

ORIGINAL ARTICLES

Mitigation Salinity Stress Effects on Barley (*Hordeum vulgare L.*) Growth, Yield and Some Physiological Aspects by Hemin

^{1,2}Abd El-Monem A.A.; ³El-Habbasha, S.F. and ³Hozayn M.

¹Botany and ³Field Crop Research Departments, Agricultural and Biological Division, National Research Centre. El-Behouth St., 12622 Dokki, Cairo, Egypt.

²Biological Department, Faculty of Science, Tabuk University, Branch Tayma, Saudi Arabia

ABSTRACT

Two field trials were carried out in the Research Farm of Faculty of Agriculture, Cairo University, Wady El-Notron Province, El Behaira Governorate, Egypt during two successive winter seasons of 2010-2011 and 2011-2012 to study the effect of different concentrations of hemin on some growth parameters, yield and yield attributes and some chemical constituents of barley irrigated with saline water under sandy calcareous soil conditions. The results showed that plant height, fresh and dry weight of plants/m², succulence and leaf area were significant positively affected by increasing the hemin concentration from 0 to 100 μ mol. Moreover, 50 μ mol hemin concentrations recorded the highest values in most studied characters with significant differences as compared with control plant (zero hemin). Plant grown under salinity stress (zero hemin) contained greater concentrations of Ca, Na, Mn and Zn elements while, the hemin treatment up to 75 μ mol resulted in significant reductions of some macro and microelements. Such reduction was parallel to the increase in Mg up to 100 μ mol. Increasing hemin concentrations significantly increased all yield attributes and economic yield (kg/fed) as compared with control plant (0 hemin). The highest recorded values of number of spikelets/spike and 100 grain weight were recorded when 75 μ mol hemin was applied as compared with other concentrations. The results showed that the proline concentration was decreased nearly to the half of that occurred when barley plants treated with 100 μ mol of hemin indicating the mitigative and protective role of hemin in counteraction salinity of irrigation water. Generally we could conclude that, hemin act as plant growth regulators in alleviating harmful effects of salinity stress.

Key words: Barley, Salinity, Hemin, Growth, Yield, Mineral content, Amino acids.

Introduction

Barley (*Hordeum vulgare L.*) is grown as a commercial crop in one hundred countries and considered one of the most important cereal crops in the world Yousufinia *et al.* (2013). It is the fourth position in total cereal production in the world after wheat, rice, and maize (FAO, 2004). Barley is considered highly salt tolerant of the agriculturally important cereals and has been grown successfully in fields that irrigation has rendered unsuitable for other crops. The salt tolerance in barley is differ between genotypes and also between different growth stages, it is most sensitive to salinity at germination and young seedling stages, and exhibits increased tolerance with age. Salt stress for barley at seedling stage has been mainly attributed to ionic effects rather than to osmotic effects (Storey and Wyn Jones 1978). The indication of good salinity tolerance at one growth stage such as germination and seedling does not necessarily mean that other stages will also have good salt tolerance. However, the levels of tolerance vary by the genotype and the developmental stage Yousufinia *et al.* (2013).

Salinity is a serious problem affecting one third of the irrigation land (Mass and Hoffman, 1977). It has been estimated that salts affected nearly 950 million ha land in the world (Babu, *et al.*, 2007). Salinity is known to retard plant growth through its effect on several facts of plants behavior like osmotic adjustment, ion uptake, protein and nucleic acid synthesis, photosynthesis, enzyme activities and hormonal balance (Dumbroff and Cooper, 1974). The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Marschner, 1995; Hasegawa *et al.*, 2000 and Ashraf and Harris, 2004).

Osmotic stress and ionic imbalance derived from salinity can affect major plant physiological and biochemical processes such as photosynthesis, protein synthesis and lipid metabolism (Abo Hamed *et al* 1999). Increasing osmotic stress in plants leads to stomatal closure, resulting in reduction of CO₂ availability and photosynthesis, thus increasing the possibility of reactive oxygen species formation Al Hakimi (2000).

