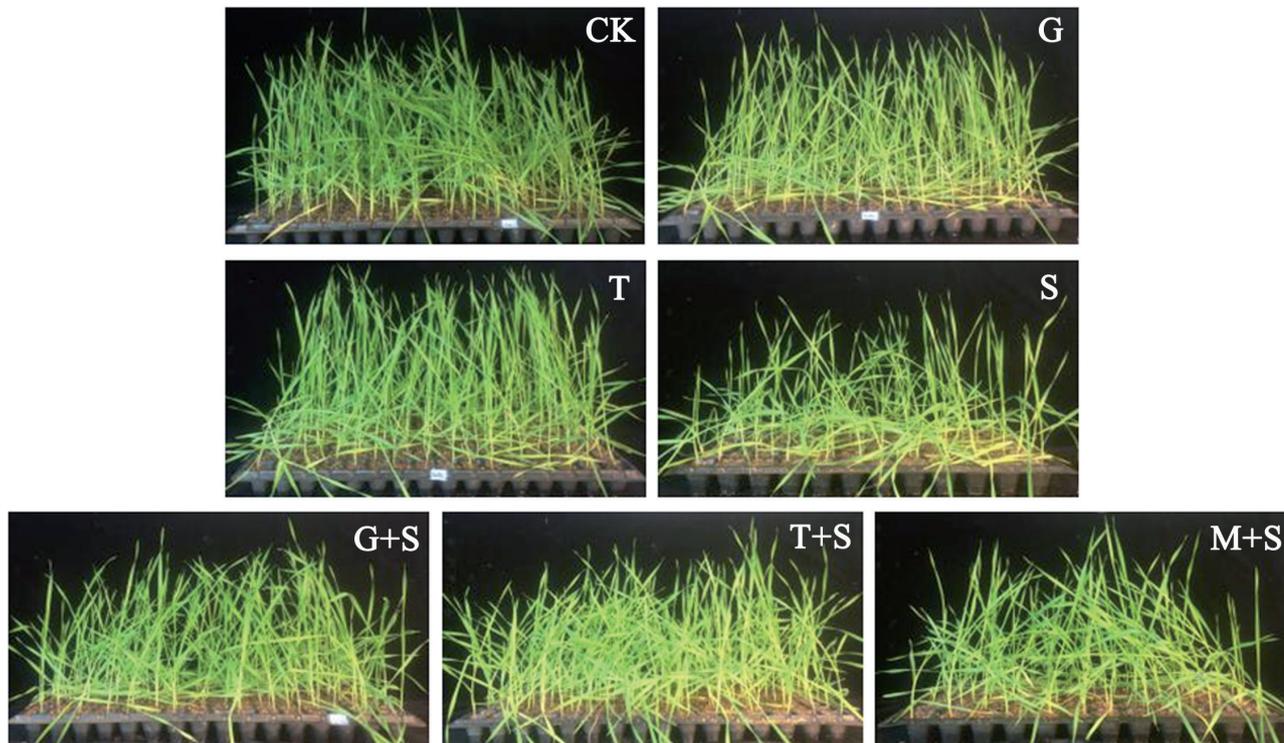


Fig.1. Effect of seven different treatments on the growth of triticale seedlings.

maintain normal photosynthesis. Mannitol did not show this effect.

Chl fluorescence: Compared with CK, F_0 was higher in G and T, while q_p , NPQ, Y, and ETR were lower in G and T, and the reduction of q_p , NPQ, Y, ETR in G were higher than that of T. Compared with CK, F_v/F_m was higher in G and lower in T, while F_m was higher in T. Compared with CK, F_0 , and NPQ were higher in S by 12 and 25%, respectively (Table 2). F_m , F_v/F_m , q_p , Y, ETR were lower in S, the reduction of q_p , Y, ETR was 17, 26, and 29%, respectively. Compared to S, F_0 was lower in G+S, T+S, and M+S, while F_v/F_m , q_p , Y, ETR were higher in G+S, T+S, M+S. Compared to S, the increment of q_p in G+S and M+S was equal, the increment of Y in M+S was higher than that of G+S and T+S, the increment of ETR in G+S, T+S and M+S was 13, 18, and 4%, respectively. Compared to S, F_m was higher in G+S, T+S and lower in M+S, while NPQ was higher in M+S and lower in G+S and T+S.

