Changes in Yield and Chloroplast Mg^{2+}-ATPase Activity of Wheat (*Triticum aestivum* L.) in Response to Soil Salinity and Nutrient Supply

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ABSTRACT

Under stress conditions such as salinity and drought, plant tolerance depends on upregulating ATPase activity. A greenhouse and a field experiments were conducted to investigate the regulation of chloroplast Mg^{+2}-ATPase activity in wheat in response to soil salinity and nutrient supply.

In the greenhouse, plants were imposed irrigation with three water salinities; 0.4, 4.0, and 8.0 dSm^{-1} with different sub-treatments of nutrient applications. Wheat yield was negatively correlated with soil salinity, presenting threshold at 6 dsm^{-1} followed by yield reduction with slope 7.4 % per unit dsm^{-1}. Salt stress decreased Mg^{+2}-ATP activity from 0.32 to 0.28 µmole P_i min^{-1} mg^{-1}chl. Application of mixed fertilizers M.F. (compost, Gypsum, P, and K) for the non stressed plants resulted in increasing both Mg^{2+}-ATPase from 0.32 to 0.40 and the relative grain yield to 120%, suggesting anabolic metabolism. At soil salinity above the threshold level, application of nutrients sharply increased Mg^{2+}-ATPase activity up to 1.06 µmole P_i min^{-1} mg^{-1}chl accompanying yield reduction; suggesting catabolic metabolism.

In the field experiment, the soil salinity was 12.3 dSm^{-1} at planting and only drainage water (EC 3.9 dSm^{-1}) was available for irrigation. The soil EC steeply reduced to 5.0 dSm^{-1} before the productive growth stages. Mg^{+2}-ATPase activity was up-regulated at flowering and was low late in the season at the grain filling stage. Foliar spray with K^+ and a biostimulant enhanced ATPase activity as well as grain yield compared to the control. Under the field condition Mg^{2+}-ATPase activity was correlated with the grain yield, which reached 8.7 Mg ha^{-1}.

The variation in wheat response in the greenhouse to that in the field may be attributed to the greenhouse conditions that impaired photosynthesis activity and induced catabolic ATP hydrolysis.

Key words: wheat, Mg^{2+}-ATPase, soil salinity, grain yield

INTRODUCTION

Salinity causes both osmotic stress triggered by an excess of salt ions in the rooting medium and ionic stress triggered by over-accumulation of salts in plant cells. Plants respond to such adverse effects by developing different mechanisms for osmotic adjustment and salt efflux from plant cells and/or compartmentation in vacuoles; depending on plant salt tolerance (Kosova *et al,* 2013). Such mechanisms are energetically very expensive (George *et al*., 2012). As a result, when plants attempt to adjust, growth and yields are reduced (Neumann, 1997: Seelig**,** 2000, Kosova *et. al*., 2013).

The chloroplast constitutes a complete unit for photosynthesis in which light energy is converted to chemical energy in forms of ATP and reducing power NADPH. Photophosphorylation (binding ADP and Pi) occurs in the stroma ones protons (H^+) are delivered by the coupling protein ATP synthase across the thylakiod membrane (Taiz and Zeiger, 1991; Dey and Harborne, 1997). Mg-ATP complex is suggested to be involved in the photophosphorylation process in the mitochondria as well as in the chloroplast (Yunis *et al*., 1983; Igamberdiev and Kleczkowski, 2015). ATP is then used to drive the synthesis of organic molecules from CO_2 and to energize many other processes such as active transport across membranes and activation of many enzymes (Dey and Harborne, 1997, Engels *et al*., 2012). The presence of Mg^{2+} dependent ATPase is established in the chloroplast envelope membranes in pea *,pisum sativum*, (Sabnis *et al*. 1970; Bouthyette and Jagendorf 1982; McCerty *et al.* 1984) and in spinach, *Spinacea oleracea* L., (Younis *et al*, 1979). The enzyme has an optimum pH between 7 and 9, and hydrolyzes a broad range of nucleoside triphosphates (McCerty, 1984).

Salt-induced slowing of plant growth is accompanied by a variety of metabolic dysfunctions in non halophytes, including inhibition of photosynthesis (Ziska et al. 1990; James *et al,* 2002), enzymatic activity (Kaiser *et al*. 1988), and absorption of minerals (Dutt et al. 1991, Hu and Schmidhater, 2005). Drought and/or high salts in the growing medium decreases leaf water potential. The low leaf water potential (osmotic stress) results in conformational change of the coupling factor protein (CF1) (Yunis *et. al*., 1979; Boyer and Younis, 1983; Matthews and Boyer, 1984), hence, reducing the binding affinity of ADP to Pi (photophosphorylation). Also, at low leaf water potential, high concentration of Mg^{2+} (above 5 mM) alters the photophosphorylation (*in vivo*) which may reflect general chloroplast response to high ion concentration (Younis and Boyer 1983).

