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Effect of Gene transfer on Salinity Tolerance, Growth, Yield, Chemical Composition and Grain Quality of Transgenic Wheat (Triticum aestivum L.) Plants

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ABSTRACT
The result of various salinity levels (0, 5000 and 8000 ppm) on growth traits, yield and its components and grain quality of transgenic (mannitol-accumulating gene [*mtlD*] for salt stress tolerance event; 79/8 and non-transgenic (cv. Giza 163) wheat plants were investigated. For substantiative this work, the two genotypes used were subjected to the afore mentioned salinity levels. When compared the event 79/8 with the cv. G-163, significant positive results were obtained with the event 79/8 under zero and 5000 ppm NaCl-salinity as compared to the level of 8000 ppm. Irrespective of the two genotype, growth traits (No. of tillers plant^{-1}, root size plant^{-1}, No. of leaves plant^{-1}, yield and its components (No. of spikes plant^{-1}, spike length, No. of spikelets spike^{-1}, No. of grains spike^{-1}, 1000-grain weight and grains weight plant^{-1}) and grain quality (nutrient status of grains, starch, protein and sugars) in 8000 ppm-NaCl-stressed plants were significantly lower than those in 5000 ppm-NaCl-stressed plants. No significant variations in all aforementioned parameters were found out between 5000 ppm-NaCl-stressed and non-NaCl-stressed plants. Regardless NaCl-stress treatments, all aforesaid characters (growth traits, yield and its components and grain quality) were significantly differed between both 79/8 event and cv. Giza 163 with preferability in behave of the former. The event 79/8 surpassed the cv. G-163 in all determined parameters. The interaction effect of NaCl-stress and also the genotype treatment was significant. Maximum positive results of all parameters under study were obtained with non-NaCl-stressed plants of the event 79/8 followed by plants of the same event under 5000 ppm NaCl stress. It has been recommended that, the transgenic genotype; salinity and drought tolerant must be used in wheat cultivation under the climatic changes occurred nowadays which proved to be increased soil salinization and droughts.
Keyword: Wheat; salinity; transgenic; genotype; yield; grain quality.

INTRODUCTION
Excessive soil salinity could be a major issue leading to the low productivity of cultivated crops. Salinity causes oxidative stress in plants by enhancing production of reactive oxygen species. Vitamin C is an efficient antioxidant which is essential for tolerance [1]. Salinity, however, is an abiotic stress, and might negatively influence the plant's morphological characteristics yet quality and amount of its phytochemical compounds of, including total phenol, total soluble sugars and its components, namely sucrose, glucose, and fructose [2]. Agriculture was established by adapting plants to grow in certain climatic conditions, thus, productivity depends on the weather conditions and typically decreases during, or following, periods of extreme weather [3]. additionally, numerous human activities have resulted in saline or drought conditions, thereby decreasing the available area of arable land. There are reports that over 800 million ha of land throughout the world are saline, covering over 6% of the total land area [4]. Further, over 20% of arable land has salinity damage [4]. Irrigation has contributed to the availability of arable land, with recent estimates suggesting that around 20% of the world's arable land is irrigated and provides 40% of food and feed [5]. However, 50% of irrigated land suffers from salinity damage [6]. Some studies predict that 30% of arable land will be lost within 25 years and around half of arable land will be unavailable by 2050 [7]. Currently, 70% of the world's fresh water is consumed by agriculture [5]. Thus, effective utilization of limited water resources is an important aspect to consider in the near future. various physical and chemical approaches exist for rising agricultural productivity in saline environments, [8]. Alternatively, researchers have been working towards developing salt-tolerant crop varieties using selective breeding techniques over the past century; however, none of those efforts has proven successful, [9-10]. Recently, transgenic technology has been perceived as a viable option for generating plants with innate ability to tolerate different level of salts, [7]. various developments have been reported in generating plants using transgenic technology where over expressing a single gene has conferred high salinity tolerance in wheat plants, [11-12]. Transgenic wheat lines had mannitol-accumulating gene [*mtlD*] for salt stress tolerance. Mannitol is considered as the most abundant sugar alcohol. It is synthesized as a primary photosynthetic product in nature in which it is represented in trace amounts in more than 100 plant species, including many crops
such as celery, olive and carrot [13-14]. Mannitol has multiple functions in bacteria, fungi, algae and plants. Because of these functions, many attempts are required in order to generate highly salt-tolerant transgenic plants. Mannitol can function as an intracellular osmolyte and protects the cell from oxidative damage by scavenging toxic oxygen intermediates, [15]. In different biological systems mannitol has been shown to serve as a compatible solute or osmoprotectant involved in stress tolerance. Although mannitol is translocated and serves as a reserve carbohydrate in celery, accelerating salinities increase mannitol levels while reduces sucrose and starch pools in leaf tissues, e.g., the ratio of mannitol to sucrose increases almost 10-fold [16]. This indicates that mannitol accumulation function in adaptation or tolerance to salinity stress. Transgenic wheat showed improved growth of mannitol-accumulating plants under salinity stress [12]. The beneficial effect of transgenic gene of mannitol-accumulating plants on tolerance salinity stress in some plants were studied by several workers [11-17-18-19-20-21-22-23]. Accordingly, the present work was intended to fulfill the growth, yield and its components and grain quality evaluation of transgenic wheat genotype, i.e. 79/8 that had *mtlD* gene in comparison with non-transgenic wheat cultivar, i.e. Giza 163

under certain NaCl-salinity levels, i.e. 0, 5000 and 8000 ppm.

2. MATERIALS AND METHODS

The present investigation was carried out in the Experimental Station of Agricultural Research Center, Giza, Egypt during the two consecutive and successive seasons of 2009/2010 and 2010/2011. The aim of this study was to investigate the effect of different salinity levels (0 ppm, 5000 ppm and 8000 ppm) on growth traits, yield and its components and grain quality of transgenic (mannitol-accumulating gene [*mtlD*] for salt stress tolerance event; 79/8 and non-transgenic (cv. Giza 163) wheat plants.