Leaf gas-exchange parameters: Compared with CK, P_N , g_s , C_i , and WUE were higher and E , L_s were lower in G and T. In G, g_s , E , and L_s were higher than that in T, while P_N ,

C_i , WUE, and WUE_i were lower in G than that in T.

Compared with CK, P_N , g_s , C_i , E , WUE, WUE_i were lower in S, while L_s was higher in S. Compared to S, P_N , g_s , C_i , E , WUE, and WUE_i were higher in G+S, T+S, and M+S (Table 3). The increase of these parameters in M+S was smaller than that of G+S and T+S. L_s in G+S, T+S, and M+S were lower than that of S; the difference between G+S and M+S was not significant, while L_s in T+S showed a small drop.

Relationships between biomass production and photosynthetic characteristics:

A correlation analysis was performed between the different photosynthetic parameters and plant biomass (Table 4). For all treatments, the total dry mass (TDM) and the total fresh mass (TFM) showed positive correlations with P_N , g_s , L_s , WUE, q_p , NPQ, ETR, and Y, and the root-to-shoot ratio (R/S) showed negative correlations with these photosynthetic parameters.

In CK, TDM showed a significant positive correlation with P_N , g_s , WUE, q_p , and NPQ, and TFM showed a significant positive correlation with L_s and ETR. In G and T, TDM and TFM showed positive correlations with P_N ,

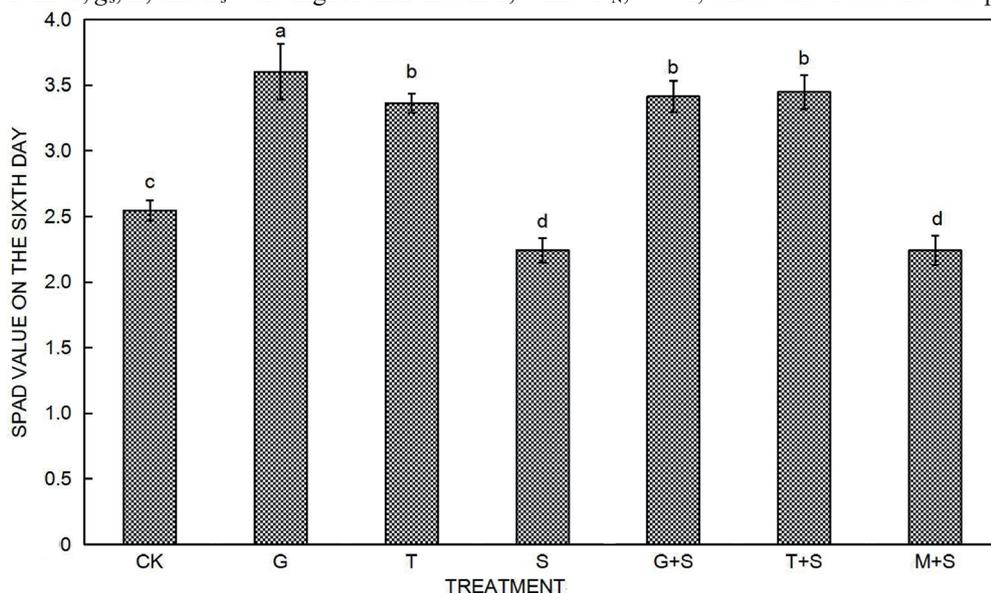


Fig. 2. Effects of seven treatments on SPAD value of triticale seedlings. CK – the control; G – glucose-pretreated; T – sucrose-pretreated; S – salt-treated; G+S – glucose-pretreated and salt-treated; T+S – sucrose-pretreated and salt treated; M+S –mannitol-pretreated and salt-treated.