Abiotic stress is a worldwide problem limiting plant growth and yield. In response to a biotic stress, plants have gradually evolved a complete set of stress able to regulate ion homeostasis to adapt to salt stress (Zhu, 2001, 2003), whereas osmoprotectants secreted by plant cells protect them against osmotic stress (Zhu, 2002; Sairam and Tyagi, 2004). Plants can adjust osmotic stress by accumulating high concentrations of compatible solutes in cytoplasm (Wu *et al.* 2013). The exogenous application of compatible solutes has been suggested as an alternative approach to improve crop productivity under saline conditions (Chen and Murata, 2002). Yield components and growth parameters also show differential responses to salinity stress (Taghipour and Salehi 2008). Ahmad *et al.* (2003) reported that the increase of sodium chloride and sodium sulfate concentration resulted in the reduction of number of tillers, length of spike, number of spikelets per spike, biomass per plant and grain yield per plant.

The hemin (product of glycine and ALA, Fig.1) attracted the attention of several researchers and have reported positive correlations between the capacity for glycine betaine and/or proline accumulation and salinity tolerance (Almansouri *et al.*, 1999; Meloni *et al.*, 2001), others have challenged the value of these solutes as positive indicators for resistance to salt stress (Heuer, 2003).

The first reaction in heme biosynthesis takes place in the mitochondrion and involves the condensation of one glycine and one succinylCoA by the pyridoxal phosphate-containing enzyme, δ -aminolevulinic acid synthase (ALAS). Delta-aminolevulinic acid (ALA) is also called 5-aminolevulinic acid. This reaction is both the rate-limiting reaction of heme biosynthesis, and the most highly regulated reaction. External application of low exogenous concentrations of glycine betaine and proline decreased salt-induced K^+ efflux from barley roots (Cuin and Shabala, 2005). Some researchers have reported positive correlations between the capacity for glycine betaine and/or proline accumulation and salinity tolerance (Almansouri *et al.*, 1999; Meloni *et al.*, 2001), others have challenged the value of these solutes as positive indicators for resistance to salt stress (Heuer, 2003).

Different forms of tetrapyrroles function in plants as electron carriers, signaling factors, and catalysts for redox reactions. Tetrapyrrole biosynthesis starts at glutamyl-tRNAGlu, and the subsequently formed 5-aminolevulinic acid (ALA) is metabolized to form tetrapyrroles through a variety of reactions (Beale and Weinstein, 1990; Fig. 1). Protoporphyrinogen oxidase (PPO) is the last enzyme before the branch in the tetrapyrrole biosynthetic pathway, and its product, protoporphyrin IX (Proto IX), is directed to the magnesium (Mg) and iron (Fe) branches for chlorophyll and heme biosynthesis, respectively Phung *et al.* (2011). Many intermediates in the porphyrin biosynthetic pathway, such as Proto IX and its various Mg²⁺ derivatives, including protochlorophyllide (Pchlde), interact with reactive oxygen species (ROS) such as singlet oxygen, which is harmful to cells and causes the peroxidation of membrane lipids (Valenzano, 1987; Dolphin, 1994; Reinbothe *et al.*, 1996). All living organisms face the danger of uncontrolled chemical reactions involving tetrapyrroles, due to their remarkable reactivity (Jung *et al.*, 2004, 2008; Kariola *et al.*, 2005; Yao and Greenberg, 2006).

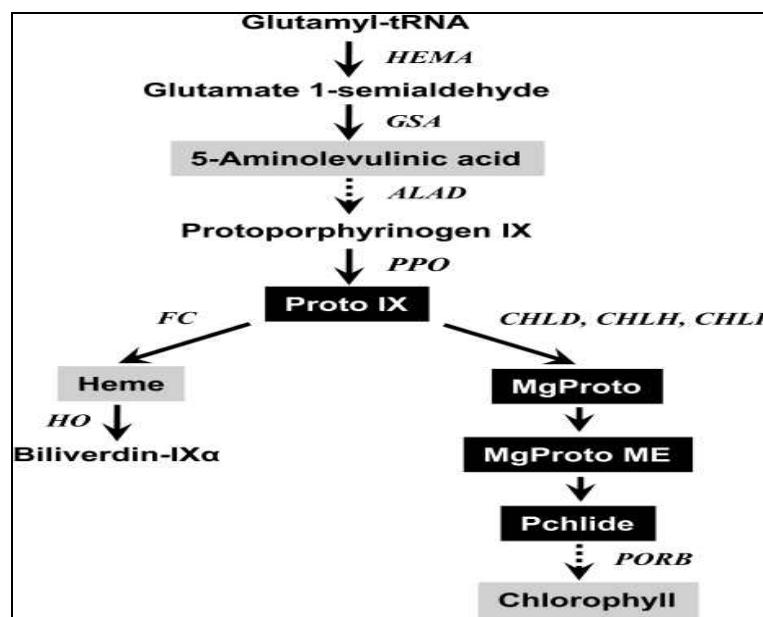


Fig. 1: The porphyrin pathway in plants showing intermediates. (Phung *et al.* 2011).