Ion specific (Na^+-specific) stress results from altered K^+/Na^+ ratios and Na^+ ion concentrations that are inimical to plants (Zhang, *et al*., 2001). Potassium plays the major role as counter ion to the light-induced H^+ flux

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Received May 28, 2017, Accepted June 19, 2017

across the thylakiod membrane in the chloroplast (Tester and Blatt, 1989). Thus, photosynthesis is strongly reduced in K-deficient leaves (Cakmak, 2005, Hawkesford *et al.,* 2012). Therefore, ability of plants to retain K^+ and to maintain K^+/Na^+ selectivity has always been considered a key feature of salt tolerance (Munns, 2002, Cuin *et al*.,2003). The tolerance of photosynthetic system to salinity may be associated with the capacity of the plant species to effectively compartmentalize salts in the vacuole (Seemann and Critchley, 1985; George *et al*., 2012).

Among approaches commonly used in agriculture to decrease Na^+ toxicity is to add nutrients such as calcium or potassium. Kaya et. al., (2001) indicated that foliar application of K and P as KH_2PO_4 significantly reduced chlorophyll and membrane damage induced by salinity. Application of Ca^{2+}-containing compounds (such as lime or gypsum) to soils is probably the most effective (Shabala, et al., 2006). An increase in external Ca^{2+} ameliorates Na^+ toxicity symptoms (Hasegawa et al., 2000), sustains K^+/Na^+ selectivity at the plasma membrane (Maathuis and Amtmann, 1999), and stimulates plasma membrane H^+-ATPase activity (Klobus and Janicka-Russak, 2004).

The present study aims to investigate how chloroplast Mg^{+2}-ATPase respond to increasing soil salinity and nutrients supply and weather this response contributes to wheat yield and its salt tolerance.

MATERIALS AND METHODS

Greenhouse pot experiment:

The greenhouse experiment was conducted on wheat (*Triticum aestivium* L.) local variety Sekha 93 which is considered a relatively salt tolerant compared to the some other local varieties (El-Hendawy, 2004). The used soil was sandy clay in texture, calcareous with total $CaCO_3$ content of 37.8%, pH of 8.05, and electrical conductivity (EC_e) of 4.5 dSm^{-1}. It was ground, dried and passed through 2 mm sieve. One kg soil was placed in a plastic pot of 15 cm diameter and 20 cm height.

After planting (AP), pots were imposed to irrigation with three different water salinities: $EC_{0.4}$ (tap water), EC_4 and EC_8 (salinized by dissolving NaCl). Each irrigation treatment was tested with and without mixed fertilizers (M.F.) consists of 15 g compost, 5 g gypsum, 100 mg K and 100 mg P per pot, applied to soil before planting (BP). The high salinity treatment (EC_8 irrigation water) was also tested with each of the 4 individual components included in the above M.F., applied at the same rate to the pots.

Wheat seeds were sown (planted) covered with fine sand, irrigated with $EC_{0.4}$ for 20 days before imposing the different irrigation treatments. Irrigation water was applied every 2-3 days by weighing to 70% of the water holding capacity. The following fertilizer nutrients, as mg pot^{-1}, were dissolved in irrigation water and supplied 30 days AP: 300 N as NH_4NO_3, 100 P as KH_2PO_4, 125 K as KH_2PO_4, Iron 1.4 mM FeEDDHA was foliar sprayed 45 days AP. Wheat was harvested at 97 days AP. The temperature in greenhouse ranged between 22 and 30 °C throughout the experiment period. The treatments were assigned at random to the pots.

Field Experiments:

The field experiment was conducted in the Experimental Research Station of the Faculty of Agriculture at Hamam region, north west Alexandria City, Egypt. The soil parent material varies between calcium carbonate and gypsum with top soil total $CaCO_3$ content of 36%. Rainfall ranges between 47-285 mm/year between October and February; but mainly in December. Temperature ranges between 9 -17 ºC in winter and 20 -32 in summer. Relative humidity ranges between 10 % in December to 60-70 % during April and May. Canal water was not available for irrigation therefore drainage water was used for irrigation. The salinity of the used drainage water for irrigation was 3.9 dS m^{-1}.

The experimental area was 1600 m^2 that uniformly received per ha 7.5 Mg manure, 50 kg N as Ammonium sulfate (21% N), 50 kg P as superphosphate 15% P_2O_5 and 120 kg sulfur mixed with top soil BP. Seeds were planted at rates of 170 kg seeds ha^{-1} (15 % more than the recommended) in an experimental randomized complete block design with four replicates for four treatments. Each block contained 4 experimental units, each 10*10 m (100 m^2). The treatments were foliar spraying as follows: Control, BS1 (400 ml of biostimulant), BS2 (800 ml of biostimulant), and 1/2 liter of K 12.4 % K_2SO_4 per 100 liter water.