Table 1. Effects of seven treatments on matter accumulation of triticale seedlings. ^a Different letters in the same columns indicate significant difference between the treatments at 0.05 level. ^bData are mean \pm SD; $n = 10$. CK – the control; G – glucose-pretreated; T – sucrose-pretreated; S – salt-treated; G+S – glucose-pretreated and salt-treated; T+S – sucrose-pretreated and salt treated; M+S – mannitol-pretreated and salt-treated.

Treatment	Shoot fresh mass [g]	Root fresh mass [g]	Total fresh mass [g]	Shoot dry mass [g]	Root dry mass [g]	Total dry mass [g]	Root-shoot ratio
CK	0.2989 \pm 0.0100 ^c	0.0388 \pm 0.0090 ^{cd}	0.3377 \pm 0.0010 ^c	0.0356 \pm 0.0030 ^c	0.0033 \pm 0.0000 ^d	0.0389 \pm 0.0030 ^c	0.1306 \pm 0.0345 ^b
G	0.4730 \pm 0.0100 ^a	0.0662 \pm 0.0060 ^{ab}	0.5392 \pm 0.0087 ^a	0.0502 \pm 0.0010 ^{ab}	0.0048 \pm 0.0000 ^b	0.0550 \pm 0.0010 ^{ab}	0.1401 \pm 0.0142 ^b
T	0.4793 \pm 0.0070 ^a	0.0803 \pm 0.0104 ^{ab}	0.5596 \pm 0.0096 ^a	0.0468 \pm 0.0012 ^b	0.0055 \pm 0.0001 ^a	0.0523 \pm 0.0013 ^b	0.1676 \pm 0.0227 ^{ab}
S	0.1362 \pm 0.0030 ^d	0.0142 \pm 0.0061 ^c	0.1504 \pm 0.0076 ^d	0.0128 \pm 0.0095 ^d	0.0023 \pm 0.0001 ^e	0.0152 \pm 0.0095 ^d	0.1043 \pm 0.0445 ^b
G+S	0.3990 \pm 0.0600 ^b	0.0585 \pm 0.0248 ^{bc}	0.4575 \pm 0.0847 ^b	0.0555 \pm 0.0007 ^a	0.0039 \pm 0.0000 ^c	0.0594 \pm 0.0007 ^a	0.1425 \pm 0.0401 ^{ab}
T+S	0.3715 \pm 0.0400 ^b	0.0843 \pm 0.0110 ^a	0.4558 \pm 0.0290 ^b	0.0482 \pm 0.0004 ^b	0.0026 \pm 0.0001 ^c	0.0508 \pm 0.0004 ^b	0.2308 \pm 0.0548 ^a
M+S	0.1379 \pm 0.0300 ^d	0.0256 \pm 0.0158 ^{dc}	0.1635 \pm 0.0404 ^d	0.0189 \pm 0.0007 ^d	0.0024 \pm 0.0001 ^f	0.0213 \pm 0.0008 ^d	0.1851 \pm 0.0983 ^{ab}

Table 2. Effects of seven treatments on chlorophyll fluorescence parameters of triticale seedlings. ^a Different letters in the same columns indicate significant difference between the treatments at 0.05 level. ^bData are mean \pm SD; $n = 10$. CK – the control; G – glucose-pretreated; T – sucrose-pretreated; S – salt-treated; G+S – glucose-pretreated and salt-treated; T+S – sucrose-pretreated and salt-treated; M+S – mannitol-pretreated and salt-treated.