The keto-amino acid, 5-aminolevulinic acid (ALA), with a molecular weight of 131, is a precursor of heme, chlorophyll, vitamin B12 and other tetrapyrrole compounds *in vivo* (Stobart and Ameen-Bukhari 1984). ALA serves as prosthetic groups of respiratory enzymes, and chlorophyll in plants (Granick 1961), and is the major photosynthetic light-harvesting pigment (Senge 1993). Low concentrations (10–300 mg/L) of ALA enhanced growth rates and photosynthesis, when applied at the 3–4 leaf stage to barley, potato, radish, garlic and kidney bean (Hotta *et al.* 1997a & b). Watanabe *et al.* (2006) found that 100 mg/L of ALA enhanced the growth and photosynthesis of grapevines, while Hotta *et al.* (1998) and Zhang *et al.* (2006) showed that ALA increased cold and salt tolerance in rice and potato. This compound therefore appears to act as a hormone-like plant growth regulator, which is effective at relatively low concentrations (10–100 mg/L). Therefore, the objective of this study was to investigate the effects of hemin (product of glycine and ALA) application on growth, yield and nutritional values of barley.

Materials And Methods

Two field experiments were carried out at the Research Farm of Faculty of Agriculture, Cairo University, Waady El Notron Province, El Behaira Governorate, Egypt during two successive winter seasons of 2010-2011 and 2011-2012 to study the effect of different concentrations (0.0, 50, 75 and 100 μ mol) of hemin on growth parameters, yield and yield attributes and some chemical constituents of barley under saline conditions. The soil texture of the experimental site was sand and some physical and chemical properties of a representative soil sample are listed in Table (1) according to the method described by Champan and Pratt (1978). Analysis of irrigation water is presented in Table (2).

Table 1: Mechanical and chemical analysis of the experimental soil site (Avarage of both seasons

Character	Mechanical analysis				Chemical analysis											
					pH	EC dS/m	CaCO ₃	Organic Matter %	Soluble cations meq/l				Soluble anions meq/l			
Depth	Sand %	Silt %	Clay %	Soil texture	Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ⁻³	HCO ⁻³	Cl ⁻	SO ⁻⁴				
0 - 30 cm	91	7.3	1.73	Sandy	8.00	0.5	12.25	0.15	0.83	0.28	3.62	2.61	--	1.36	3.52	2.46
30 - 60 cm	89.5	8.61	1.88	Sandy	8.20	0.7	10.52	0.23	0.86	0.35	2.95	2.77	--	1.5	3.39	2.04

Table 2: Chemical analysis of irrigation water

Characters	pH	EC dS/m	Soluble cations meq/l				Soluble anions meq/l			
			Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ⁻³	HCO ⁻³	Cl ⁻	SO ⁻⁴
	7.80	5.00	26.30	0.71	4.45	13.20	0.02	4.52	35.10	5.02

The soil was ploughed twice, ridged and divided into plots. During seed preparation, 100 kg/fed calcium superphosphate (15.5% P₂O₅) and 50 kg/fed potassium sulphate (48 % K₂O) were applied. 60 kg N/fed as ammonium sulfate (20.6% N) was added in four equal doses began after three weeks and the other doses were applied weekly till flowering initiation. The hemin treatments were sprayed at 30 and 45 days after sowing and allocate randomly in Randomized Complete Block Design (RCBD) with three replications. Each plot consisted of 15 rows (20 cm spacing) of 3.5 meter length, i.e. 10.5 m² (1/400 faddan), with seed rate of 40 kg/faddan. Planting date was 17th and 20th November in first and second seasons, respectively. Sprinkler irrigation took place immediately after sowing, then every one week intervals according to agronomic practices in the district.

Data recorded

Growth parameters:

During both growing seasons, wheat plants from each plot -in one square meter- were cut from ground surface at 60 days after sowing. Plant height (cm), total fresh and dry weight (g/m²) and water contents (%) were determined. Leaf area (cm²) was estimated according to the method described by Fowler and Rasmusson (1969). The harvested shoots were then dried to constant weight at 70° and the values of succulence (ratio of fresh weight/dry weight) were calculated according to Tiku equation (1975).