The biostimulant was a manure extract (1:3 manure : water ratio) enriched with micronutrients (2% of each Fe, Zn, and Mn as sulfate salt). The final product contained 5.6% dissolved organic carbon in addition to the soluble nutrients extracted from the manure after 3 weeks of incubation (nutrient content was not measured) All treatments were foliar sprayed twice at 59 and 83 days after planting (AP). During the experimental period 150 Kg N ha^{-1} as urea (46% N) was soil applied at 2 times for all plots. After maturity, wheat plants were harvested at 145 days AP.

The wheat plants grown in the greenhouse as well as in the field were subjected to the determination of yield and yield components. Also, plant samples were collected, washed with tap water then with distilled water, and oven-dried at 75 ºC for 48 hr. Subsamples were then ashed at 500 ºC for 6 hr, the ash was then

dissolved in dilute hydrochloric/nitric acid mixture, and filtered (Chapman and Pratt, 1961). The concentration of potassium and sodium were determined by flame photometry in the filtrate (Page *et al.*, 1982).

Chlorophyll and Mg^{2+}-ATPase measurements:

The newly completely developed wheat leaves were separated at flowering stage in the greenhouse experiment and at the flowering and grain filling stages in the field experiment. Leaves were washed with tap water then by distilled water. Functionally intact chloroplasts were isolated according to the protocol of Heldt (2005). From the isolated chloroplast, 20µl was pipette into centrifuge tube containing 10 ml of 80 % acetone, shaken and centrifuged at 5000 r.p.m for 10 minutes. The chlorophyll concentration in the supernatant was determined spectrophotometrically according to Arnone (1949) by measuring the absorbance at 700, 663, 652 and 645 nm against a blank of 80% aceton.

The latent Mg^{2+} dependent ATPase of chloroplasts was activated by incubating with Dithiothreitol (DTT) for 15 minutes in ice. Suspended chloroplasts at chlorophyll concentration of 1 mg ml^{-1} were incubated with 15 mM DTT in ice for 15 minutes before assay of the activity (Younis *et al.* 1979). Twenty to fifty µl of chloroplasts suspensions containing 20-50 µg chlorophyll was pipetted into a centrifuge tube containing 125 µl of 54.4 mM Tris-HCl pH 8. Then, 5 µl of 0.2 M $MgCl_2$ and 50µl ATP 0.2 M was added and the mixture was incubated for 5 min at 37 ºC in water bath. The reaction was stopped by addition of 200 µl of ice-cold 50% trichloroacetic acid and the tubes were centrifuged at 5000 r.p.m for 5 minutes to remove the chloroplast debris. The contents were transported to clean tubes, and then 800µl of the reagent added to each tube of the enzyme assay. The reagent was prepared fresh immediately before use by diluting 10 N H_2SO_4, 10% ammonium molybedate to one – tenth the concentration and adding crystals of $FeSO_4.7H_2O$ to make 5 % solution. The solution was shaken for dissolving all the crystals. Inorganic phosphate in the supernatant was assayed according to method of Taussky and Shorr (1953). The absorbance of the resulting blue color, at 740 nm, was compared to a standard curve of known phosphate concentration between 0.1 to1.2 µmole of KH_2PO_4.

Statistical analysis

The obtained results were statistically analyzed for least significant difference, Tukey test (LSD, $p < 0.05$), using PC-SAS software, SAS Institute.

RERSULTS

Wheat response to salt stress in greenhouse:

Table 1 showed that increasing salinity in the greenhouse pot experiment significantly decreased shoot dry matter through reduction of tillers number, leaf surface area, and spike formation (data not shown). The dry matter yield as well as grain yield significantly decreased with salinity increase. High concentrations of external Na^+ inhibit K^+ uptake by wheat as indicated by sharp decrease in K^+/Na^+ ratio (Table 1). The K^+/Na^+ ratio was generally higher in grains than in shoots or roots. With respect to high salinity stress (EC_8), K^+/Na^+ was around 0.2 in shoots of the EC_8 treatment and increased to 1.0 with the addition of gypsum. This could be due to the slow release of Ca^{2+} from the added gypsum which reduced the detrimental effect of high Na^+. Similarly, K^+ added to EC_8 treatment increased the K^+/Na^+ ratio from 0.2 to around 0.8.

Grain yield data and Mg^{2+}-ATPase are given in Fig .1. The $EC_{0.4}$ treatment had a relatively high grain yield that increased with M.F. Salt stress (EC_4 and EC_8 treatments) resulted in a reduction of the grain yield that unexpectedly further reduced with MF (Fig. 1).