.1. Plant Expression Vector

The genetic construct pAB4 (8.53 kb; [24] Ramadan et al., 2013), containing the *E. coli mtlD* gene (encoding mannitol 1-phosphate dehydrogenase), was used as plant expression vector. The *mtlD* gene was functioned under the control of maize ubiquitin (*ubi*) promoter (Christensen et al., 1992) [25] and *NOS* terminator. The plasmid contains *bar* gene (encoding the phosphinothricin acetyltransferase) as a selectable marker for Basta herbicide resistance [26] (De Block et al., 1987) driven by *CaMV35S* promoter, with maize *Adh1* intron in the 5`nontranslated region and terminated by *NOS* terminator. Several intermediate construction steps were done in order to obtain the plasmid pAB4. The *Pcab* promoter of pcabMTLD plasmid was replaced by maize ubiquitin promoter (*P-ubi*) and *ubi1* intron of pAHC17 plasmid to obtain pAB3. *Hin*DIII fragment of *bar* gene cassette (2.09 kb), obtained from pAB1 (Ramadan et al., 2013) [24], was integrated into the unique *Hin*DIII site of pAB3 to obtain pAB4 (8.53 kb).

2.2. Wheat Transformation

Immature embryos were isolated from field grown bread wheat (*Triticum aestivum* L.) cv. Giza 163 and pre-cultured for 1 - 4 days in the dark on modified MS medium [27] (Weeks et al., 1993). The protocol used for transforming wheat plants was performed according to Sivamani et al. (2000) [28]. Primary transformants were transferred to the biocontainment greenhouse of AGERI, ARC, Egypt and tested using leaf painting assay with a 0.1% aqueous solution of Glufosinate 200™ (AgrEvo USA, NJ, USA) containing 20% glufosinate ammonium.

2.3. Molecular Analysis of Putative mtlD Transgenic

Genomic DNA was extracted from one selected putative transgenic, resistant during leaf painting to the herbicide Basta, as well as the wild type control, using DN easy TM Plant Mini kit (Qiagen Inc., cat. no. 69104). PCR was performed by the amplification of the partial-length *bar* (400 bp) and full-length *mtlD* (1.08 kb) genes using specific primers with the following sequences:

bar-F5`TACATCGAGACAAGCACGGT3`
bar-R5`GTGCCCTTGACCGTACTGCA3`
mtlD-F5
`CGAGATCTAACAATGAAAGCATTACATTTTGGCGC3`
mtlD-R5`GGGATATCTTATTGCATTGCTTTATAAGCGG3`

The reaction conditions were optimized and mixtures (50-µl total volume) composed of dNTPs (0.2 mM), MgCl2 (1.5mM), 1x buffer, primer (0.2 µM), DNA (100 ng) and Taq DNA polymerase (2 units). Amplification was carried out in a Hybaid PCR Express programmed for 40 cycles as follows:

94°C/4 min for primary denaturation (1 cycle); 94°C/1 min for denaturation, 55°C/1 min for annealing with *bar* gene and 58°C/1.2 min for annealing with *mtlD* gene, 72°C/2 min for extension (38 cycles); 72°C/8 min (1 cycle); 4°C (infinitive). Agarose (1.2%) was used for resolving PCR products. A Lambda phage DNA/*Bst*EII digest was used as a standard DNA (8.45, 7.24, 6.37, 5.69, 4.82, 4.32, 3.68, 2.32, 1.93, 1.37, 1.26, 0.70, 0.12 kb). The run was performed at 80 V in Bio-Rad submarine (8 cm X 12 cm), and bands were detected on UV-transilluminator (Data not shown). Genomic Southern analysis (Southern, 1975) was carried out for the selected T0 transgenic (Data not shown).

2.1. Method of planting:

Plastic pots of 20 cm diameter were used for planting all aforesaid wheat genotypes. Each pot filled with 12 kg acid then distilled water-washed sand. The sand soil used was firstly washed with commercial HCl 10% for 24 h to remove all anions and cations, then was washed with distilled water for several times to remove the excess of acid. One wheat grain of used genotype was sown in each container. Sowing was done at the first of November in the 2009/2010 season and at the first of December in the 2010/2011 season.

2.2. Nutritive solution used:

Modified Hoagland solution suggested by [29] was used as nutrient supplement. For salinity treatments, the nutritive solution was adjusted by using pure NaCl salt to obtain salinity levels; 5000 and 8000 ppm. Irrigation of different treatments as well as the control was applied at 10 days intervals during November to February then, diminished to a week interval. The following Table describes the Hoagland nutritive solution used in this study.

Used wheat cultivars:

Transgenic wheat cultivar; 79/8 (as salt-tolerants; having *mtlD* gene that encodes mannitol-1-phosphate dehydrogenase. Product of this gene, found in mannitol pathway, in bacteria *E. coli*, but its found that this product accumulate mannitol against drought and salinity) as well as one non-transgenic wheat cultivar; Giza 163 obtained from Agricultural Research Center were used in this study.

Solution A

Salt	Amount(g)	Distilled water(ml)	Use in preparation of nutritive solution (ml)
KH_2PO_4	34.022	100	1
KNO_3	25.275	1000	5
$Ca(NO_3)_2$	41.0225	1000	5
$MgSO_4$	30.975	1000	2

Solution B

Salt	Amount (g)	Iron solution (g)
H_3BO_3	0.715	Iron tartarate 1.25 g+ distilled water 1000 ml
$MnCl_2$	0.452	
$ZnSO_4.7H_2O$	0.055	
$CuSO_4.5H_2O$	0.02	
$H_2MoO_4.H_2O$	0.005	
Distilled water 1000 ml, use 1 ml		

Experimental design:

A split-plot with 15 replicates was the experimental design used. Main plots were salinity levels; 0, 5000, and 8000 ppm. Genotypes; transgenic and non-transgenic wheat were occupied sub-main plots.

Data recorded

2.3. Growth traits, Yield and its components:

Sample was obtained at 75 days after sowing of all treatments. At the sampling date, three pots of each treatment were taken, and plants were obtained carefully (a moderate stream of tap water was used for removing plants from the soil of pot) and cleaned from adhering dirt's by tap water then by distilled water to study the following traits (No. of tillers plant^{-1}, No. of leaves plant^{-1}, root size (cm^3) plant$^{-1)}$. At the end of maturity stage, spikes were collected to determine yield and its

components (No. of spike plant^{-1}, spike length (cm), No. of spikelets spike^{-1}, No. of grains spike^{-1}, 1000- grain weight (g), weight of grains plant^{-1}) as well as the grain quality. All these parameters were estimated after air-drying spikes for 48 hours.