Treatments	F_0	F_m	F_v/F_m	qp	NPQ	Y	ETR
CK	0.3077 \pm 0.0017 ^c	1.3480 \pm 0.0066 ^a	0.8048 \pm 0.0017 ^{ab}	0.6033 \pm 0.0121 ^a	0.7537 \pm 0.0061 ^c	0.3847 \pm 0.0154 ^a	44.0000 \pm 1.0000 ^a
G	0.3191 \pm 0.0007 ^d	1.2870 \pm 0.0085 ^c	0.8062 \pm 0.0016 ^c	0.4574 \pm 0.0125 ^c	0.5543 \pm 0.0235 ^c	0.3000 \pm 0.0113 ^d	34.0000 \pm 1.0000 ^d
T	0.3187 \pm 0.0009 ^d	1.3480 \pm 0.0114 ^a	0.8015 \pm 0.0035 ^b	0.4970 \pm 0.0125 ^d	0.5683 \pm 0.0145 ^c	0.3233 \pm 0.0210 ^{cd}	36.6670 \pm 0.5774 ^c
S	0.3489 \pm 0.0018 ^a	1.2740 \pm 0.0164 ^c	0.7546 \pm 0.0012 ^f	0.5107 \pm 0.0146 ^d	0.9493 \pm 0.0204 ^b	0.3010 \pm 0.0052 ^d	34.0000 \pm 1.0000 ^d
G+S	0.3198 \pm 0.0036 ^d	1.3110 \pm 0.0195 ^b	0.7824 \pm 0.0036 ^c	0.5403 \pm 0.0140 ^c	0.6957 \pm 0.0170 ^d	0.3417 \pm 0.0101 ^{bc}	38.6670 \pm 0.5774 ^b
T+S	0.3306 \pm 0.0015 ^b	1.3110 \pm 0.0132 ^b	0.7754 \pm 0.0023 ^d	0.5743 \pm 0.0160 ^b	0.7580 \pm 0.0046 ^c	0.3553 \pm 0.0150 ^b	40.3330 \pm 1.5275 ^b
M+S	0.3271 \pm 0.0022 ^c	1.2503 \pm 0.0011 ^d	0.7594 \pm 0.0027 ^e	0.5453 \pm 0.0150 ^c	1.3293 \pm 0.0132 ^a	0.3147 \pm 0.0186 ^d	35.6670 \pm 1.1547 ^{cd}

Table 3. Effects of seven treatments on gas-exchange parameters of triticale seedlings. ^a Different letters in the same columns indicate significant difference between the treatments at 0.05 level. ^bData are mean \pm SD; $n = 10$. CK – the control; G – glucose-pretreated; T – sucrose-pretreated; S – salt-treated; G+S – glucose-pretreated and salt-treated; T+S – sucrose-pretreated and salt-treated; M+S – mannitol-pretreated and salt-treated.

Treatments	P_N	g_s	C_i	E	L_s	WUE _s	WUE _i
CK	3.5040 \pm 0.0953 ^b	15.1300 \pm 1.1763 ^a	245 \pm 3.0000 ^b	0.2790 \pm 0.0113 ^a	0.3875 \pm 0.0100 ^{ab}	12.5800 \pm 0.8083 ^b	0.2322 \pm 0.0119 ^a
G	3.6200 \pm 0.1375 ^b	16.0100 \pm 1.4405 ^a	253 \pm 5.0000 ^{ab}	0.2680 \pm 0.0218 ^{ab}	0.3675 \pm 0.0175 ^{bc}	13.5825 \pm 1.4334 ^{ab}	0.2271 \pm 0.0181 ^a
T	3.8000 \pm 0.0700 ^a	15.8700 \pm 1.3363 ^a	259 \pm 4.3580 ^a	0.2560 \pm 0.0092 ^b	0.3525 \pm 0.0109 ^c	14.8506 \pm 0.3166 ^a	0.2406 \pm 0.0217 ^a
S	0.2560 \pm 0.0092 ^c	4.0200 \pm 1.7183 ^c	236 \pm 1.0000 ^c	0.1210 \pm 0.0118 ^c	0.4100 \pm 0.0025 ^a	2.1325 \pm 0.2606 ^c	0.0760 \pm 0.0433 ^c
G+S	1.2100 \pm 0.1011 ^c	8.8700 \pm 1.3329 ^b	253 \pm 6.9280 ^{ab}	0.1510 \pm 0.0079 ^{cd}	0.3675 \pm 0.0173 ^{bc}	8.0200 \pm 0.6418 ^c	0.1398 \pm 0.0347 ^b
T+S	1.1700 \pm 0.0507 ^c	9.9400 \pm 0.9583 ^b	251 \pm 5.1960 ^{ab}	0.1680 \pm 0.0115 ^c	0.3725 \pm 0.0130 ^{bc}	6.9739 \pm 0.2430 ^c	0.1181 \pm 0.0075 ^{bc}
M+S	0.5000 \pm 0.0794 ^d	5.3700 \pm 0.9601 ^c	253 \pm 5.5670 ^{ab}	0.1370 \pm 0.0090 ^{de}	0.3675 \pm 0.0139 ^{bc}	3.6846 \pm 0.8276 ^d	0.0939 \pm 0.0108 ^c