Table 1. Shoot dry matter and K^+/Na^+ ratio of wheat (*Triticum aestivium* L.) grown in greenhouse for 97 days, irrigated with EC0.4, 4, and 8 dSm^{-1} salinized water, and supplied with mixed or individual fertilizer (15 g compost, 5 g gypsum, 100 mg P, 100 mg K)

Treatment	Shoot [g pot^{-1}]	K^+/Na^+ ratio		
		Root	Shoot	Grains
$EC_{0.4}$	11.26^{b}	0.6	6.5	9.1
$EC_{0.4}$ + M.F.	13.15^{a}	0.8	7.3	9.2
EC_4	9.09^{c}	0.3	0.3	2.3
EC_4 + M.F.	8.22^{cd}	0.2	0.3	2.3
EC_8	5.53^{f}	0.2	0.2	2.9
EC_8 + M.F.	5.54^{f}	0.2	0.3	2.3
EC_8 + Gyp.	5.67^{ef}	0.7	1.0	3.7
EC_8 + Com.	5.89^{ef}	0.2	0.3	1.2
EC_8 + P	5.80^{ef}	0.3	0.2	2.6
EC_8 + K	6.56^{ef}	0.4	0.8	4.3

Means with the same letters are not significantly different
(Tukey, $P < 0.005$).

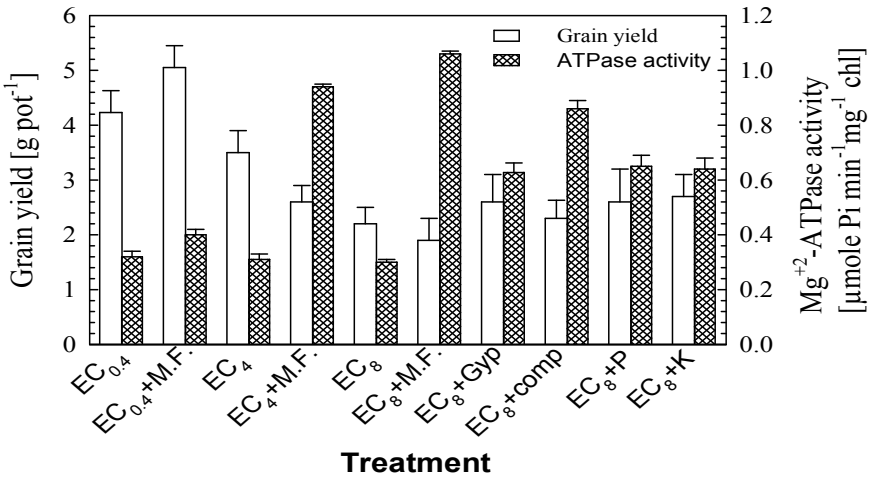

Fig. 1. Changes in grain yield and Mg^{2+}-ATPase activity at flowering growth stage of wheat grown in greenhouse for 97 days irrigated with EC 0.4, 4.0, 8.0 dSm^{-1} salinized water and supplied with mixed fertilizers (M.F.) or individual (compost, gypsum, P, K)

Response to applied nutrients is expected to be less under high saline conditions without lowering yield below the control. On the other hand, application of each component individually for EC_8 treatment slightly enhanced the grain yield compared to EC_8 without or with the M.F.

Mg^{2+}-ATPase activity (at flowering) was 0.32 µmole Pi min^{-1} mg chl^{-1} for $EC_{0.4}$ decreased to 0.31 and 0.28 µmole Pi min^{-1} mg chl^{-1} for the EC_4 and EC_8 treatments, respectively (Fig. 1). In other words, salt stress slightly decreased chloroplast Mg^{2+}-ATPase. In contrast, the application of M.F. sharply increased activity to 0.94 and 1.06 µmole Pi min^{-1} mg chl^{-1} for the EC_4 and EC_8 treatments, respectively. Thus, due to application of M.F., Mg^{2+}-ATPase activity has been increased with salinity. For the M.F. treatments between $EC_{0.4}$ and EC_4, ATPase activity increased in proportion to the increase in soil EC.

In order to get general guideline in this experiment, the relative yield was plotted versus soil salinity; measured after wheat harvest (Fig.2). Also in this figure, the relative increase in Mg^{+2}-ATPase activity due to the nutrients application ((ATPase with nutrient/ATPase without)*100) was plotted vs. soil EC.