2.3. Anatomical study:

For anatomical study, samples from the second season were taken at the age of 75 days from sowing of different wheat genotypes used in this study. Samples for wheat genotypes were taken from the fully expanded leaf (4th leaf from the top as indicated by a primary experiment) including internodes. All samples were killed and fixed in F.A.A. solution (10 ml formalin + 5 ml glacial acetic acid + 50 ml ethyl alcohol 95 % + 35 ml distilled water) for 72 hours, then washed in 50 % ethyl alcohol, dehydrated, cleared in n-butyl alcohol series and embedded in paraffin wax of 56-58ºC m.p. Cross sections of 25µ thick were cut, using a rotary microtom, adhesive on slides by "Haupt's adhesive" then stained with the Crystal violet– Erythrosin combination, cleared in carbol xylene and mounted in Canada balsam [30]**.**

3.6.3. Chemical analysis of different plant parts and harvested grains:

3.6.3.1. Leaf photosynthetic pigments determination:

Chlorophyll a, b and total carotenoids were extracted by homogenization of leaf sample in 80% acetone. The absorbance of the clear extract was measured at 663, 646 and 470 nm. The concentrations of chlorophyll a, chlorophyll b and carotenoids were calculated (mg g-1 fresh weight) to the formulae of **[31]** as follows:

Chl. a=12.21 E663-2.81 E646 µg-1

Chl. b=20.13 E646-5.03 E663 µg-1

Total carot. =1000 E470-3.27 Chl.a-104 chl.b/229 µg-1

3.6.3.2. Enzymes activity determination:

A - α-amylase:

α-amylase activity was determined by the method described by [32] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml. phosphate buffer (pH 6.0). Extract was centrifuged for 15 min. at 400 rpm. The activity of α-amylase was determined in the supernatant using soluble starch as a

substrate and 0.1 ml of the extract. Enzyme activity was expressed (nm / 5 min / 0.5 g fresh leaves) as changes in the optical density (O.D.) at 620 nm.

B - Peroxidase:

Peroxidase activity was determined as outlined by **[33]** as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.0). Extract was centrifuged for 15 min. at 400 rpm. The activity of enzyme was measured in the supernatant using reaction mixture consisted of 1.5 ml of

phosphate buffer, 1.5 ml of H2O2 (20 volume), and 1.5 ml of 0.04 M catechol solution as substrate and 0.1 ml of the extract. Enzyme activity was expressed (nm /5 min / 0.5 g fresh weight of leaves) as changes in the O.D. at 470 nm.

C – Catalase:

Catalase activity was determined by the method of [34] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 7.0). Extract was centrifuged for 15 min. at 400 rpm. Catalase activity was measured in the supernatant using 1.9 ml of reagent grade water, 1.0 ml of H2O2 as substrate and 0.1 ml of extract. Enzyme activity was expressed (nm / 5 min / 0.5 g fresh weight of leaves) as changes in the O.D. at 240 nm.

D - Polyphenol oxidase;

Polyphenol oxidase activity was determined by the method described by **[35]** as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.0). The reaction mixture contained 2 ml of 1% catechol solution as substrate, 0.2 ml of enzyme extract and rest of 0.05 M sodium phosphate buffer (pH 6.8) in volume of 4 ml. Enzyme activity was expressed (nm / 5 min / 0.5 g fresh weight of leaves) as changes in the O.D. at 430 nm.

E - Ascorbic acid oxidase:

Ascorbic acid oxidase activity was determined by the method mentioned by [36] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.2). The sample cuvette contained 1.0 ml of sodium phosphate buffer (pH 6.2), 0.2 ml of ascorbic acid 10-3 molar as substrate, 0.1 ml of enzyme extract and 1.7 ml of distilled water. Enzyme activity was expressed (nm / 5 min / 0.5 g fresh weight of leaves) as changes in the O.D. at 265 nm.

F - Superoxide dismutase (SOD):

Superoxide dismutase activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) and the change in absorbance was measured at 560 nm [37]. The reaction mixture consisted of 25 mM phosphate buffer (pH 7.8), 65 µM NBT, 2 µM riboflavin, enzyme extract, and TEMED and the reaction mixture was exposed to light of 350 µmol m-2 s-1 for 15 min.

2.4. Chemical analysis of harvested grains:

2.4.1. Sugars determination:

Reducing, non-reducing and total sugars were extracted from harvested grains by using 80% ethanol. Sugar fractions were colorimetrically determined (mg g^{-1} dry weight) using phosphomolybdic acid reagent as described by, [38].

2.4.2. Protein determination:

Nitrogen was determined (mg g^{-1} dry weight) in powdered dry material of harvested grains by Orange G dye colorimetric method according to, [39]. Protein calculated (mg g^{-1} dry material) by multiplying nitrogen by 6.25 for wheat roots and shoots and by 5.83 for grains.

2.4.3. Macro-and microelements determination:

The wet digestion of 0.1g of fine dry material of harvested grains of each treatment was done with sulphuric and perchloric acid mixture as mentioned, [40]. Phosphorus was colorimetrically determined using chlorostannus molybdo-phosphoric blue color method in sulphuric acid system as described by, [41]. Potassium and sodium (%) were determined using a Perkin-Elmer, Flam photometer, [42]. Calcium, magnesium and chlorine (%) were determined using a Perkin-Elmer, Model 3300, Atomic absorption Spectrophotometer, [43]**.**

2.4.4. Starch determination:

Starch of harvested wheat grains of each treatment was extracted by using ethanol (80%) then, hydrolyzed by using concentrated HCl. Starch (%) was determined by using Fehling (A+B) reagent and methylene blue as an indicator as described by, [38].

2.5. Statistical analysis:

The obtained data of this study are statistically analyzed according to the design used. The least significant difference test (LSD) at p=0.05 level was used to verify the difference between treatments as mentioned by [41].

3. RESULTS

3.1. Growth traits

Data shown in "Table 1" clearly show that, irrespective of genotypes treatments, growth parameters (No. of tillers plant^{-1}, root size plant^{-1}, No. of leaves plant^{-1}) in 8000 ppm-NaCl-stressed plants were significantly lower than 5000 ppm-NaCl-stressed and non-NaCl-stressed plants. No significant differences

in studied growth traits were found out between NaCl (5000 ppm)-stressed plants and non-salinity-stressed plants. The same trends were observed over two growing seasons (2009/2010 and 2010/2011).

Regardless salinity treatments, data in "Tables 1" reveal that all mentioned growth characters were significantly differed between plants of the tested event; 79/8 and cultivar Giza-163 with preferability in behave of plants of the event79/8. The mentioned growth traits of plants of both two events were significantly surpassed those of the cultivar Giza-163. The same trend was observed over both growing seasons.

The interaction effect of NaCl stress and genotypes treatments was significant "Tables 1". Maximum growth parameters were observed in non-NaCl-stressed condition with transgenic plants of the event 79/8 followed by plants of the same event under 5000 ppm-NaCl-stress. The trend of these results was in accordance in both 2009/2010 and 2010/2011 seasons.

