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Effect of Chitosan on Growth, Yield and Certain Salinity Stress-Related Metabolites in Two Barley Cultivars Contrasting in Salt Tolerance

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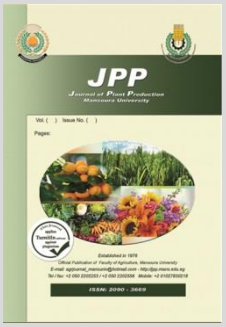

ABSTRACT

The present study aimed to elucidate the effect of chitosan on germination, growth, yield and certain salinity stress-related metabolites in two barley cultivars contrasting in salt tolerance namely cvs. Giza 129 (salt susceptible) and Giza 136 (salt tolerant). Salinity stress treatments were commenced 30 days after sowing via soil irrigation with either NaCl or $CaCl_2$ each at three levels 0, 3000 mgL^{-1} (4.6875 dS m^{-1}) and 6000 mgL^{-1} (9.375 dS m^{-1}). The obtained results indicated that both types of salinity at 6000 mgL^{-1} decreased germination percentage, growth parameters, total chlorophylls, relative water content and yield whereas increased mean germination time as well as carotenoids, proline and total soluble sugars (TSS). Chitosan (CHS) treatment at 200 mgL^{-1} increased yield and its components in plants growing under normal conditions, and alleviated the negative effects of salinity on characters that were negatively-affected in salinity-stressed plants. On the other hand, there was an additive effect between salinity and CHS on inducing the content of carotenoids, proline and TSS. The damaging effect of salinity was more pronounced in case of NaCl compared with $CaCl_2$, and the alleviative effects of CHS was more pronounced at 200 mgL^{-1}. Salinity tolerance of cv G 136 may be attributed to higher seed germination potential along with higher intrinsic contents from proline and TSS.

Keywords: Barley, Hordeum vulgare; Chitosan, Cultivars, Salt tolerance

INTRODUCTION

Barley is the fourth most important cereal crop in the world after wheat, maize, and rice and dominates other grains in some developing countries having arid and semi-arid climates where it is the only cereal and only staple food resource. Even in more developed countries, it is also very important species not only for animal feed but also for malt industry. Barley is known to be a salt tolerant plant (Norlyn and Epstein 1982), however large variation in salt tolerance exists among cultivars (Bhatti *et al*., 1976). It has been argued that tolerant cultivars have more efficient antioxidant system that help them to overcome salinity-induced oxidative stress (Chawla *et al*., 2013; Abedini and Daie-Hassani 2015).

Soil salinity is one of the major environmental constraints limiting crop production in many parts of the world and is predicted to increase due to global climate change (FAO, 2011). The situation is worst in arid and semi-arid regions, characterized by water deficiency and high temperature, aggravating the effects of salinity. Salinity stress induces a multitude of responses in plants including morphological, physiological, biochemical, and molecular changes (Ambede *et al*., 2012; Abreu *et al*., 2013). It causes ionic imbalance, which results in ionic toxicity, osmotic stress, and generation of reactive oxygen species (ROS; Chawla *et al*., 2013). Accumulation of Na^+ under salinity stress competes with K^+ binding in proteins, causing inhibition of protein synthesis (Pardo and Quintero, 2002).

High concentrations of NaCl in the roots environment reduce the water potential, and making it more difficult for plants to absorb water. In leaves, high salt levels cause stomatal closure, impairment of electron transport and the photosynthetic apparatus, leading to reduced photosynthesis and productivity (Abreu *et al*., 2013; Deinlein *et al*., 2014). High salinity also induces the formation of ROS within plant cells, and their over accumulation results in oxidative damage of membrane lipids, proteins and nucleic acids (Gill and Tuteja, 2010).

It has been confirmed that under saline condition, some chemicals could alleviate the negative effects of salt stress on plants. Chitosan (CHS) is a natural, safe, and cheap biopolymer produced from chitin, the major constituent of arthropods exoskeleton and fungi cell walls and the second renewable carbon source after lignocellulosic biomass.

Biological responses of plants to CHS are dependent on its structure, concentration, species and developmental stage.

Chitosan has a great potential for enhancing crop production due to its effects on plants such as stimulating growth of plant and seed germination (Luan *et al*., 2006); increasing chlorophyll content and photosynthetic efficiency (Limpanavech *et al*., 2008); enhancing nitrogen fixation in legumes (Dzung and Thang, 2004); increasing nutrient uptake and reducing stress of plants (Dzung *et al*., 2011); thereby enhancing plant productivity (Bukrudeen *et al*., 2010; Dzung, 2010). Other biochemical and molecular changes observed in plants fed with CHS include callose deposition (Faoro and Iriti, 2007), inhibition of plasma membrane H^+-ATPase (Amborabé *et al*., 2008), chromatin alterations (Iriti and Faoro, 2009), synthesis of alkaloids (Orlita *et al*., 2008), and phytoregulators, jasmonic acid and

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abscisic acid (Iriti and Faoro, 2008). Moreover, CHS also alleviates biotic stress on plants (El Hadrami *et al*., 2010).