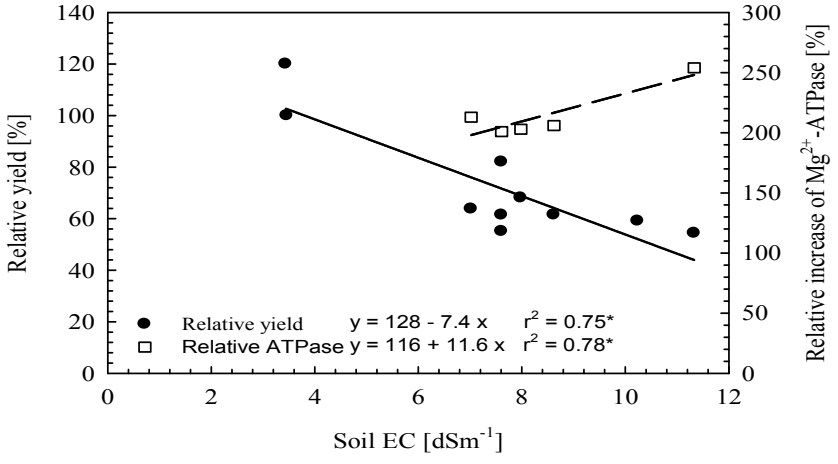

Fig. 2. Relative grain yield of wheat grown in greenhouse for 97 days and relative increase of Mg^{2+}-ATPase (flowering) due to nutrient application as influenced by soil salinity

Employment of the threshold and slope concept (Mass, 1996) indicated that wheat threshold for salinity is presumed to be around 6 dSm^{-1} followed by a yield reduction with slope of 7.4% per unit dSm^{-1}. It seems that below the threshold level, nutrients application (M.F.) increased both wheat yield and Mg^{2+}-ATPase by nearly same magnitude (see Fig. 1). While application of nutrients above the threshold level resulted in yield reduction associated with sharp increase in relative Mg^{+2}-ATPase between EC 7 and 11 dSm^{-1}, with slope 11.6 per unit dsm^{-1} (Fig. 2).

Wheat response to salt stress in the field:

Field soil salinity control:

The field soil was highly saline (12.3 dS m^{-1}) at planting (Fig. 3), and only saline drainage water (EC 3.9 dS m^{-1}) was available for irrigation with. Amount of leaching needed depends on plant tolerance to salinity and the salinity of the irrigation water. For a wheat crop having a salt tolerance threshold of 6 dSm^{-1} and irrigated with 4 dSm^{-1} water; its mean root zone salinity would be around 4 dSm^{-1} with a leaching requirement expected to be around 0.2.

Figure 3 shows that soil (0-25 cm) salinity was 12.3 dSm^{-1} before wheat planting, which was delayed 15 days so that the effective rainfall would reduce soil salinity and coincide with the sensitive growth stages of wheat. However, the scanty rainfall failed to reduce soil salinity at the initial growth stage. After 69 days till harvest, top soil salinity as well as mean root zone salinity was reduced to around 5.0 dSm^{-1} (Fig 3); due to the cumulative of 107 mm rainfall.

Wheat yield, Chlorophyll concentration, and Mg+2-ATPase:

Yield components determined at different stages of wheat development are given in Table 2. Grain yield was significantly higher for the 3 foliar treatments compared to the control. Yield index ranged from 0.30 to 0.35, the maximum yield index (0.35) as well as the maximum grain yield were obtained for the BS_2 treatment. Chlorophyll concentration in leaves at flowering stage ranged from 2.47 to 3.74 mg ml^{-1}. The results showed that foliar application significantly increased the chlorophyll concentration in wheat leaves for the measurement at flowering (Table 2). However, at grain filling stage there was no specific trend.

Figure 4 illustrates the changes in Mg^{2+}-ATPase activity and grain yield as a result of foliar treatments. Mg^{2+}-ATPase activity ranged from 1.70 to 2.67 at flowering and from 0.348 to 0.656 µmole Pi min^{-1} mg chl^{-1}at grain filling. Osmotic stress might be caused by soil salinity before the high rainfall rate (see Fig. 3). Mg^{2+}-ATPase was much higher at flowering compared to that at grain filling. Salinity and or water stress induced enhanced or reduced Mg^{2+}-ATPase activity can't be exclusively answered in this experiment especially at flowering stage. Moreover, Mg^{2+}-ATPase activity responded favorably to the foliar treatments in the following order: K^{+}> BS_2> BS_1> Control.

Under field conditions, Figure 5 shows that grain yield was increased with increase in Mg^{2+}-ATPase activity, in response to foliar treatments. In other words, with proper soil salinity (lower than the threshold level especially at the sensitive growth stages) and nutrient management, ATP synthesis can be enhanced to improve wheat yield.

Table 2. Chlorophyll and yield components of wheat (*Triticum aesetivium* L.) grown in the field experiment for 145 days

Parameter	Treatment			
	Control	BS1	BS2	K^{+}
Chlorophyll, flowering. (mg m^{-1} suspended chloroplast)	2.47	2.89	3.64	3.74
Chlorophyl, grain filling	3.49	3.40	3.00	3.58
Plant height, harvest, (cm)	80.2^{b}	90.0^{a}	85.0^{ab}	86.5^{a}
Leaf area, booting stage (cm^2)	30.7^{ab}	39.8^{a}	34.7^{ab}	25.9^{b}
No. of spikelets/spike, harvest	17.9^{a}	18.0^{a}	18.1^{a}	17.9^{a}
Straw yield (Mg ha^{-1})	19.6^{a}	18.6^{ab}	16.5^{b}	17.7^{ab}
Grain yield (Mg ha^{-1})	7.21^{c}	7.9^{b}	8.7^{a}	8.3^{b}
Harvest index	0.30	0.30	0.35	0.32

Means with the same letters are not significantly differed (Tukey $P < 0.05$).