Chemical composition of shoot:

Plant samples were taken from each plot at 60 days after sowing, dried at 70°, and grounded using stainless steel equipments to determine K, Mg, Ca, Na, Fe, Mn and Zn concentration. Plant nutrients were determined as

follows: Total nitrogen by using the micro kjeldahl method (AOAC1980). Phosphorus, potassium and micronutrients were extracted by using dry ashing technique according to Cottenie *et al.*, (1982). Phosphorus was photometrically determined using vanadate method and measured by spectrophotometer, while potassium was measured by flam photometer. Micronutrients and magnesium was measured using atomic absorption spectrophotometer.

Yield and yield components:

At the harvest, one square meter from each plot was counted to determine number of spikes/m². Plant height (cm), spike length (cm), spike weight (g), spike grain weight (g), number of spikelets/spike and 100-grain weight were determined from randomly selected 20 tillers from each plot. The whole plot was harvested once and threshed to determine seed, straw and biological yields (ton/fed) as well as harvest index (grain yield/total biological yield) and crop index (grain yield/total straw yield) were calculated.

Nutritional value of grains:

The dried grains were finally ground to K, Mg, Ca, Na, Fe, Mn and Zn concentration as mentioned by Cottenie *et al.*, (1982). Total nitrogen percentage was determined according to the method described by A.O.A.C. (1975) and the crude protein content was calculated by multiplying total nitrogen concentration by factor of 5.75. Identification and determination of the amino acid composition of barely grains was carried out by using HPLC (Eppendorf, Germany) according to Gehrke *et al.* (1985).

Statistical analysis:

Data was analyzed using an Analysis of variance of Randomized Complete Block Design (MSTAT-C v. 3.1., 1988). Since the trend was similar in both seasons, Bartlett's test was applied and the combined analysis of the two growing seasons was done. LSD ($P < 0.05$) was used to compare treatment means.

Results And Discussion

Growth parameters:

Data presented in Table (3) show that plant height, fresh and dry weight of plants/m², succulence and leaf area, were significant positively affected by increasing the hemin concentration from 0 to 100 μ mol. Application of 50 μ mol hemin concentration recorded the highest values in most studied characters with significant differences in plant height, fresh and dry weight of plants/m² and leaf area ,while the highest value of succulence was recorded by the hemin concentration at 75 μ mol without significance differences between different hemin concentration in this last character.

Table 3: Effect of hemin concentration on some growth characters of barley plants irrigated with saline water at 60 days after sowing. (Combined data of both seasons).

Character		Plant height (cm)	Weight (g/m ²)		Succulence	LA (cm ²)
Treatment			Fresh	Dry		
Hemin concentration (μ mol)	0.0	62.00	652.00	212.00	3.13	7.76
	50	79.80	1002.67	338.67	2.97	12.32
	75	74.40	908.00	307.92	2.98	10.45
	100	66.40	655.33	242.00	2.72	9.25
	F-Sig.	**	**	**	NS	**
LSD at 5%		2.86	83.72	31.65	NS	0.70
CV		3.22	8.27	9.14	16.42	5.56

Ns, **, non significant and statistically different at 1% level, respectively.

Among the most important factors responsible of retarded growth, under the influence of salinity, is the decline in photosynthetic performance (Saffan, 2008). Thus, salinity usually causes a reduction in the leaf area which generally leads to a drastic reduction in net CO₂ assimilation (Franco *et al.* 1991 and Benzioni *et al.* 1992). The increased thickness of the leaf under salinity stress may also reduce the photosynthetic performance via decrease of CO₂ diffusion in the mesophyll cells (Laisk & Loreto 1996 and Lauteri *et al.* 1997). The osmotic effect resulting from soil salinity may cause disturbances in the water balance of the plant (Saffan, 2008), including a reduction of turgor and on inhibition of growth, as well as stomatal closure and reduction of photosynthesis (Poljakoff Mayber 1982). At the whole plant level the effect of stress is usually perceived as a decrease in photosynthesis and growth (Rahdari and Hoseini 2012), and is associated with alteration in carbon and nitrogen metabolism (Yordanov *et al.*, 2003).