The physiological and molecular bases for CHS effect on plants have been elucidated. Chitooligosaccharides are cell molecular signals that induce and regulate defensive, symbiotic as well as developmental and growth processes in plants (Sy and Dzung, 2010). An initial oxidative burst with hydrogen peroxide (H_2O_2) accumulation was observed in different plants supplied with CHS (Iriti and Varoni, 2015). It is thought that this can lead to the induction of plant defense enzymes, and to the synthesis of secondary metabolites, such as polyphenolics, lignin, flavonoids, and phytoalexins (Hamel and Beaudoin, 2010).

The present study aimed to elucidate the effect of chitosan on germination, growth, yield and certain salinity stress-related metabolites in two barley cultivars contrasting in salt tolerance.

MATERIALS AND METHODS

Plant materials, experimental conditions, and application of treatments

Two experiments were conducted at the Greenhouse and in the Labs. of the Agric. Bot. Dept., Fac. of Agric., Mansoura Univ., Egypt. Grains of barley (*Hordeum vulgare* L.), cvs. Giza 129 (salt susceptible) and Giza 136 (salt tolerant) were secured from Field Crops Research Institute, Agric. Res. Center, Egypt. Grains were disinfested by immersion in a 2.5 % solution of sodium hypochlorite for 10 min and washed thoroughly with distilled water and sown on November 21, 23 during the two successive growing seasons 2015/2016 and 2016/2017, respectively. Sowing was carried out in bottom-perforated black plastic bags containing 15 kg of air-dried soil at the rate of 10 grains/bag. Representative soil samples were taken from the experimental site during both seasons and analyzed according to Black *et al*. (1965) and presented in Table (1). Thinning was made 15 days after sowing (DAS) to leave 6 uniform seedlings/ bag.

Salinity stress treatments were commenced concomitantly with sowing via soil irrigation with equal amount of either NaCl or $CaCl_2$ each at three levels 0, 3000 (4.6875 dS m^{-1}), 6000 (9.375 dS m^{-1}) $mgL^{-1,}$ and this amount was adjusted progressively to accommodate plant growth. Chitosan was added as seed soaking at either 0, 200 or 400 mgL^{-1} for 12 h before sowing. The experiments were laid out in a randomized complete block design with three replications. Plants were fertilized with 2.5 g ammonium sulfate (20.6% N), 3.59 g calcium superphosphate (15.5% P_2O_5) and 1.25 g potassium sulfate (48% k_2O) per bag.

Fertilization with calcium superphosphate was done before sowing, whereas N fertilization was applied in two equal doses, at 20 and 30 DAS. Fertilization with potassium sulphate was applied at the beginning of the heading stage. All agricultural practices were applied according to the normal recommended for barley by ARC, Egypt.

Table 1. Physical and chemical analysis of the used soil (average of the two growing seasons).

CS %	FS %	S %	C %	$CaCO_3$ %	OM %	TN%	AP ppm	EK ppm	TSS %
11.2	27.6	26.0	35.2	2.7	2.0	0.11	14	213	0.20

***CS, Coarse sand; FS, Fine sand; S, Silt; C, Clay; OM, Organic matter; TN, total N; AP, available P; EK, exchangeable K; TSS, total soluble solutes**

Recoded parameters and analyses

Ten DAS, germination percentage was recorded as evidenced by emergence of the radical. In addition, germinating grains were recorded daily for 10 days to determine mean germination time according to Ellis and Roberts (1980) as MGT = Σnd/T, where n is the no. of grains newly germinated on the day of counting (d) and T is the total no. of germinated seeds during the 10-days germination period. At 75 DAS, plant samples were collected to determine plant height, plant fresh and dry weight as well as leaf area. In addition, total soluble sugars (TSS) in the shoots and photosynthetic pigments, relative water and proline contents in the flag leaf were determined. Leaf area was determined as: Leaf area = Length x Width x 0.75 (Quarrie and Jones, 1979). TSS were extracted in 80% ethanol and determined according to Homme *et al.* (1992).

Weight of 0.5 g leaf tissues was grounded in 80% acetone and leaf photosynthetic pigments were determined according to Lichtenthaler (1987). Leaf relative water content was determined according to Beadle *et al.* (1993) according to the formula:

$$\mathbf{RWC} = \frac{\mathbf{fresh\ weight - dry\ weight}}{\mathbf{turgid\ weight - dry\ weight}}\ \mathbf{x\ 100}$$

Proline content was estimated spectrophotometrically at 520 nm according to the method of Bates *et al.* (1973). At maturity, 125 DAS, yield and its components were recorded.

Statistical analysis:

Data of the two growing seasons were subjected to combined analysis of variance using MSTAT-C software. Significance of differences between treatments means were compared with Duncan's multiple range test at the 0.05 probability level.

RESULTS AND DISCUSSIONS

Results

Germination capacity

Germination capacity of cv Giza 136 (G 136) was higher than that in cv Giza 129 (G 129) as shown in Table (2). Germination percentage (G %) was significantly higher in cv G 136 whereas mean germination time (MGT) was higher in cv G 129. Germination percentage was not affected by the lower level (3000 mg L^{-1}) from either NaCl or $CaCl_2$ whereas decreased in response to the higher level (6000 mg L^{-1}). On the other hand, all salinity levels delayed germination as evidenced by increasing MGT. The higher level of NaCl proved to be more deleterious than that of $CaCl_2$ in decreasing G % and delaying germination.