BS1: 400 ml of biostimulant, BS2: 800 ml of biostimulant, K^{+}: 12.4% K per 100 L.

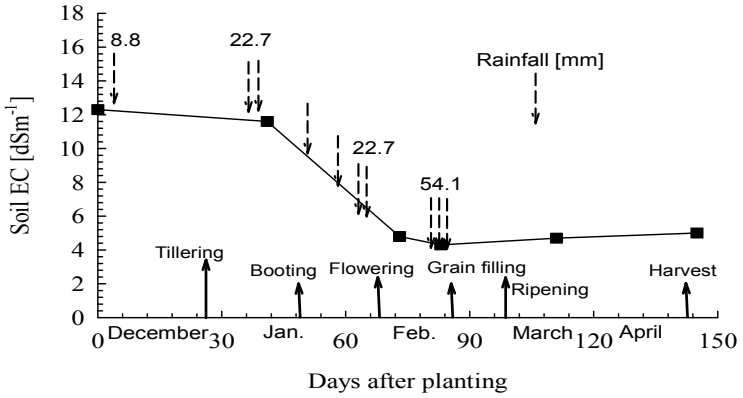

Fig. 3. Changes in salinity of the field soil irrigated with drainage water (EC = 3.9 dSm^{-1}) and received 107.3 mm rainfall during the 145 days of wheat growth season

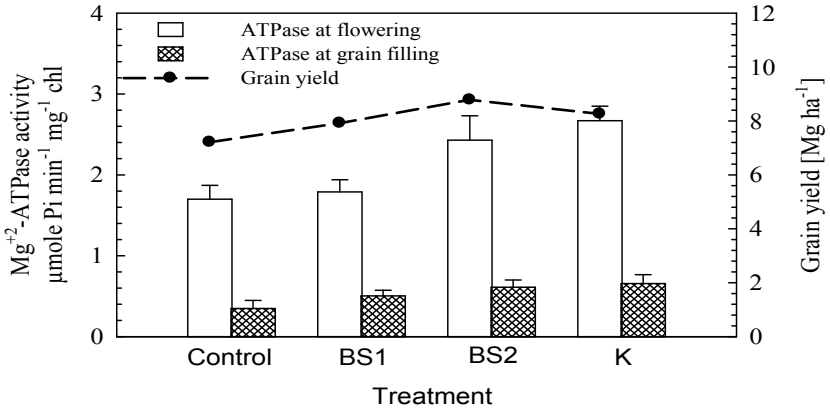

Fig. 4. Changes of Mg^{2+}-ATPase and grain yield of wheat grown in the field for 145 days as influenced by folair treatments (BS1: 400 ml biostimulant, BS2: 800 ml biostimulant, K: 1/2 L of 12.5 % potassium per 100 L)

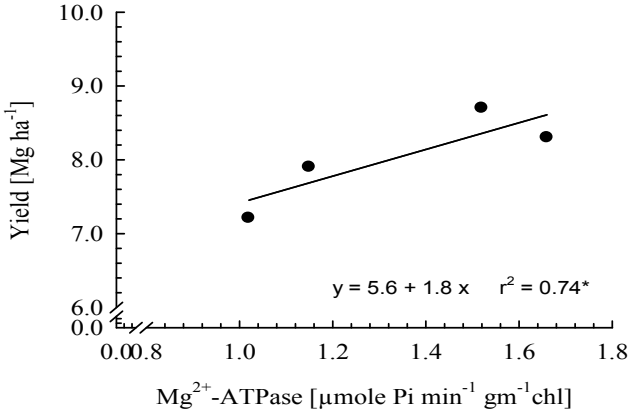

Fig. 5. Relationship between Mg^{2+}-ATPase at flowering growth stage and the grain yield of wheat grown in the field for 145 days growth season