Table 1.
Effect of different salinity levels on some growth traits of both transgenic and non-transgenic wheat plants in 2009/2010 and 2010/2011 seasons

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		No. of tillers plant^{-1}	Root size (cm^3)	No. of leaves plant^{-1}	No. of tillers plant^{-1}	Root size (cm^3)	No. of leaves plant^{-1}
0	G. 163	2.67	5.8	7.33	2.33	8.3	10.33
	79/8	3.33	6.5	8.33	3.67	9.3	12.67
5000	G. 163	2.00	4.8	7.00	1.67	6.7	9.00
	79/8	3.00	6.1	8.00	3.33	9.0	11.33
8000	G. 163	1.00	3.2	4.67	1.33	4.6	7.00
	79/8	2.33	5.1	6.67	2.67	6.3	10.67
$LSD_{0.05}$		**0.51**	**0.9**	**0.92**	**0.42**	**1.3**	**1.88**
Means of salinity (ppm)	0	3.22	6.4	8.11	3.22	9.2	12.22
	5000	2.78	5.8	7.78	2.89	8.4	11.22
	8000	2.00	4.7	6.34	2.33	6.0	9.67
$LSD_{0.05}$		**0.46**	**0.7**	**0.70**	**0.36**	**0.9**	**1.54**
Means of genotypes	G. 163	1.89	4.6	6.33	1.78	6.5	8.78
	79/8	2.89	5.9	7.67	3.22	8.2	11.56
$LSD_{0.05}$		**0.35**	**0.6**	**0.58**	**0.28**	**0.7**	**1.32**

Table 2.
Effect of different salinity levels on some yield components of both transgenic and non-transgenic wheat plants in 2009/2010 and 2010/2011 seasons

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		No. of spikes plant^{-1}	Spike length (cm)	No. of spikelets spike^{-1}	No. of spikes plant^{-1}	Spike length (cm)	No. of spikelets spike^{-1}
0	G. 163	2.1	9.7	12.4	2.2	9.4	14.6
	79/8	3.2	11.2	13.1	3.1	10.9	20.4
5000	G. 163	1.7	8.8	10.9	1.9	8.6	12.5
	79/8	3.0	11.1	12.8	3.0	10.7	20.1
8000	G. 163	0.6	4.1	5.0	0.0	0.0	0.0
	79/8	1.6	7.9	8.4	0.0	0.0	0.0
$LSD_{0.05}$		**0.5**	**0.9**	**1.1**	**0.4**	**1.0**	**1.4**
Means of salinity (ppm)	0	3.0	11.0	13.1	3.0	10.7	20.1
	5000	2.8	10.6	12.5	2.9	10.4	19.2
	8000	1.4	6.9	7.7	0.6	2.8	3.6
$LSD_{0.05}$		**0.3**	**0.6**	**0.7**	**0.2**	**0.7**	**1.1**
Means of genotypes	G. 163	1.5	7.5	9.4	1.4	6.0	9.0
	79/8	2.6	10.1	11.4	2.0	7.2	13.5
$LSD_{0.05}$		**0.2**	**0.5**	**0.5**	**0.2**	**0.5**	**0.9**

3.2. Yield and its components and grain quality

3.2.1. Grain yield and its components

Regardless genotypes treatments, "Tables 2 and 3" show that wheat grain yield and its components (i.e. No. of spikes plant^{-1}, spike length, No. of spikelets spike^{-1}, No. of grains spike^{-1}, 1000-grain weight and grains weight plant^{-1}) of 8000 ppm-NaCl-stressed plants were significantly lesser than 5000 ppm-NaCl-stressed and non-NaCl-stressed plants. However, no significant differences were recorded between both 5000 ppm-NaCl-stressed and non-NaCl-stressed plants. The trend of the obtained findings was in accordance over both growing seasons.

Irrespective of NaCl stress treatments, wheat grain yield and its components of the event 79/8 plants were significantly higher than the cv. G-163 plants which in turn yielded significantly higher grain yield and its components when compared with the cv. G-163 (non-transgenic plants) as shown in "Tables 2 and 3". The results of 2009/2010 season were in accordance with those of 2010/2011 one.

It was found as presented in "Tables 2 and 3" that under non-NaCl-stressed or NaCl stressed conditions, the event 79/8plants have maximum grain yield and its components. The cv. G-163 recorded minimum grain yield and its components under the two salinity levels of 0 and 5000 ppm. Our results show that the cv. G-163 and the event 79/8 failed to give any of yield components.

Table 3.
Effect of different salinity levels on some yield components of both transgenic and non-transgenic wheat plants in 2009/2010 and 2010/2011 seasons

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		No. of grains spike^{-1}	1000-grain weight (g)	Grains weight plant^{-1} (g)	No. of grains spike^{-1}	1000-grain weight (g)	Grains weight plant^{-1} (g)
0	G. 163	10.3	20.7	0.50	11.3	20.5	0.63
	79/8	12.6	22.1	0.56	17.7	22.6	0.82
5000	G. 163	8.9	18.8	0.39	9.4	18.9	0.54
	79/8	12.3	21.9	0.53	17.3	22.1	0.80
8000	G. 163	1.9	5.6	0.08	0.0	0.0	0.0
	79/8	7.1	12.4	0.24	0.0	0.0	0.0
$LSD_{0.05}$		**1.1**	**1.4**	**0.09**	**1.2**	**1.3**	**0.11**
Means of salinity (ppm)	0	12.1	22.2	0.58	17.2	22.6	0.80
	5000	11.5	21.5	0.53	16.4	21.9	0.76
	8000	5.6	10.4	0.21	2.8	5.3	0.16
$LSD_{0.05}$		**0.7**	**0.9**	**0.06**	**0.9**	**0.8**	**0.07**
Means of genotypes	G. 163	7.0	15.0	0.32	6.9	13.1	0.39
	79/8	10.7	18.8	0.44	11.7	14.9	0.54
$LSD_{0.05}$		**0.6**	**0.7**	**0.05**	**0.7**	**0.6**	**0.05**

3.2.2. Grain quality; nutrients, starch, protein and sugars contents

"Tables 4 ,5 and 6" show that, In the time in which grain quality (i.e. N,P, K, Ca, Na, Cl, starch, protein and sugars contents) revealed no significant differences under both non-NaCl-stressed and 5000 ppm-NaCl-stressed conditions, it varied significantly under 8000 ppm-NaCl-stressed condition as compared to both non-NaCl-stressed and 5000 ppm-NaCl-stressed conditions. N, P, K, Ca, starch and protein contents of grains of 8000 ppm-NaCl-stressed plants were significantly lesser than those of both 5000 ppm-NaCl-stressed and non-NaCl-stressed plants. Na, Cl and sugars contents were behaved reverse trend. As for genotypes treatments, data in "Tables 4, 5 and 6" reveal that the event 79/8 plants had the highest grain quality among genotype used in this study. N, P, K, Ca, starch, protein and sugars contents were significantly higher, but Na and Cl contents were significantly lesser in grains of the event 79/8 plants as compared to the cv. G-163 plants. Plants of the event 79/8 produced grains with higher quality as compared to those of the cv. G-163. The interaction effect of NaCl stress and genotypes treatments on grain quality (i.e. N, P, K, Ca, starch, protein and sugars contents) showed maximum values in non-NaCl-stressed followed by 5000 ppm-NaCl-stressed then 8000 ppm-NaCl-stressed plants in the first season; 2009/2010. Na and Cl contents behaved reverse trend "Table 5". In the 2010/2011 season, grain quality of the cv. G-163 and the event 79/8 don't recorded due to plants failed to produce any of yield components.