Both levels of CHS did not significantly affect either G % or MGT in plants growing under normal conditions. In salinity-stressed plants, CHS increased G % whereas decreased MGT compared with control (Table 2). However, the difference was not significant in case of plants stressed with the lower salinity level. So, CHS counteracted the effects of salinity at 3000 mg L^{-1} on both G % and MGT. In

all treatments involving salinity, values of G % were higher whereas those of MGT were lower in cv G 136 compared with cv G 129.

The interaction effect between cultivars and treatments showed no significant effect regarding G %. On the other hand, the interaction was significant regarding MGT. Within all treatments involving both salinity and CHS, the higher MGT was recorded in cv G 129 plants stressed with NaCl 6000 mgL^{-1} and treated with CHS at 400 mgL^{-1} whereas the lowest MGT was recorded in cv G 136 plants stressed with $CaCl_2$ 3000 mgL^{-1} and treated with CHS at 400 mgL^{-1}.

Table 2. Effects of chitosan on germination percentage and mean germination time (MGT) of salinity-stressed barley cultivars Giza 129; G129 and Giza136; G136 (combined analysis of the two growing seasons).

Parameters	Germination %			MGT (d)		
Treatments	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**
Cont	99.4	99.5	99.4a	1.62	1.66	1.64h
NaCl 3000 mgL^{-1}	99.5	98.4	98.9a	1.68	2.04	1.86f
NaCl 6000 mgL^{-1}	89.2	87.7	88.4d	2.80	3.40	3.10d
$CaCl_2$ 3000 mgL^{-1}	99.6	98.7	99.1a	1.70	1.94	1.82gf
$CaCl_2$ 6000 mgL^{-1}	93.6	92.1	92.8c	2.64	3.15	2.89b
CHS 200 mgL^{-1}	99.4	99.7	99.5a	1.65	1.66	1.65h
CHS 400 mgL^{-1}	98.8	99.5	99.1a	1.70	1.72	1.71gh
NaCl 3000 mgL^{-1} + CHS 200 mgL^{-1}	99.6	99.1	99.3a	1.66	1.70	1.68h
NaCl 3000 mgL^{-1} + CHS 400 mgL^{-1}	99.5	99.3	99.4a	1.68	1.78	1.73gh
NaCl 6000 mgL^{-1} + CHS 200 mgL^{-1}	94.4	92.5	93.4c	2.52	2.98	2.75c
NaCl 6000 mgL^{-1} + CHS 400 mgL^{-1}	95.0	93.2	94.1c	2.63	3.26	2.94b
$CaCl_2$ 3000 mgL^{-1} + CHS 200 mgL^{-1}	99.5	99.4	99.4a	1.72	1.74	1.73gh
$CaCl_2$ 3000 mgL^{-1} + CHS 400 mgL^{-1}	99.9	99.3	99.6a	1.60	1.84	1.72gh
$CaCl_2$ 6000 mgL^{-1} + CHS 200 mgL^{-1}	97.3	94.8	96.0b	2.15	2.75	2.45e
$CaCl_2$ 6000 mgL^{-1} + CHS 400 mgL^{-1}	98.4	95.0	96.7b	2.26	2.94	2.60d
Mean	97.5A	96.5B		2.00B	2.30A	
LSD 5 %:						
Cultivars		0.53			0.04	
Treatments		1.46			0.12	

Growth attributes

Control plants of cv G 136 had higher values of leaf area/plant, plant height, as well as plant fresh and dry weight compared with those of cv G 129 (Table 3). This was also true when the mean of each cultivar overall all treatments is considered, though the difference in mean leaf area was insignificant. Salinity stress caused by either NaCl or Ca Cl_2 decreased growth parameters at 6000 mgL^{-1} except plant dry weight. The magnitude of decrease in growth parameters was higher in case of NaCl compared with $CaCl_2$. On the other hand, salinity was stimulatory to dry weight, either with or without CHS. In this regard, the lower level of salinity (3000 mgL^{-1}) stimulated the recorded growth attributes, but the effect was insignificant.

In unstressed plants, CHS treatments did not significantly affect the growth parameters (Table 3). On the other hand, growth parameters in salinity-stressed plants that were treated with CHS were, generally, of higher values compared with those in salinity-stressed only plants, especially in plants exposed to the higher salinity level, though the differences were not significant.

The effect of the interaction between cvs and treatments was significant only regarding plant dry weight. Within treatments in which plants received the combined treatments, the highest dry weight was recorded in cv G 136 plants stressed with NaCl at 3000 mgL^{-1} and treated with CHS at 200 mgL^{-1} whereas the lowest dry weight was recorded in cv G 129 plants stressed with NaCl at 6000 mgL^{-1} and treated with CHS at 400 mgL^{-1}.