Application of hemin increased growth parameters under saline conditions as compared with the control treatment. These results are in good harmony with those obtained by Hotta *et al.* (1997a) and Watanabe *et al.* (2006) who found that application of ALA at low concentrations increased the growth over the control on kidney bean, barley, potato and garlic plants. These authors assumed that the higher production was related to an increase in photosynthetic rate and CO₂ fixation and reduced release of CO₂ in darkness. Moreover Xu *et al.*, 2010 found that application of ALA increased growth of kudzu plant through increasing leaf chlorophyll concentration, photosynthetic rate and stomatal conductivity.

Chemical composition of shoot:

Data presented in Table (4) show significant differences among different spraying of hemin concentrations in macro (Ca, Mg, K and Na) and micro (Fe, Mn and Zn) nutrients of barley shoot at 60 days after sowing. The untreated plant (zero hemin) contained greater Na, Mn and Zn elements while, spraying plants with 50 μ mol hemin gave the highest value of Ca, Mg, K and Fe compared to other treatments. Generally, shoot Na contents decreased gradually with increasing hemin concentration from 0 to 100 μ mol. Meanwhile, the most of macro and micro elements decreased after 50 μ mol.

Table 4: Effect of hemin concentration on some chemical composition of barley shoot grown under saline condition at 60 days after sowing in 2011/12 season

Character		Macronutrients (Mg/100g)				Micronutrients (ppm)		
Treatment		Ca	Mg (ppm)	K	Na	Fe	Mn	Zn
Hemin concentration (μ mol)	0.0	1.02	4.63	1.53	2.37	0.75	0.83	0.05
	50	1.03	4.80	1.80	2.25	0.84	0.82	0.03
	75	0.67	4.53	1.36	1.92	0.57	0.80	0.03
	100	0.50	3.74	1.35	1.66	0.45	0.80	0.03
F-Sig.		**	*	**	**	**	**	**
LSD at 5%		0.08	0.64	0.22	0.29	0.03	0.01	0.01
CV		4.71	7.22	7.34	7.02	1.98	0.61	14.63

Ns, **, non significant and statistically different at 1% level, respectively.

The increases in the Na, Mg, Mn and Zn in the untreated plants are considered a normal result for increasing this element in the irrigation water (Table 2). The effect of high ambient NaCl concentrations on the internal concentrations of macronutrient elements have been extensively studied. Na, K and Cl have been measured, most not ably those of Ca, N and P because these nutrients are of interest and required in large quantities to sustain adequate plant growth and their concentrations in the soil are often limiting to plant growth. Given that the dominant salt in saline soils is NaCl, both Na⁺ and Cl⁻ ions will occur in high concentrations. However, the contribution of Cl⁻ to growth reduction under salt stress is less well understood than that of Na⁺ in broad acre crops. This reflects the fact that most research on salt tolerance in cereals has focused on Na⁺ with little regard to Cl⁻ toxicity (Teakle and Tyerman, 2010). Both Na⁺ and Cl⁻ should be given equal consideration since they are both metabolically toxic to plants if accumulated at high concentrations in the cytoplasm (Tavakkoli *et al.*, 2010a, b).

Hemin application decreased the uptake of Ca, K, Na, Fe, Mn, and Zn in plant shoot under saline condition as compared with control plant. This may be as a result of osmoregulation process of hemin as growth regulators. This result support the findings of Zhang *et al.* (2006) who showed that ALA increased cold and salt tolerance in rice and potato, and added that this compound therefore appears to act as a hormone-like plant growth regulator, which is effective at relatively low concentrations (10–100 mg/L). The increase in Mg as a result of hemin application at 75 and 100 μ mol is considered a normal result because hemin is a precursor of chlorophyll which has Mg as central atom (Fig 1).

Yield attributes:

Data presented in Table (5) show the effect of hemin treatment on yield characters (plant height, number of spikes/m², weight of spike, grains weight/spike and number of spikelets/spike), grown under saline condition. Increasing hemin concentrations significantly increased all yield attributes as compared with the untreated control. However, application of 50 μ mol hemin concentration gave the highest values of plant height (75.17 cm), number of spikes/m² (450), weight of spike (2.22 g) and grain weight/spike (1.90 g), number of spikelets/spike (17.80) and 100 grain weight (11.62 g) compared with other concentrations.