Table 4. Correlation coefficients among biomass, gas exchange, and chlorophyll fluorescence parameters in seven treatments of triticale seedlings. ^a TDM: total dry mass; TFM: total fresh mass; R/S: root-to-shoot ratio. ^b The correlations were calculated using the three replicates of each treatment. ^c Probabilities (* $P \leq 0.05$; ** $P \leq 0.01$) are shown. CK – the control; G – glucose-pretreated; T – sucrose-pretreated; S – salt-treated; G+S – glucose-pretreated and salt-treated; T+S – sucrose-pretreated and salt treated; M+S –mannitol-pretreated and salt-treated.

Treatment	Parameter	P_N	g_s	L_s	WUE	q_p	NPQ	Y	ETR
CK	TDM	0.98*	0.99**	0.50	0.96*	1.00**	0.98*	0.81	0.50
	TFM	0.66	0.59	1.00**	0.24	0.41	0.65	0.91	1.00**
	R/S	-0.67	-0.6	-1.00**	-0.26	-0.43	-0.67	-0.92	-1.00**
G	TDM	0.98*	0.36	0.92	0.12	1.00**	0.48	0.99**	0.47
	TFM	0.30	0.96*	0.48	1.00**	0.14	0.91	0.19	0.92
	R/S	-0.16	0.73	0.03	0.88	-0.32	0.64	-0.27	0.64
T	TDM	0.94	0.93	0.74	0.89	0.80	0.77	1.00**	0.32
	TFM	0.92	0.91	0.78	0.86	0.76	0.73	0.99**	0.27
	R/S	-0.80	-0.79	-0.91	-0.71	-0.58	-0.54	-0.94	-0.03
S	TDM	0.96*	1.00**	0.89	0.99**	0.84	0.96*	0.86	0.89
	TFM	0.79	0.90	0.65	0.88	0.58	0.79	0.60	0.65
	R/S	-0.81	-0.92	-0.68	-0.89	-0.61	-0.81	-0.63	-0.68
G+S	TDM	0.97*	0.85	0.90	0.89	0.40	0.38	0.92	0.90
	TFM	0.99*	0.79	0.85	0.83	0.50	0.47	0.87	0.85
	R/S	-0.96*	-0.86	-0.91	-0.90	-0.37	-0.35	-0.93	-0.91
T+S	TDM	0.39	1.00**	0.59	0.66	0.04	0.28	0.85	0.90
	TFM	0.73	0.94	0.87	0.30	0.44	0.65	0.56	0.65
	R/S	-0.77	-0.92	-0.90	-0.24	-0.49	-0.70	-0.51	-0.61
M+S	TDM	0.94	0.49	0.98*	0.91	0.81	0.86	0.96*	0.99*
	TFM	0.93	0.50	0.98*	0.91	0.81	0.87	0.96*	0.98*
	R/S	-0.97*	-0.39	-0.95*	-0.95*	-0.88	-0.80	-0.99*	-1.00**

g_s , L_s , q_p , NPQ, Y, and ETR. In S, TDM showed significant positive correlations with P_N , g_s , WUE, and NPQ. In G+S, TDM and TFM showed a significant positive correlation with P_N . In T+S, TDM showed a significant positive correlation with g_s . In M+S, TDM and TFM showed significant positive correlations with L_s , Y, and ETR.