Table 3. Effects of chitosan on growth parameters of salinity-stressed barley Cultivars Giza 129; G129 and Giza136; G136 (combined analysis of the two growing seasons).

Parameters	Plant height (cm)			Leaf area/plant (cm^2)			F.W. (g)			D.W. (g)		
Treatments	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**
Cont	73.4	71.4	72.4[ab]	28.4	25.3	26.8abc	25.7	23.8	24.7abcd	3.56	2.72	3.14c
NaCl 3000 mgL^{-1}	75.0	73.6	74.3ab	22.4	22.2	22.3def	29.0	22.4	25.7abc	5.78	3.49	4.64de
NaCl 6000 mgL^{-1}	64.3	57.5	60.9e	16.5	15.5	16.0g	22.4	14.5	18.4f	5.68	3.26	4.47gh
$CaCl_2$ 3000 mgL^{-1}	76.1	75.3	75.7a	25.8	24.5	25.1abcde	30.8	22.8	26.8ab	5.48	4.21	4.84b
$CaCl_2$ 6000 mgL^{-1}	67.6	62.4	65.0de	20.2	18.6	19.4fg	24.6	16.8	20.7ef	6.81	3.48	5.14a
CHS 200 mgL^{-1}	72.5	70.8	71.6ab	28.1	27.7	27.9a	27.2	20.1	23.6abcde	3.28	2.96	3.12k
CHS 400 mgL^{-1}	71.0	68.7	69.8bc	27.2	27.7	27.4ab	25.3	22.2	23.8abcde	3.27	2.56	2.91l
NaCl 3000 mgL^{-1} + CHS 200 mgL^{-1}	75.0	72.3	73.7ab	25.3	23.7	24.abcde	31.0	21.6	26.3ab	5.53	3.51	4.52fg
NaCl 3000 mgL^{-1} + CHS 400 mgL^{-1}	71.4	70.5	70.9bc	22.3	24.6	23.4cde	32.0	22.0	27.0a	5.25	3.55	4.40h
NaCl 6000 mgL^{-1} + CHS 200 mgL^{-1}	61.4	59.5	60.4e	19.7	18.2	18.9fg	24.0	19.0	21.5def	5.18	3.29	4.23i
NaCl 6000 mgL^{-1} + CHS 400 mgL^{-1}	62.7	63.2	62.9de	18.8	16.6	17.7g	23.7	19.5	21.6def	4.22	3.19	3.70j
$CaCl_2$ 3000 mgL^{-1} + CHS 200 mgL^{-1}	77.4	74.0	75.7a	26.3	28.0	27.1abc	32.8	20.9	26.8ab	4.96	4.24	4.60ef
$CaCl_2$ 3000 mgL^{-1} + CHS 400 mgL^{-1}	75.8	73.2	74.5ab	25.6	25.1	25.3abcd	31.2	21.8	26.5ab	4.60	4.29	4.45gh
$CaCl_2$ 6000 mgL^{-1} + CHS 200 mgL^{-1}	68.7	64.5	66.6dc	25.1	23.1	24.1bcde	25.0	20.1	22.5cde	5.16	4.41	4.78bc
$CaCl_2$ 6000 mgL^{-1} + CHS 400 mgL^{-1}	66.4	63.5	64.9de	22.9	20.2	21.5ef	26.2	21.0	23.6bcde	5.13	4.29	4.71cd
Mean	70.5A	68.0B		23.6A	22.7A		27.4A	20.5B		4.92A	3.56B	
LSD 5 %:												
Cultivars		1.7			1.3			1.2			0.03	
treatments		4.6			3.7			3.3			0.09	

Photosynthetic pigments

Leaves of cv G 136 contain higher concentrations from chlorophyll a (chl a), total chlorophylls (tchls) as well as higher chl a/b ratio. On the other hand, leaves of cv G 129 contain higher concentration from carotenoids (Table 4). However, the difference in chl b concentration between the two cultivars was insignificant.

Salinity stress due to NaCl treatments decreased tchls whereas increased carotenoids (Carots) concentration at both levels. Similar results were recorded in response to $CaCl_2$, but only at the higher level. Generally, all salinity types and levels showed no significant effect on chl a/b ratio.

Application of CHS, generally, did not significantly affect either chl a, chl b, tchls, or chl a/b ratio, whereas increased carots concentration in both cvs. Treatment of salt-stressed plants with CHS increased chl a, tchls and carots concentrations compared with CHS-untreated plants, and the enhancing effect of CHS was more pronounced at its lower adopted level (Table 4). On the other hand, CHS treatments did not affect chl b concentration in salt-stressed plants. It is worth mentioning that salinity and CHS treatments as well as their interactions increased carots concentration whereas, generally, did not significantly affect chl a/b ratio (Table 4).

Table 4. Effects of chitosan on leaf photosynthetic pigments of salinity-stressed barley cultivars Giza 129; G129 and Giza136; G136 (combined analysis of the two growing seasons).