Table 4.
Effect of different salinity levels on grain N, P and K (% DW) of both transgenic and non-transgenic wheat plants in 2009/2010 and 2010/2011 seasons

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		N	P	K	N	P	K
0	G. 163	1.40	0.20	1.52	1.37	0.22	1.61
	79/8	1.53	0.23	1.69	1.60	0.26	1.79
5000	G. 163	1.28	0.18	1.38	1.26	0.19	1.49
	79/8	1.52	0.22	1.67	1.58	0.27	1.77
8000	G. 163	0.98	0.09	0.89	-	-	-
	79/8	1.17	0.14	1.29	-	-	-
$LSD_{0.05}$		**0.20**	**0.03**	**0.22**	**0.24**	**0.03**	**0.21**
Means of salinity (ppm)	0	1.54	0.23	1.67	1.58	0.26	1.77
	5000	1.49	0.22	1.62	1.54	0.25	1.72
	8000	1.16	0.14	1.19	0.51	0.08	0.57
$LSD_{0.05}$		**0.14**	**0.02**	**0.15**	**0.16**	**0.03**	**0.16**
Means of genotypes	G. 163	1.22	0.16	1.26	0.88	0.14	1.03
	79/8	1.41	0.20	1.55	1.06	0.18	1.19
$LSD_{0.05}$		**0.11**	**0.02**	**0.12**	**0.12**	**0.02**	**0.13**

Table 5. Effect of different salinity levels on grain Ca, Na and Cl (% DW) of both transgenic and non-transgenic wheat plants in 2009/2010 and 2010/2011 seasons

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		Ca	Na	Cl	Ca	Na	Cl
0	G. 163	0.35	0.43	0.23	0.33	0.39	0.21
	79/8	0.39	0.33	0.20	0.40	0.35	0.18
5000	G. 163	0.32	0.49	0.27	0.29	0.44	0.24
	79/8	0.37	0.34	0.20	0.40	0.36	0.18
8000	G. 163	0.12	0.58	0.37	-	-	-
	79/8	0.26	0.45	0.29	-	-	-
$LSD_{0.05}$		**0.05**	**0.05**	**0.04**	**0.05**	**0.04**	**0.03**
Means of salinity (ppm)	0	0.38	0.35	0.20	0.39	0.35	0.19
	5000	0.37	0.37	0.21	0.37	0.38	0.20
	8000	0.24	0.48	0.30	0.10	0.14	0.07
$LSD_{0.05}$		**0.03**	**0.03**	**0.02**	**0.03**	**0.03**	**0.02**
Means of genotypes	G. 163	0.26	0.50	0.29	0.21	0.28	0.15
	79/8	0.34	0.37	0.23	0.27	0.24	0.12
$LSD_{0.05}$		**0.02**	**0.03**	**0.02**	**0.03**	**0.03**	**0.02**

Table 6.Effect of different salinity levels on grain quality (starch, protein and total soluble sugars; % DW) of both transgenic and non-transgenic wheat plants in 2009/2010 and 2010/2011 seasons

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		Starch	Protein	Sugars	Starch	Protein	Sugars
0	G. 163	61.9	8.16	1.76	59.0	7.99	1.84
	79/8	66.0	8.92	1.98	64.1	9.33	2.16
5000	G. 163	53.1	7.46	1.90	52.2	7.35	2.04
	79/8	64.0	8.86	2.08	63.3	9.21	2.27
8000	G. 163	30.2	5.71	2.29	-	-	-
	79/8	48.4	6.82	2.53	-	-	-
$LSD_{0.05}$		**8.2**	**0.84**	**0.21**	**7.4**	**1.20**	**0.24**
Means of salinity (ppm)	0	66.3	8.98	2.08	64.1	9.19	2.14
	5000	62.6	8.69	2.18	61.2	8.96	2.25
	8000	46.2	6.74	2.55	19.5	2.99	0.99
$LSD_{0.05}$		**5.0**	**0.56**	**0.15**	**4.8**	**0.84**	**0.16**
Means of genotypes	G. 163	48.4	7.11	1.98	37.1	5.11	1.29
	79/8	59.5	8.20	2.20	42.5	6.18	1.48
$LSD_{0.05}$		**3.9**	**0.48**	**0.12**	**3.4**	**0.66**	**0.14**

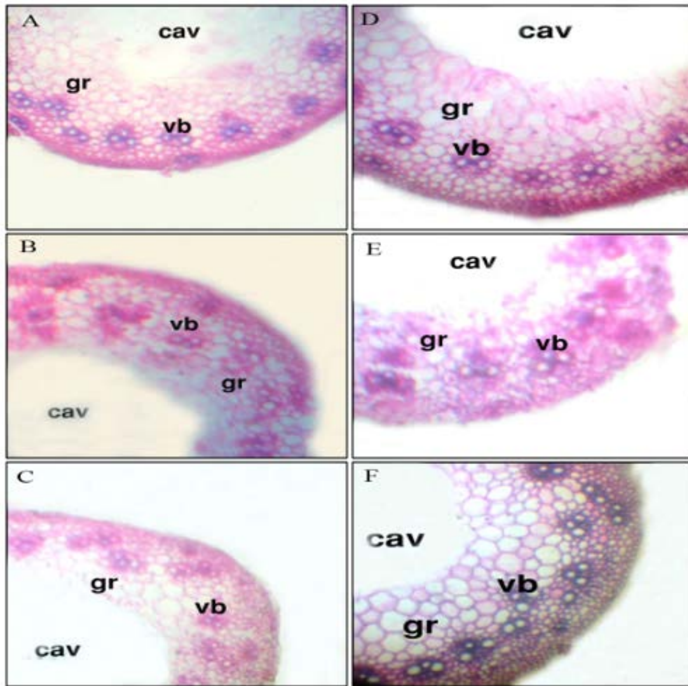
4.2. Anatomical structure.