Discussion

In plants, as in many other organisms, soluble sugars are essential primary metabolites. They play major roles as regulatory molecules controlling gene expression, plant physiology, metabolism, the cell cycle, and plant development (Koch 1996, Paul and Foyer 2001, Gibson 2005). The most common and significant effect of salt stress in non-halophytes is the inhibition of growth. Soil salinization is widely prevalent; thus, considerable attention has been paid to the study of salt resistance in plants. The effects of exogenous sugars on seedling growth seem to be complex. Glucose and sucrose are known to inhibit photosynthesis and seedling development at high concentrations, such as 300 mM (Koch 1996, Zhou 1998), but low to moderate concentrations of sugars can enhance the growth of *Arabidopsis* plantlets in terms of Chl accumulation and root growth (Paul and Foyer 2001, Gibson *et al.* 2001, Martin *et al.* 2002, Sulmon *et al.* 2004, 2006). Baena-González and Sheen (2008) found that glucose and sucrose, as soluble sugars, acted as signals

controlling maize responses to biotic and abiotic stress. Boriboonkaset (2007) found that exogenous sucrose and glucose as the main carbon source in the culture media of rice played a direct role in stimulating growth and development and functioned as a defense mechanism against salt stress. However, systematic studies of the ability of exogenous sugars to alleviate the inhibitory effects caused by salt stress in triticale were lacking until this study.

Our results were similar to those of previous studies; the root fresh mass and dry mass of triticale decreased under salt stress, the pretreatment with glucose and sucrose could maintain or enhance these traits, and the pretreatment with mannitol did not relieve the reductions of root fresh mass and dry mass. Schmidt *et al.* (2015) found that the increased production of papaya dry biomass was caused by the application of an exogenous carbon source. Campostrini *et al.* (2008) also found that shoot dry matter was increased by sucrose in the culture medium. Roots are the first parts affected by excessive salinity; reduction of root growth and alteration of development induced by salinity have been observed in many plant species (Córdoba *et al.* 2001, Cavalcanti *et al.* 2007, Farissi *et al.* 2013, Yan *et al.* 2016). Salt stress also reduces shoot growth by suppressing leaf initiation and expansion, as well as internode growth, and by accelerating leaf senescence and abscission. Salt stress rapidly reduces the leaf growth rate (Munns 1993) due to a reduction in the number of elongating cells and/or the

rate of cell elongation (Szalai and Janda 2009). Our study showed that the root-to-shoot ratio in triticale decreased under salt stress, and application of glucose, sucrose, and mannitol reduced this salt stress damage. Exogenous glucose and sucrose enhanced the salt resistance of plants and promoted growth and dry matter accumulation. The same concentration of mannitol did not cause this effect; it showed that the ameliorating effect of exogenous sugar on salt stress does not occur because of osmosis.

Salt stress influences photosynthesis due to the reduction in Chl content (Kiani-Pouya and Rasouli 2014). Salt stress may have a deleterious impact on chloroplasts due to the influx of Na^+ and Cl^- , which directly exert toxic effects, resulting in the degradation of Chl (Kiani-Pouya and Rasouli 2014). Parvaneh *et al.* (2012) found that, in plant resistance against salinity, increasing the proline, sugar, and Chl contents in leaves is useful for retaining and improving photosynthetic performance in plants. Exogenous applications of such agents enhanced the Chl contents in maize and soybean (Mason *et al.* 1928). Sulmon *et al.* (2004) found that both glucose and sucrose promoted Chl accumulation in some mutant seedlings, particularly, at lower (40 mM) concentrations, compared to growth in the absence of sugars. This effect had been previously observed by Gibson *et al.* (2001), who reported that a 150 mM sucrose treatment increased Chl content. Sugars accumulated in leaves have direct effects on the expression of photosynthetic genes and Chl. Zhang *et al.* (2005) found that compared with non-transgenic plants, transgenic plants accumulated more trehalose, maintained their Chl content, and showed enhanced tolerance to salt. In this paper, the results are consistent with those of previous studies. Our results showed that glucose and sucrose can increase the Chl content and promote photosynthesis in triticale under normal conditions. Glucose and sucrose can alleviate the damage to chlorophyll caused by salt stress and maintain or enhance photosynthesis.