Table 5: Effect of hemin concentration on barley yield attributes grown under saline condition at 60 days after sowing (combined data of both seasons)

Character		Plant height (cm)	Spike (s) character				Spikelets no/spike	100 grain weight (g)
Treatment			number/m ²	Length (cm)	Weight (g)	Grains weight (g)		
Hemin concentration (μ mol)	0.0	64.40	360.00	13.80	1.81	1.43	13.80	11.23
	50	75.17	450.00	15.20	2.22	1.90	17.80	11.62
	75	73.60	414.00	14.70	2.07	1.73	17.20	11.59
	100	71.40	408.00	14.40	1.92	1.59	17.00	11.30
	F-Sig.	**	**	NS	**	**	**	NS
LSD at 5%		5.21	22.47	1.16	0.10	0.04	1.74	0.41
CV		5.82	4.38	6.35	3.71	2.22	8.42	2.85

Ns, **, non significant and statistically different at 1% level, respectively.

The lowest yield attributes of barley plants under the untreated treatment reflected the detrimental effect of salinity of the irrigation water (Table 2). This also closely related to the decrease in growth parameters showed in table 3. The high mineral ion content of macro and micronutrient in control plants (0 hemin) recorded in table 4 could be used as indicator to osmotic imbalance which reflected on the decrease in yield attributes in barley plants grown under salinity conditions. This connection, Ahmad *et al.* (2003), reported that the increase of sodium chloride and sodium sulfate concentration resulted in the reduction of number of tillers, length of spike, number of spikelets per spike, biomass per plant and grain yield per plant. Moreover, salinity is one of the most serious stress factors that limit crop production. It can disrupt the plants' metabolic functions and can be easily noticed on the entire plant subsequently leading to decrease in productivity or even plant death.

Yield (ton fed⁻¹):

Data presented in Table (6) show effect of increasing hemin concentration on grain, straw and biological (ton/fed) as well as harvest and crop index. The data revealed significant increase on grain, straw and biological, when hemin concentration increased from 0 to 100 μmol as compared with the untreated control plant (0 hemin). Harvest and crop indexes increased with insignificant values by increasing hemin concentration up to 75 μmol.

Table 6: Effect of different hemin concentration on grain, straw and biological yield/fed of barley grown under saline conditions (combined data of both seasons)

Character		Yield (ton fed ⁻¹)			Harvest index (%)	Crop index (%)
Treatment		Grain	Straw	Biological		
Hemin concentration (μ mol)	0.0	1.20	3.65	4.86	24.74	32.96
	50	1.59	4.76	6.36	25.15	33.71
	75	1.55	4.65	6.20	25.03	33.51
	100	1.39	4.62	6.01	22.45	29.02
	F-Sig.	**	**	**	NS	NS
LSD at 5%		0.13	0.40	0.39	2.63	4.65
CV		7.27	7.07	5.19	8.58	11.49

Ns, **, non significant and statistically different at 1% level, respectively.

The low values of economic yields (grain straw and biological) in the untreated plants (0 hemin) may be attributed to the increased irrigation water salinity (Table 2), the decrease in growth criteria (Table 3) and the decrease in the yield attributes (Table 5). In this regard considerable decrease in grain yield of cereals following the irrigation with saline water has been observed. The negative effects of salinity on growth were reported by Hamdy *et al.*, (2005). Excessive salts injure plants by disturbing the uptake of water into roots and interfering with the uptake of competitive nutrients (David Franzen, 2007). Soil salinity is a considerable problem adversely affecting physiological and metabolic processes, finally diminishing growth and yield (Ashraf and Harris, 2004). Recently, Turki *et al.* (2012) concluded that salt treatment (100 mM of NaCl solution) depressed growth and yield production in 45 common and durum wheat varieties. The reduction in grain yield might be caused by the salinity, which induced reduction of photosynthetic capacity leading to less starch synthesis and accumulation in the grain.

Increasing yield attributes and economic yields as a result of hemin application may be attributed to the increase in plant growth (Table 3) and decreasing uptake on mineral ions specially K, Na. It also may be due to increasing Mg concentration (Table 4) which accompanied with increasing photosynthetic pigment. In this connection, Hotta *et al.* (1997a) and Watanabe *et al.* (2006) found that the yield of plants, including barley, garlic, kidney bean, potato, radish and grape, could be improved by 10-60% by ALA foliar treatment at low concentration (30-300 mg/L). Moreover, Xu *et al* (2010) concluded that, this study has provided interesting results on the positive impact of spraying with low concentrations of ALA on growth and chemical composition and yield of kudzu plants.