4.2.1. Stem structure:

Data in Table (5) and figure (1) show that the untreated transgenic genotype; 79/8 of wheat plants with saline water irrigation gave the highest increase of stem structure measurements i.e. section diameter of stem (1625 and 1562.5 µ), thickness of ground tissue (400 and 425 µ) and average diameter of cells in ground tissue (44.4 and 50.0 µ) as compared to nontransgenic cultivar Giza-163 (1400, 350 and 38.8 µ, respectively). Also, cleared that the increasing levels of salinity markedly decreased the measurements of anatomy of stem characters under genotype of wheat plants. The reduction was 28.6 % in section diameter of stem, 42.9 % in thickness of ground tissue and 35.6 % in average diameter of ground tissue cells, at the treatment, cv. Giza-163 + 8000 ppm; 8.9, 21.4 and 1.13%, respectively, in cv. Giza-163 + 5000 ppm, as compared to the cv. Giza-163 + zero salinity. While, reached to (26.4, 37.5 and 13.1%, respectively) in the line 79/8 + 8000 ppm and (18.7, 12.5 and 1.8%, respectively) in the line 79/8 + 5000 ppm, in comparison to untreated line 79/8 plants. Generally, the obtained data cleared that the transgenic 79/8 was more salt tolerant as compared to non-transgenic cv. Giza-163 of all stem anatomy characters.

Table (7): Effect of different levels of salinity on stem anatomy of both transgenic and non-transgenic wheat plants in 2010/2011seasons

Salinity levels (ppm)	**Genotype**	**Section diameter of stem (µ)**	**Thickness of ground tissue (µ)**	**No. of cells in ground tissue**	**Diameter of cells in ground tissue (µ)**	**Diameter of vascular bundle (µ)**	**No. of vascular bundle**	**Diameter of *Mx (µ)**
0	G. 163	1400	350	9	38.8	95	22	20
	79/8	1563	400	9	44.4	95	23	25
5000	G. 163	1275	275	8	34.4	90	19	18
	79/8	1270	350	8	43.6	70	20	20
8000	G. 163	1000	200	8	25.0	70	18	18
	79/8	1150	250	8	31.3	75	20	25



1mm

Fig. (1): Transections of both transgenic and non-transgenic genotype of wheat stem treated with salinity:
A- zero +G.163 D- zero +79/8
B- 5000ppm +G.163 E- 5000ppm +79/8
C- 8000ppm +G.163 F- 8000ppm+79/8
(vb = vascular bundle, gr= ground tissue and cav= cavity).

4.3. Chemical constituents

4.3.1. Leaf photosynthetic pigments

Considerable variation in chlorophyll a, b and total carotenoids was observed among the treatments of NaCl stress and genotypes; cv. Giza-163 and line 79/8 (Table 7). NaCl-stressed plants under 8000 ppm irrespective of studied genotypes showed a significant reduction in examined chlorophylls and carotenoids than non-NaCl-stressed plants and 5000 ppm-NaCl-stressed plants. The differences between studied pigments of non-NaCl-stressed and 5000 ppm-NaCl-stressed plants were insignificant. Similar trends were observed in both the 2009/2010 and 2010/2011 seasons. Data shown in (Table 7) demonstrate that, leaves of the genotypes, 79/8 (transgenic plants) revealed significantly increase in chlorophyll a, b and total carotenoids as compared to leaves of the cv. Giza- 163 (non-transgenic plants). The maximum content of chlorophylls and carotenoids was observed in leaves of the line 79/8 the same trends were seen in 2009/2010 and 2010/2011 seasons.

The data pertaining to interaction effect of NaCl stress and genotypes clearly indicated indefective role of transgenic plants (79/8 genotype) for improving chlorophylls and carotenoids and the line 79/8 was found to be more efficient in mitigating the NaCl-stress by increasing chlorophylls and carotenoids (Table 7). NaCl-stressed 79/8 plants had significant increases in chlorophyll a, chlorophyll b and total carotenoids than NaCl-stressed Giza-163 plants. It is worth mentioning here that as compare to non-NaCl-stressed and 5000 ppm-NaCl-stressed Giza-163 plants showed significant reduction in studied chlorophylls and carotenoids whereas the same treatments with 79/8 genotype significantly increased chlorophylls and carotenoids with preferability in behave of the former. Thus, it appears that the studied genotype, transgenic plants have in defective role for improving chlorophylls and carotenoids and have been more resistant to salinity stress than the cv. Giza-163 as shown by the alleviation of the reduction in chlorophylls and carotenoids under NaCl stress. The increment in chlorophyll a, chlorophyll b and total carotenoids for the line 79/8 under non-NaCl-stress was particularly significant as compared to all other treatments. The trend in the two studied seasons, 2009/2010 and 2010/2011 was true. The highest values of photosynthetic pigments in leaves of transgenic wheat plants might be attributed to the enhanced nutritional status of these plants (Tables 10-17) since; nitrogen (N) and magnesium (Mg) are two of the essential components of chlorophyll. Besides, the presence of iron and manganese is necessary for biosynthesis of chlorophylls. In addition, the enhanced photosynthetic pigments in leaves of the transgenic plants may be partly due to a higher dilution rate of the total Na+ content resulted in the higher contents of osmolytes in the leaves of transgenic plants, as the growth parameters of the transgenic plants were significantly higher than the non-transgenic plants under salt stress.

Table (7): Effect of different salinity levels on leaf pigments (mg g1 ˉF.W.) of both transgenic and non-transgenic wheat plants

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		Chl. a mg/g	Chl. b mg/g	Carotenoids Mg/g	Chl. a mg/g	Chl. b mg/g	Carotenoids mg/g
0	G. 163	0.38	0.25	0.17	0.46	0.27	0.20
	79/8	0.43	0.29	0.19	0.53	0.31	0.24
5000	G. 163	0.31	0.20	0.15	0.40	0.23	0.17
	79/8	0.42	0.27	0.19	0.50	0.29	0.23
8000	G. 163	0.19	0.12	0.07	0.26	0.15	0.10
	79/8	0.28	0.18	0.12	0.38	0.20	0.16
$LSD_{0.05}$		**0.07**	**0.06**	**0.04**	**0.08**	**0.05**	**0.04**
Means of salinity (ppm)	0	0.42	0.28	0.19	0.51	0.30	0.22
	5000	0.39	0.26	0.18	0.47	0.28	0.21
	8000	0.26	0.17	0.11	0.35	0.19	0.15
$LSD_{0.05}$		**0.05**	**0.04**	**0.02**	**0.05**	**0.03**	**0.03**
Means of genotypes	G. 163	0.29	0.19	0.13	0.37	0.22	0.16
	79/8	0.38	0.25	0.17	0.47	0.27	0.21
$LSD_{0.05}$		**0.03**	**0.03**	**0.02**	**0.03**	**0.02**	**0.02**

4.3.2. Activities of antioxidant enzymes

Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbic acid oxidase (AAO), polyphenol oxidase (PPO) and α-amylase activities varied significantly in response to NaCl stress, cv. Giza-163 and genotype 79/8 as shown in Tables (8 and 9).