Parameters	Chl a (mg g^{-1} FW)			chl b (mg g^{-1} FW)			tchls (mg g^{-1} FW)			Chl a /chl b			Tcarots (mg g^{-1} FW)		
Treatments	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**
Cont	1.47	1.30	1.38cd	o.43	0.50	0.46bc	1.90	1.80	1.85b	3.54	2.61	3.07cd	0.60	0.52	0.56f
NaCl 3000 mgL^{-1}	1.28	1.18	1.23e	o.40	0.43	0.41cde	1.68	1.60	1.64cde	3.30	2.74	3.02cd	0.62	0.66	0.64de
NaCl 6000 mgL^{-1}	0.94	0.86	0.96f	o.32	0.26	0.29f	1.26	1.12	1.19f	3.00	3.41	3.20cd	0.64	0.72	0.68cd
$CaCl_2$ 3000 mgL^{-1}	1.38	1.34	1.36cd	0.53	0.55	0.54ab	1.91	1.89	1.90b	2.70	2.44	2.57d	0.58	0.65	0.61ef
$CaCl_2$ 6000 mgL^{-1}	0.98	0.93	0.95f	0.37	0.32	0.34def	1.35	1.25	1.30f	2.78	2.92	2.85cd	0.56	0.71	0.64de
CHS 200 mgL^{-1}	1.43	1.37	1.40bc	0.37	0.43	0.40cde	1.80	1.80	1.80bc	3.95	3.25	3.60abc	0.67	0.62	0.64cde
CHS 400 mgL^{-1}	1.47	1.30	1.38cd	0.32	0.40	0.36def	1.79	1.70	1.74bcd	4.65	3.39	4.02ab	0.69	0.58	0.63de
NaCl 3000 mgL^{-1} + CHS 200 mgL^{-1}	1.45	1.34	1.39bc	0.45	0.48	0.46bc	1.90	1.82	1.86b	3.27	2.87	3.07cd	0.68	0.72	0.70abc
NaCl 3000 mgL^{-1} + CHS 400 mgL^{-1}	1.52	1.30	1.41bc	0.43	0.42	0.42cd	1.95	1.72	1.83b	3.55	3.22	3.39abc	0.69	0.71	0.70abc
NaCl 6000 mgL^{-1} + CHS 200 mgL^{-1}	1.38	1.22	1.30cde	0.29	0.36	0.32ef	1.67	1.58	1.62de	4.72	3.57	4.15a	0.71	0.79	0.75a
NaCl 6000 mgL^{-1} + CHS 400 mgL^{-1}	1.24	1.20	1.22e	0.35	0.34	0.34def	1.59	1.54	1.56e	3.58	3.62	3.60abc	0.74	0.73	0.74ab
$CaCl_2$ 3000 mgL^{-1} + CHS 200 mgL^{-1}	1. 57	1.45	1.51ab	0.59	0.57	0.58a	2.16	2.02	2.09a	2.68	2.55	2.62d	0.65	0.71	0.68bcd
$CaCl_2$ 3000 mgL^{-1} + CHS 400 mgL^{-1}	1.68	1.60	1.64a	0.56	0.52	0.54ab	2.24	2.12	2.18a	3.04	3.11	3.07cd	0.66	0.68	0.67cd
$CaCl_2$ 6000 mgL^{-1} + CHS 200 mgL^{-1}	1.32	1.19	1.25de	0.42	0.38	0.40cde	1.74	1.57	1.65cde	3.26	3.27	3.26bcd	0.70	0.80	0.75a
$CaCl_2$ 6000 mgL^{-1} + CHS 400 mgL^{-1}	1.26	1.16	1.21e	0.37	0.35	0.36def	1.63	1.51	1.57e	3.51	3.31	3.41abc	0.72	0.77	0.74a
Mean	1.35A	1.25B		0.41A	0.42A		1.77A	1.67B		3.43A	3.08B		0.66B	0.69A	
LSD 5 %:															
Cultivars		0.04			0.03			0.06			0.27			0.02	
Treatments		0.12			0.08			0.16			0.76			0.05	

Proline, RWC and TSS

Leaves of cv G 136 contained significantly higher concentrations from proline and TSS, but comparable RWC content compared with the leaves of cv G 129 (Table 5).

Both types of salinity decreased RWC, whereas increased proline and TSS concentrations. Both applied levels of CHS did not significantly affect either RWC, proline or TSS. In all treatments involving the combination between salinity and CHS, RWC was decreased whereas proline and TSS were increased. Nevertheless, RWC, proline and TSS were higher in salinity-stressed, CHS-treated plants compared with salinity-stressed only plants (Table 5). The interaction effect was significant regarding both proline and TSS. The highest proline concentration was recorded in the leaves of cv G 136 plants treated with the combination of the higher level of NaCl and CHS, whereas the highest TSS concentration was recorded in the leaves of cv G 136 plants treated with $CaCl_2$ at 6000 mgl-1 in combination with CHS at 200 mgL^{-1}.

Table 5. Effects of chitosan on biochemical constituents of salinity-stressed barley cultivars Giza 129; G129 and Giza136; G136 (combined analysis of the two growing seasons).