DISCUSSION

Wheat (*Triticum aestivum* L.) falls in the field crop category as a relatively salt tolerant, with threshold of 6 dSm^{-1} (Ayer and Westcot, 1985; Rhoades *et al*.,1992; Mass, 1996). High Soil salinity adversely affected crop physiological parameters, water use, yield, and overall plant growth (Arfan *et al*., 2007). In the greenhouse experiment, wheat yield significantly decreased with soil EC increase (Table1 and Fig.2). In agreement with Mass (1996), the salinity tolerance threshold was around EC 6.0 dSm^{-1} with yield reduction following the threshold 7.4 % per unit dSm^{-1} (Fig.3), compared to 7.1% per unit dSm^{-1} as represented by Mass (1996). The salinity exerted it detrimental effect on wheat yield because of osmotic stress and ion toxicity. The latter can be clearly shown by the strong reduction in K^+/Na^+ ratio in both root and shoot (Table1). Both osmotic adjustment and Na^+ exclusion are energetically very expensive physiological processes (George *et al*., 2012), resulting in growth and yield reduction (Seelig**,** 2000; Munns 2002; Kosova *et al*., 2013). Wheat is a classical "salt excluder" characterized by low rates of sodium transport to the shoot thus keeping mesophyll cells as Na^+-free as possible (Munns and James, 2003; James *et al*., 2011). The K^+/Na^+ ratio was higher in grains than in shoot or in root at all salinity levels (Table1) suggesting higher re-translocation of K^+ relative to that of Na^+. This was true for shoot compared to the root only below the salinity threshold level (6.0 dSm^{-1}).

The reduction in grain yield with increasing salinity ($EC_{0.4}$, EC_4, EC_8) as dominant growth factor (Fig.2) was associated with a slightly decrease in chloroplast Mg^{2+}-ATPase (from 0.32 to 0.28 µmol P_i mg^{-1} min^{-1}chl). This is possibly, due to the negative effect of low K^+/Na^+ ratio and osmotic stress. At high salinity and low K supply a reduction in photosynthetic capacity, cytochrome f, and ATP synthesis were reported in spinach (Chow *et al*., 1990). Water potential (Ψ_w) in leaves decreases with salt stress (Kirnak *et al*., 2001). The Ψ_w decrease is associated with large losses in chloroplast activities (Potter and Boyer, 1973), notably electron transport and photophosphorylation (Younis et. al., 1979 and 1983,). ATP synthesis involves the binding of ADP and P_i to CF_1 and the release of ATP in the medium. Presumably, CF_1 in the EC_4 and EC_8 treatments of this study were impaired by salt stress.

Calcium and potassium are playing important roles under salinity stress (Zhu, 2003; Georga, *et al*., 2012, Chow *et al*., 1990). Ca^{2+} plays a fundamental role in regulating root potassium nutrition and salt tolerance (Liu and Zhu, 1997; Kinrade, 1999; Renault and Afifi, 2009) and increase PM-H^+-ATPase activity (Hu-Cheng et al., 2003, Morgan et. al., 2014). Supplementing wheat with M.F. (compost, Gypsum, P, and K) below the threshold level ($EC_{0.4}$ treatment, soil EC 3.7) increased the relative yield to 120% as well as Mg^{2+}-ATPase activity from 0.32 to 0.40 µmole Pi min^{-1} mg^{-1} chl; reflecting constructive metabolism (anabolism) and ATP synthesis. In contrast, supplementing the EC_4 and EC_8 treatments (soil salinity above the threshold level) with M.F. reduced the relative grain yield (Fig.2) while Mg^{+2}-ATPase activity sharply increased up to 1.06 µmole Pi min^{-1} mg^{-1} chl, suggesting destructive metabolism inducing futile ATPase activity. Nutrients supplied in the M.f. above the threshold level may have contributed to proton gradient dissipation through indirect proton transport. Presumably, the ATP synthase switched its catalytic activity from ATP synthesis to ATP hydrolysis. Also, application of each component included in the M.F. to $EC_{8.0}$ resulted in an increase of ATPase activity (Fig. 4).

In this study, the conditions in the green house differed than that in the field. Wheat growth period in the field was 145 days, within the normal range, compared to 97 days in greenhouse, as greenhouse temperature was 7 to 10 °C higher than outdoors. Enzyme-catalyzed reactions like all thermochemic reactions increase in rate with rise in temperature (Street and Cockburn, 1972). With temperature above 30 °C, inhibition of proton transport was reported for V-type ATPase of maize root (Brauer *et al*., 2000). In green house, plants were subjected to salinity stress from 20 days AP up to harvest, whereas, soil salinity in the field was reduced from 12.3 dSm^{-1} early in the season to 5.0 dSm^{-1} (from 69 day AP till harvest) due to cumulative 107 mm rainfall. Higher rate of seeds (115% of recommended) was planted to overcome the effect of soil salinity on germination, and planting was delayed so that effective rain (~10 cm) falling in Jan and Feb would coincide with the wheat sensitive growth stages (Fig. 5). The sensitive stage in cereal crops is the early productive stage. If plants are stressed at the spike or panicle differentiation stage, grain yield is significantly reduced (Maas, 1996). Morever, the field soil was gypsic calcareous so that adequate Ca^{+2} supply is expected to be available for wheat throughout the season. In such soils, plants can tolerate about 2.0 dSm^{-1} salinity higher than their threshold level (Rhoades et al., 1992).