Stressed plants at 5000 ppm NaCl had significantly higher activities of SOD, POD, CAT, AAO, PPO and α-amylase than non-NaCl-stressed plants and 8000 ppm-NaCl-stressed-plants (Tables 8 and 9). The same trends were observed over both growing seasons; 2009/2010 and 2010/2011. Data in Tables (8 and 9) show that, plants of the line 79/8 showed slightly increase in the activity of SOD, POD, CAT, AAO, PPO and α-amylase as compared to plants of the cv. Giza-163 The same trends were observed in both growing seasons; 2009/2010 and 2010/2011. The interaction effect of NaCl stress and genotypes on the activities of all antioxidant enzymes under study as presented in Tables (8 and 9) showed maximum activities in plants of the line 79/8 under 5000 ppm NaCl which was significant as compared to plants of the cv. Giza-163 under the same NaCl stress level. However, the minimum activities of SOD, POD, CAT, AAO, PPO and α- amylase were noted in 8000 ppm-NaCl-stressed plants of cv. Giza-163 and genotype79/8 in most cases. Both two seasons; 2009/2010 and 2010/2011 revealed the same trend. The trend observed in 2009/2010 season was in accordance with that observed in 2010/2011 season. Antioxidant enzymes particularly SOD enzyme catalyses the dismutation of superoxide anion radicals (O2-) with great efficiency resulting in the production of H2O2 and O2 (**[45].** which improves the scavenging systems of cell and reduces the accumulation of free radicals.

Table (8): Effect of different salinity levels on superoxide dismutase, peroxidase and catalase (nm min-1 g-1 fresh leaf) of both transgenic and non-transgenic wheat plants

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		Super oxide dismuta se nm min/g	Per oxidas-e nm min/g	Cata lase nm min/g	Super oxide dismuta se nm min/g	Per oxidase nm min/g	Cata lase nm min/g
0	G. 163	0.12	0.17	0.22	0.15	0.13	0.19
	79/8	0.16	0.21	0.25	0.21	0.15	0.26
5000	G. 163	0.15	0.19	0.29	0.19	0.16	0.24
	79/8	0.22	0.26	0.36	0.28	0.20	0.32
8000	G. 163	0.13	0.10	0.15	0.13	0.10	0.12
	79/8	0.21	0.15	0.19	0.21	0.15	0.21
$LSD_{0.05}$		**0.03**	**0.05**	**0.04**	**0.05**	**0.03**	**0.05**
Means of salinity (ppm)	0	0.15	0.20	0.24	0.19	0.14	0.24
	5000	0.20	0.24	0.34	0.26	0.19	0.30
	8000	0.18	0.13	0.18	0.19	0.14	0.18
$LSD_{0.05}$		**0.02**	**0.03**	**0.03**	**0.03**	**0.02**	**0.03**
Means of genotypes	G. 163	0.13	0.15	0.22	0.16	0.13	0.18
	79/8	0.20	0.21	0.27	0.23	0.17	0.26
$LSD_{0.05}$		**0.02**	**0.02**	**0.02**	**0.03**	**0.02**	**0.03**

Table (9): Effect of different salinity levels on α- amylase, ascorbic acid oxidase and polyphenol oxidase (nm min-1 g-1 fresh leaf) of both transgenic and non-transgenic wheat plants

Salinity level (ppm)	Genotype	2009/2010 season			2010/2011 season		
		α-amy lase nm min/g	Asco-rbic acid oxidase nm min/g	Poly-phenol oxidase nm min/g	α –amy lase nm min/g	Asco rbic acid oxidase nm min/g	Poly phenol Oxidase nm min/g
0	G. 163	0.28	0.13	0.18	0.24	0.16	0.21
	79/8	0.36	0.15	0.20	0.32	0.19	0.28
5000	G. 163	0.32	0.16	0.21	0.29	0.19	0.28
	79/8	0.41	0.21	0.27	0.38	0.26	0.39
8000	G. 163	0.18	0.07	0.09	0.17	0.13	0.15
	79/8	0.25	0.10	0.15	0.25	0.17	0.20
$LSD_{0.05}$		**0.07**	**0.04**	**0.04**	**0.06**	**0.03**	**0.03**
Means of salinity (ppm)	0	0.34	0.15	0.20	0.29	0.19	0.27
	5000	0.39	0.19	0.24	0.36	0.23	0.35
	8000	0.25	0.10	0.13	0.23	0.16	0.19
$LSD_{0.05}$		**0.05**	**0.02**	**0.03**	**0.04**	**0.02**	**0.03**
Means of genotypes	G. 163	0.26	0.12	0.16	0.23	0.16	0.21
	79/8	0.34	0.15	0.21	0.32	0.21	0.29
$LSD_{0.05}$		**0.04**	**0.02**	**0.02**	**0.03**	**0.02**	**0.02**