Parameters	RWC %			Proline (mg g^{-1} DW)			TSS (mg g^{-1} DW)		
Treatments	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**
Cont	85.8	87.2	86.5a	9.19	7.2	8.1h	32.1	29.0	30.5g
NaCl 3000 mgL^{-1}	77.5	76.4	76.9de	16.5	13.6	15.0fg	46.3	35.1	40.7f
NaCl 6000 mgL^{-1}	69.0	67.1	68.0f	23.4	19.3	21.4bc	73.6	51.8	62.7c
$CaCl_2$ 3000 mgL^{-1}	80.2	78.4	79.3bcd	15.6	11.3	13.4g	50.1	36.0	43.0ef
$CaCl_2$ 6000 mgL^{-1}	71.6	68.0	69.8f	20.4	17.5	19.0cde	80.7	55.1	67.9bc
CHS 200 mgL^{-1}	87.1	90.9	89.0a	7.8	8.6	8.2h	36.4	32.1	34.2g
CHS 400 mgL^{-1}	85.0	91.3	88.2a	7.0	6.7	6.8h	33.1	32.0	32.6g
NaCl 3000 mgL^{-1} + CHS 200 mgL^{-1}	80.4	82.1	81.2bc	18.5	16.4	17.4def	52.3	42.3	47.3de
NaCl 3000 mgL^{-1} + CHS 400 mgL^{-1}	81.2	83.4	82.3b	20.3	18.7	19.5cd	55.6	39.8	47.7de
NaCl 6000 mgL^{-1} + CHS 200 mgL^{-1}	75.6	75.4	75.5e	26.1	22.0	24.1ab	82.0	60.1	71.1b
NaCl 6000 mgL^{-1} + CHS 400 mgL^{-1}	80.3	76.8	78.5cde	26.9	24.5	25.7a	79.1	54.9	67.0bc
$CaCl_2$ 3000 mgL^{-1} + CHS 200 mgL^{-1}	83.7	80.4	82.0b	18.8	13.4	16.1efg	57.3	45.1	51.2d
$CaCl_2$ 3000 mgL^{-1} + CHS 400 mgL^{-1}	83.6	79.6	81.6bc	20.3	15.7	18.0def	52.1	43.1	47.6de
$CaCl_2$ 6000 mgL^{-1} + CHS 200 mgL^{-1}	76.6	74.3	75.4e	23.6	20.1	21.9bc	87.9	65.1	76.5a
$CaCl_2$ 6000 mgL^{-1} + CHS 400 mgL^{-1}	77.4	75.3	76.3de	24.4	18.0	21.2bc	81.1	60.3	70.7b
Mean	79.6A	79.1A		18.6A	15.5B		60.0A	45.4B	
LSD 5 %:									
Cultivars		1.2			1.1			1.9	
Treatments		3.3			3.1			5.3	

Yield and its components

Number of spikes/plant, grains weight/spike, 100-grains weight and grains yield/plant in cv G 136 surpassed those in cv G 129 (Table 6). Salinity stress by either salt type at both levels decreased all yield components, except 100-grains weight which decreased only at the higher level (6000

mgL^{-1}). On the other hand, CHS treatments increased all yield components at its lower adopted level (200 mgL^{-1}). In salinity-stressed plants, CHS treatments alleviated the depressing effect of salinity on yield components, where the values in salinity-stressed, CHS-treated plants were higher than those in salinity-stressed only plants.

The interaction effect was significant regarding all estimated yield components except 100-grains weight. The highest grains yield/plant was recorded in plants of cv G 136 that were treated with NaCl at 3000 mgL^{-1} in combination with CHS at 200 mgL^{-1}, whereas the least grain yield was recorded in plants of cv G 129 that were stressed by NaCl at 6000 mgL^{-1} in combination with CHS at 400 mgL^{-1}.

Table 6. Effects of chitosan on yield and its components of salinity-stressed barley cultivars Giza 129; G129 and Giza136; G136 (combined analysis of the two growing seasons).