The field experiment conditions have led to anabolic ATP synthesis. Mg^+-ATPase activity up-regulation was noted at flowering stage and was low late at grain filling. Grain yield was increased with increases in ATPase activity measured at flowering; in response to the foliar

treatments. Sharma and Mani (1990) reported that rice grain yield per panicle was significantly positive with ATPase activity. Such results indicate that supplementary foliar application may improve wheat ATPase activity and yield at proper soil salinity stress. Kaya et al (2001) suggested that supplying P and K^+ via leaves can reduce the adverse effects of high salinity on plant growth and physiological development.

The variation in wheat response to salinity in greenhouse and that in the field can be attributed to the greenhouse conditions that impaired photosynthesis and induced catabolic ATP hydrolysis. Futile Mg^{2+}-ATPase activity sharply increased between EC 7 and 10, that might result in both drastic reduction in relative yield and account for the yield slope beyond the salt tolerance threshold.

ACKNOWLEDGEMENT

The authors are highly grateful to the late Prof. Fathi Amer, Soil and Water Science Dept. Faculty of Agriculture, Alexandria University, for his contribution to this work. The authors are also highly grateful to Prof. Hassan Yunis, Pesticide Chemistry & Technology Dept. Faculty of Agriculture, Alexandria University, for his great help in the determination of Mg^{2+}-ATPase.

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الملخص العربي

التغيرات في المحصول ونشاط انذيم Mg^{2+}-ATPase في نبات القمح وتأثره بملوحة التربة والإمداد بالعناصر

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المحافظة على ضبط إنزيم ATPase تحت ظروف الإجهاد، مثل الإجهاد الملحى والإجهاد المائى، يساعد النبات على تحمل ظروف الإجهاد. وقد هدف هذا البحث الى دراسة التغيرات الحادثة فى نشاط إنزيم Mg^{2+}-ATPase فى كلوروبلاست أوراق نبات القمح النامى تحت ظروف ملحية فى تجربة إصص (الصوبة الزجاجية) وكذلك فى الحقل.

فى تجربة الصوبة تم تعرض النباتات الى ثلاثة مستويات من الملوحة ($0.4, 4.0, 8.0\ dSm^{-1}$) مع إضافة سماد مختلط (كمبوسط، جبس زراعى، فوسفور، بوتاسيوم) أو أحد هذه المكونات منفردة تحت الظروف عالية الملوحة EC_8. وقد أوضحت نتائج تجربة الصوبة إنخفاض فى المحصول مرتبطا بمستوى ملوحة التربة بحد أقصى لتحمل الملوحة عند 6.0 dSm^{-1} ومعدل إنخفاض قدره ٧.٤ % لكل وحدة ملوحة تزيد عن ذلك. وقد أدى الإجهاد الملحى الى إنخفاض نشاط الإنزيم من ٠.٣٢ الى ٠.٢٨ ميكرومول Pi/دقيقة/مجم كلوروفيل. وقد أدت إضافة المغذيات تحت ظروف الملوحة الأقل من الحد الأقصى لتحمل النبات الى زيادة فى نشاط الإنزيم من ٠.٣٢ الى ٠.٤ ميكرومول Pi/دقيقة/مجم كلوروفيل رافقته زيادة فى المحصول النسبى الى ١٢٠%. بينما أدت إضافة المغذيات عند مستويات الملوحة الأعلى من الحد الحد الأقصى للتحمل الى نقصا فى المحصول يقابله زيادة واضحة فى مستوى الإنزيم وصلت الى ١.٠٦ ميكرومول Pi/دقيقة/مجم كلوروفيل. يعكس ذلك استهلاكا للطاقة الحيوية داخل النبات فى عمليات مقاومة لا جدوى منها وذلك عندما تصبح الملوحة هى العامل المحدد.

أجريت التجربة الحقلية بمزرعة كلية الزراعة، جامعة الإسكندرية، بمنطقة الحمام. وقد كانت ملوحة التربة عند الزراعة $12.5\ dSm^{-1}$ مع توفر مياه الصرف الزراعى فقط للرى (3.9 dSm-1). أدى الرى وهطول الأمطار الى انخفاض الملوحة الى $5.0\ dSm^{-1}$ ولك قبل المراحل الإنتاجية فى نبات القمح. وقد أوضحت النتائج مستوى مرتفع للإنزيم فى مرحلة طرد السنابل مقارنة بمرحلة ملىء الحبوب وكذلك زيادة فى مستوى الإنزيم مع معاملات الرش بالمغذيات. وقد وجدت علاقة ارتباط فى هذة التجربة بين مستوى الإنزيم فى أوراق القمح عند مرحلة طرد السنابل والمحصول الناتج الذى بلغ ٨.٧ طن/هكتار فى أفضل المعاملات.

إتضح كذلك من النتائج أن الإختلافات بين استجابة نبات القمح فى تجربة الصوبة وتجربة الحقل قد يرجع الى مستويات الملوحة ودرجة الحرارة المؤثرة على النبات فى الحالتين.