PSII is considered the primary site of injury to the photosynthetic apparatus during the stress and senescence processes (Sharkey *et al.* 2010). PSII injury can lead to obvious changes in Chl fluorescence. Chl fluorescence has been used as a powerful and reliable method for assessing changes in the function of PSII and for assessing the primary photosynthetic processes under environmental stress conditions (Maxwell and Johnson 2000, Hajiboland *et al.* 2010). Analysis of Chl fluorescence parameters allows insights into the status of PSII. Stress applied to green plants, which directly or indirectly affects the photosynthetic metabolism, is likely to change the yield of Chl fluorescence (Dabrowski *et al.* 2017). PSII efficiency in light, electron transport chain efficiency, the efficiency of PSII open reaction centers in light, and the maximum quantum yield of PSII were reduced, while nonphotochemical quenching increased during salt stress (He *et al.* 2009, Zhang *et al.* 2009). Some studies have shown that exogenous agents improve the efficiency of PSII in different plants during stress or senescence. Sulmon *et al.* (2004) found that exogenous sucrose induced the expression of the *psbA* gene to support rapid repair of PSII function. We found that the application of glucose

and sucrose to triticale seedlings caused increases in F_m , F_v/F_m , Y , ETR, and decreases in F_0 , under salt stress. The F_v/F_m value is a measure of accumulated photo-oxidative damage to PSII (Garg *et al.* 2002); under optimal growth conditions, this ratio is approximately 0.85 in many plants, but it markedly declines during stress. For the regulatory mechanism of improving Chl fluorescence of photon-counting histogram (PSII), a reasonable explanation is the increase of the Chl content. Our study showed that compared to S, q_p was higher and NPQ was lower in G+S and T+S, while NPQ was higher in M+S, which indicated that glucose and sucrose can enhance the resistance of triticale. The increased excitation energy dissipated in the form of heat (NPQ) to protect the photosynthetic apparatus from damage.

The results obtained from our study showed that P_N , g_s , C_i , E , WUE, and WUE_i decreased under salt stress. Salinity decreases the availability of CO_2 at its fixation site due to the impacts of toxic ions (Na^+ and Cl^-) on the photosynthetic apparatus and the disturbances in the function of leaf stomata (Reddy *et al.* 1998). In addition, P_N can be affected by stomatal or nonstomatal factors (Farooq *et al.* 2009). The decrease in the photosynthetic rate seems to result from CO_2 limitation since C_i was reduced with the imposition of salt stress. When sucrose was omitted from the culture medium and the medium was enriched with CO_2 , Khan *et al.* (2002) detected photosynthetic carbon assimilation in all assessment periods.

The application of exogenous sugar can mitigate salt injury. Siringam *et al.* (2012) found that exogenous sucrose could effectively improve photosynthesis in rice under salt stress. These previous results are consistent with our report here, where the application of glucose and sucrose increased P_N and C_i and delayed the reduction rate of E and g_s , compared to S. Zhang *et al.* (2016) also found that adding glucose significantly enhanced the growth rate and carrying capacity of *Ochromonas* compared to photoautotrophic growth and that photosynthesis also increased with the application of glucose during the exponential growth stage.

Conclusion: Salt stress inhibited growth and reduced photosynthesis in triticale, causing a reduction in biomass. The application of glucose and sucrose alleviated the injury caused by salt stress, increasing SPAD value, F , F_v/F_m , F_m , P_N , and C_i . These sugars also decreased NPQ, L_s , and WUE_i , reduced the decrease in F_0 , E , and g_s and improved photosynthetic capacity, resulting in a greater fresh and dry matter accumulation.

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