Parameters	No. of spikes/plants			Grains wt/spike			100 grains wt(g)			Grains wt/plant		
Treatments	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**	**G136**	**G129**	**Mean**
Cont	6.7	4.7	5.7b	2.76	2.06	2.41b	3.74	3.49	3.61bc	18.48	9.70	14.09b
NaCl 3000 mgL^{-1}	5.0	3.3	4.1gh	1.77	1.06	1.41ig	4.10	3.21	3.65bc	8.84	3.51	6.17h
NaCl 6000 mgL^{-1}	3.7	2.6	3.1j	1.52	1.10	1.31j	2.39	1.90	2.14g	5.63	2.86	4.24i
$CaCl_2$ 3000 mgL^{-1}	5.4	3.3	4.3fg	1.91	1.83	1.87g	4.06	3.21	3.64bc	10.30	6.02	8.16g
$CaCl_2$ 6000 mgL^{-1}	4.9	3.0	3.9h	1.60	1.40	1.50i	2.67	2.20	2.43fg	7.96	4.21	6.09h
CHS 200 mgL^{-1}	7.3	5.7	6.5a	3.80	2.19	2.99a	4.09	3.84	3.96a	27.72	12.47	20.09a
CHS 400 mgL^{-1}	7.0	4.3	5.6bc	2.86	1.99	2.42b	3.84	3.70	3.77ab	20.02	8.56	14.29b
NaCl 3000 mgL^{-1} + CHS 200 mgL^{-1}	6.8	4.0	5.4cd	2.64	1.75	2.19cd	3.70	3.44	3.57bc	17.97	6.98	12.47c
NaCl 3000 mgL^{-1} + CHS 400 mgL^{-1}	5.3	3.7	4.5ef	2.49	1.59	2.04ef	3.47	3.41	3.44c	13.39	5.87	9.63ef
NaCl 6000 mgL^{-1} + CHS 200 mgL^{-1}	4.8	3.6	4.2gh	2.45	1.55	2.00efg	3.13	2.48	2.80de	11.78	5.59	8.69fg
NaCl 6000 mgL^{-1} + CHS 400 mgL^{-1}	4.0	3.2	3.6i	2.32	1.11	1.71h	3.00	2.30	2.65ef	9.34	3.57	6.45h
$CaCl_2$ 3000 mgL^{-1} + CHS 200 mgL^{-1}	6.0	4.3	5.1d	2.60	1.69	2.14de	3.73	3.53	3.63bc	15.58	7.26	11.42d
$CaCl_2$ 3000 mgL^{-1} + CHS 400 mgL^{-1}	5.3	4.0	4.6e	2.47	2.15	2.31bc	3.85	3.40	3.62bc	13.10	8.63	10.86d
$CaCl_2$ 6000 mgL^{-1} + CHS 200 mgL^{-1}	6.4	3.8	5.1d	2.19	1.84	2.02ef	3.24	2.93	3.08d	14.20	7.01	10.60de
$CaCl_2$ 6000 mgL^{-1} + CHS 400 mgL^{-1}	6.0	3.4	4.7e	2.06	1.75	1.91fg	3.30	2.73	3.02d	12.38	5.97	9.17f
Mean	5.6A	3.7B		2.36A	1.67B		3.49A	3.05B		13.78A	6.55b	
LSD 5 %:												
Cultivars		0.10			0.05			0.10			0.36	
Treatments		0.28			0.14			0.29			0.99	

Discussion

Results of the present study revealed differential germination (table 2), growth (Table 3), biochemical constituents (Tables 4, 5) and yield (Table 6) between cvs G 136 and G 129. In this context, it has been postulated that differential salt tolerance among barley cultivars is due to differential Na ion transport from roots to shoots, with a more sensitive cultivars having a higher cytosolic Na concentration compared with resistant ones (Flowers and hajibagheri, 2001).

Data of the present study revealed that at the same salinity level, the detrimental effects of salinity stress was more pronounced in case of NaCl salinity compared with $CaCl_2$, which is consistent with the results of previous studies (Ayad, 2010; Kheloufi *et al*., 2016). The higher stress magnitude imposed by NaCl may be due to the stress being both osmotic and ionic in case of NaCl whereas it is mainly osmotic in case of $CaCl_2$ (Neumann, 1997). In addition, Trajkova *et al*. (2006) argue that Na rather than Cl is the primary cause of salt damage.

In the present study, the higher level of salinity decreased germination percentage and delayed germination. Salt-induced inhibition of seed germination is due to inhibition of seed imbibition as a consequence of reduced osmotic potential of the germination medium (khan and Weber, 2008). Salt-induced inhibition of seed imbibition leads to reduced activity of protein biosynthesis enzymes (Dantas *et al*., 2007) hence, reduced cell division, as well as other enzymes catalyzing the utilization of seed reserves (Othman *et al*., 2006).

Results also indicated that salinity at the higher level attenuated growth of barley plants and chitosan treatment alleviated salinity effects. Inhibition of growth in response to salinity stress was attributed to inhibition of cell division and cell expansion which are the underpinnings of growth (Munns and tester, 2008). Mitigation of salinity stress by CHS application was previously reported (Guan *et al*., 2009; Zeng and Luo, 2012; Jabeen and Ahmad, 2013; Peykani and Sepehr, 2018). It has been suggested that CHS enhances plant growth through enhancing water as well as nutrients uptake via strengthening of cell osmotic pressure (Guan *et al*., 2009).

In accordance with the obtained results, it has been reported that salinity had negative impact on leaf photosynthetic pigments (Polash *et al*., 2019). Salinity-induced chlorophyll decrement was attributed to inhibition of its synthesis coupled with acceleration of its breakdown via chlorophyllase action (Santos, 2004). Results of the present study revealed that CHS alleviated the effects of salinity stress on chlorophyll content. Previous studies indicated an enhancing effect of CHS on chlorophyll content in plants subjected to various types of stress (Dzung *et al*., 2011; Zeng and Luo, 2012). It has been suggested that CHS releases amino compounds that contribute to stimulation of chlorophyll synthesis (Chibu and Shibayana, 2001). In addition, CHS may lead to increased content from both nitrogen and potassium in plant shoots (Hidangmayum *et al*., 2019) hence, higher number of chloroplasts per cell and higher chlorophyll content (Possingham, 1980).