CAT enzyme is an important antioxidant system that catabolises hydrogen peroxide, a precursor of reactive oxidants ([47] and reacts with H2O2 directly to form water and oxygen (**[48]; [49]**). POD enzyme catalyses H2O2 dependent oxidation of substrate (RH2). According to [50], inhibition of lipid peroxidation may be one of the mechanisms responsible for the anti-senescence effects of triazoles. POD is one of the major systems for the enzymatic removal of H2O2 in plants. The increased activities of POD in plants suggest the protective role of the enzyme in drought stress. Furthermore, NR is a very labile enzyme [51]. NaCl-stressed transgenic plants; the genotypes 235/3 and 79/8 showed higher SOD, POD, CAT, AAO, PPO and α-amylase activities than cv. Giza-163 under the same stress. This may be attributed to the transferred gene in
upregulating the SOD, POD, CAT, AAO, PPO and α-amylase activities in transgenic wheat plants under NaCl stress. This transferred gene may establish very efficient antioxidative defense mechanism for detoxifying and scavenging of toxic oxygen species through an adoptive mechanism involving upregulation of antioxidative enzymes such as SOD, POD, CAT, AAO, PPO and α-amylase. With significant preferment of transgenic wheat plants, results showed that using transgenic plants for cultivating salinity-affected soils and economic yields are obtainable due to that transferred gene minimizes the negative effects of salinity stress (particularly 5000 ppm NaCl) with evidence of enhancing leaf photosynthetic pigments (Table 7), plant nutritional status (Tables 10-17) and different photosynthates (Tables 18-25) of the genotype, 79/8 which positively reflected on the growth and yield.[52] reported that higher activity of antioxidant enzymes i.e. superoxide dismutase, glutathione reductase and catalase caused lower H2O2 production, lipid peroxidation and higher membrane stability. Beneficial effect of higher osmolyte contents of transgenic wheat plants under study i.e. sugars (Tables 18 and 19), amino acids among them free proline (Tables 20 and 21) and potassium (Tables 10 and 11) is reflected in stabilization of essential enzyme proteins resulting in higher activity of studied antioxidant enzymes (Tables 8 and 9) under salinity stress. Plants posses' antioxidant systems in the form of antioxidant enzymes and metabolites as antioxidants under study can increase these enzymes and antioxidants under various environmental stresses to tolerate these stresses such as transgenic plants under study.

4. DISCUSSION

It is clear from the results of the present study that, the improved effect on growth parameters under study by using the transgenic plants of 79/8 events may be due to the enhanced photosynthetic pigments, activities of antioxidant enzymes, nutritional status of plants and different photosynthates particularly osmolytes of these events which positively reflected on the growth traits under salinity stress, ([18];[60];[61]).The *mtlD* transgenic wheat lines exhibited improved growth parameters under saline conditions especially at 5000 ppm NaCl. Marked differences in studied growth characters were observed between the *mtlD* transgenic wheat genotypes and non-transgenic plants. The growth reduction of non-transgenic wheat plants in saline medium is mainly attributed to the Na^+-specific toxicity. Improved growth of the *mtlD* transgenic plants can be explained on the basis of the bacterial, *E. coli, mtlD* gene for mannitol accumulation, [53]. The accumulation of this sugar alcohol in plant cells plays an essential role in osmotic stress adaptation, [54] The accumulation of mannitol in transgenic wheat plants with *mtlD* had increased salt tolerance as proved by, [11] who observed improvement of some growth traits in transgenic wheat with *mtlD* compared with non-transgenic plant under 150 mM NaCl. Also, [52] found that dry weight of transgenic tobacco with *mtlD* was not reduced under salt stress compared with 40% reduction in non-transgenic tobacco. In our study, the transgenic plants exhibited counteractive effect against the deleterious effects of NaCl-salinity particularly under 5000 ppm NaCl. This suggestion may be due to that these plants of tested event; 79/8 have the capability to stimulate plant growth with more osmolytes thus grant plants an obvious vigor in growth enable them to resist the adverse effects of NaCl-salinity by a way or another. The *mtlD* transgenic wheat lines exhibited improved grain yield and its components in saline soils. We think that transgenic plants of both two events under study have capability to alleviate the deleterious effects of salinity via induction of plant growth associated with an explicit vigor in growth due to their *mtlD* gene which reflected in yield and its components. In general, the increase in the percentages of yields and their components of event; 79/8 as good indicators for stress tolerance as conferred by stress-related transgenes. This way of estimating salt stress tolerance had a better influence in the present study.
The improved yield and grain quality of the *mtlD* transgenic wheat genotypes in saline media with no yield penalty under 5000 ppm NaCl, observed in this study, provide direct evidence on usefulness of mannitol accumulation technology for increasing osmotic suction of transgenic plants and improving wheat productivity in saline soils.

In this concern, [18] showed that the bacterial, *E. coli, mtlD* gene for mannitol accumulation minimizes the negative effects of NaCl stress particularly 5000 ppm with evidence of enhancing plant proline, soluble sugars, and potassium element and antioxidant enzymes leading to maximization of grain yield accompanied with higher quality.

The highest grain contents from N, P, K, Ca, starch, protein and sugars) revealed to the effect mannitol accumulation technology of on producing vigorous plants with large and widespread roots "Table1" which promote rooting process and root distribution and consequently the amounts of mineral elements absorbed by roots and translocated into the different parts of the plant. The increase in protein content of grain may be attributed to the increment in total nitrogen percent of grains.
It is clear that the negative effects of salinity may be due to several specific structure changes that disturb water balance in plant as it reduced differentiation and
development of vascular tissues. Salinity effects on vascular tissues seem to be a structural adaptation that reduces water conduction. Possibly in relation to lower transport losses [53]. **[57]** explains the negative effect of water stress on vascular tissues of stem or leaf blade structure by inhibited cambial differentiation. Also, decreasing parenchymatous cells of stem or leaf blade mesophyll. This was mainly due to the decrease in cell size because inhibition of cell elongation. The obtained data in Tables (5and 6) and Figures (1 and 2) show increasing in measurements of stem or leaf blade anatomy characters of transgenic genotype (79/8) of wheat plants which grown under either with or without salinity levels in comparison to non-transgenic cv. Giza-163. These results may be due to the accumulated osmolytes compounds (such as mannitol, soluble sugars, proline, amino acids and phenols) which increasing the osmotic pressure of root plant cells and consequently increasing its ability to absorption more nutrients from the saline soil solution such as (N, P, K, Fe, Mn and Zn) which play an important role in the formation of protoplast, different organic compounds, enzymes and plant hormones (auxins and cytokenins) and to its great action on accelerating both cell division and enlargement as well as its effect on apical meristem

cambium activity [58] and meristematic tissues development [18]. [59] revealed that, leaves of the salt tolerant genotypes have thicker mesophyll than the salt sensitive ones. The chlorenchyma cells of the mesophyll have thin walls and living protoplast, and consequently are more sensitive to salinity. Our results show that, transgenic genotype (79/8) have metaxylem vessels wider than those of the non-transgenic cv. Giza-163. This feature could be considered as a responsible criterion for salt tolerance, as it compensates the harmful effect of salinity especially, that a great decrease in diameter of metaxylem vessels saline condition. Finally, from the present results, it could be concluded that the transgenic genotypes; salinity and drought tolerant must be used in wheat cultivation under the climatic changes occurred nowadays which proved to be increased soil salinization and droughts. Moreover, increased growth and grain yield as well as improved grain quality and its chemical constituents of grain., so that the used mannitol accumulation technology could be increase wheat productivity with high quality grains.

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