It is evident from the present study that RWC in salt-stressed, CHS-treated plants was significantly higher than that in salt-stressed only plants. Similar results were reported by Yahyaabadi *et al*. (2016). Maintenance of RWC in salt-stressed plants by CHS may be due to its effects in adjusting

cell osmotic pressure through elevating jasmonic acid biosynthesis (Farouk and Ramadan, 2012) as well as to its antitranspirant effect (Abu-Muriefah, 2013).

Salinity stress led to accumulation of proline and TSS and the treatment with CHS exacerbated this effect (Table 5). These findings are in line with the results of previous studies (Peykani and Sepehr, 2018). According to Hasegawa (2013), the major function of these osmolytes is to maintain osmotic balance hence, continuous water influx. Proline is not only an osmoprotectant, but also a ROS-quencher and a redox balance stabilizer (Hidangmayum and Dwivedi, 2018). The enhancing effect of CHS on TSS content may be due to its enhancing effect on chlorophyll content (Table 4), thereby enhancing photosynthetic activity. On the other hand, the enhancing effect of CHS on proline content may be due to its enhancing effect on amino acids biosynthesis (Li *et al*., 2017).

Grain yield was decreased in response to salinity, whereas it was increased in response to CHS treatment at 200 mgL^{-1} either in plants growing under normal or salinity stress conditions (Table 6). Salinity-induced yield loss is due to both osmotic and ionic stress, thereby injury and/or death of leaves which decrease leaf photosynthetic area, leading to a lower supply of photosynthates and reduced productivity (Polash *et al*., 2019). In addition, salinity-induced decrease in grains weight/plant may be due to a decrease in spikelet differentiation duration, leading to a decrease in number of spikelets/spike and a decrease in number of grians/spike (Javed *et al*., 2003). Meanwhile, the decrease in 100-grains weight in response to salinity stress was attributed to a shortening of grain filling period (Javed *et al*., 2003).

The effect of CHS on enhancing yield of stress-affected plants recorded in the present study is in line with the results of previous studies (Zeng and Luo, 2012; Bistgani *et al*., 2017). Yield increment due to CHS treatment in salt-stressed plants was attributed to increased stomatal conductance and net photosynthetic CO_2-fixation activity (Khan *et al*., 2002). In addition, the enhancing effect of CHS on flowering (Utsunomiya and Kinai, 1994) may also contribute to its effect on enhancing yield in stressful environments.

CONCLUSIONS

It could be concluded that salinity stress led to a depressive effects on germination and plant growth of barley, whereas increased germination time, proline content and total soluble sugars as well as carotenoids in the two studied barley cultivars depending on salt type, level applied as well as cultivar affected. Cultivation of cv G 136 is recommended in soils affected by high salt concentrations, as it is, according to the results of the present study, more adapted to saline conditions compared with cv G 129. Moreover, application of chitosan proved to be a favorable agent to mitigate salt stress in barley plant. In this case, the concentration of 200 mgL^{-1} is more effective than higher ones, in light of the current study's conditions.

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تأثير الكيتوزان على النمو، المحصول و بعض المحتويات البيوكيماوية ذات العلاقة بالاجهاد الملحى فى صنفين من الشعير يتمايزان فى قدرتهما على تحمل الملوحة

هبة محمد ابراهيم و سالى عرفة

قسم النبات الزراعى، كلية الزراعة، جامعة المنصورة، 35516، المنصورة، ج.م.ع

يهدف البحث لدراسة تأثير الكيتوزان على النمو والمحصول والمحتويات البيوكيماوية ذات العلاقة بالاجهاد الملحى فى صنفين من الشعير هما جيزة 129 (صنف حساس للملوحة)، جيزة 136 (صنف مقاوم للملوحة) عوملا بملحى كلوريد الصوديوم و كلوريد الكالسيوم بعد 30 يوم من الزراعة بتركيزات 0 ،3000 ،6000 جزء فى المليون. وأوضحت النتائج أن استخدام كلا الملحين عند التركيز المرتفع سبب نقصاً فى نسبة الانبات ، قياسات النمو ومحتوى الكلوروفيلات الكلية والماء النسبى والمحصول بينما أدى الى سرعة الانبات وزيادة فى محتوى الكاروتنيدات والبرولين والسكريات الذائبة الكليه. كما أدت المعامله بالكيتوزان بتركيز 200 ملجرام /لتر لزيادة المحصول ومكوناته فى النباتات المنزرعه فى الظروف العادية وقللت من أثر الملوحه على النباتات المعرضه للاجهاد الملحى. ولقد كان للكيتوزان أثر اضافى للملوحه فى زيادة الكاروتنيدات والبرولين والسكريات الذائبة الكلية. وهذا الأثر كان أكثر وضوحا فى حالة معاملة التداخل بين كلوريد الصوديوم والكيتوزان بتركيز 200 ملجرام / لتر مقارنه بكلوريد الكالسيوم. و أوضحت النتائج أن القدرة على تحمل الملوحه للصنف جيزة 136 تعزى الى قوة الانبات وزيادة محتوى النبات من البرولين والسكريات الذائبه الكليه.