Chemical composition of yielded barley grains:

Macro and micro elements:

Data presented in Table (7) illustrate the effect of increasing the hemin concentration from 0 to 100 μ mol on some macro (Ca, Mg, K and Na), micro (Fe, Mn and Zn) nutrients and grain protein content. The data show insignificance effects on the most of the studied characters by increasing hemin concentration except Mg, Na and Fe. Treatments of 50 and 75 μ mol of hemin (without significant between them) recorded the highest values of Ca, Mg, K and Fe concentrations. Also, hemin application at 50 μ mol recorded the highest value (without significant difference) of protein percentage as compared with other treatments.

The low protein values of yielded barley grains grown under saline conditions reflect the inhibitory effect of salinity on plant growth, yield and its attributes (Tables 3,4,5). Consequently, concentration of protein was not clearly affected in the control plants. These results are confirmed the results obtained by Turki *et al* (2012) who found that the decrease in grain yield of wheat and durum might be caused by the salinity, which induced reduction of photosynthetic capacity leading to less starch synthesis and accumulation in the grain. In addition the results showed that salt accumulation increased protein content in five varieties and one accession of durum wheat. This variation maybe related to the relatively stable nitrogen metabolism under salt stress, which might contribute to the higher protein concentration.

Table 7: Effect of increasing hemin concentration on chemical composition of yielded barley grains grown under saline conditions in 2011/12 season

Character		Macronutrients (Mg/100g)				Micronutrients (ppm)			Grain protein (%)
Treatment		Ca	Mg (ppm)	K	Na	Fe	Mn	Zn	
Hemin concentration (μ mol)	0.0	0.15	2.67	0.35	0.99	0.22	0.83	0.06	14.05
	50	0.16	2.80	0.38	0.90	0.35	0.81	0.05	16.00
	75	0.19	2.84	0.43	0.84	0.69	0.81	0.05	14.70
	100	0.17	2.82	0.43	0.80	0.44	0.81	0.05	14.00
	F-Sig.	NS	*	NS	*	**	NS	NS	NS
LSD at 5%		NS	0.12	NS	0.12	0.11	NS	NS	NS
CV		15.75	2.15	9.3	6.72	12.57	2.25	18.82	5.02

NS, *, **, non significant and statistically different at 5% and 1% level, respectively.

The hormone like growth regulators effect of hemin in alleviating the harmful effect of salinity may be reflected in the mineral contents of yielded grains and the grain protein percent which appeared slight increase as compared with control plant (0 hemin). In this connection Hotta *et al.* (1998) and Zhang *et al.* (2006) showed that ALA increased cold and salt tolerance in rice and potato. This compound therefore appears to act as a hormone-like plant growth regulator, which is effective at relatively low concentrations (10–100 mg/L).

Amino acid contents:

Data presented in Table 8 showed the effect of spraying barley plants grown under saline conditions with different concentrations of hemin. The results cleared that, proline (non-essential) and arginine (essential) contents decreased gradually with increasing hemin concentration up to 100 mol. Reverse the trend was recorded in the lysine contents. Contents of others amino acids increased under different hemin concentration compared to untreated plant. The results also cleared that spraying barley with 50 μ mol hemin recorded the highest value of the most of determined amino acids. Regarding total essential and non-essential amino acids, the results cleared that all hemin concentrations resulted in decrease in essential, non essential and total amino acid contents as compared with control plants (0 hemin). At the meantime the ratio of essential to non essential amino acids were increased in response to hemine concentration.

The high amount of proline in control grains (0 hemin) grown under saline condition exhibit the suitable way for plant to overcome the deleterious effect of salinity. It is worthy to note that proline concentration was decreased nearly to the half of that recorded when barley plants treated with 100 μ mol of hemin indicating the metigative and protective role of hemin in counteraction salinity of irrigation water. These results are in a good harmony with those obtained by Sakamoto and Murata, (2000); Shabala and Cuin, (2006). They concluded that, metabolic acclimation via the accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress. Many plant species accumulate significant amounts of glycine betaine, proline, and polyols in response to high salinity (Rhodes and Hanson, 1993; Bohnert *et al.*, 1995; Di Martino *et al.*, 2003). Stress occurs from two ways due to increase of proline, which includes increasing synthesis of proline enzymes levels and decreasing of destructive proline enzymes actions (Rontein *et al.*, 2002). Amino acid proline resulted from proteins degradation whose response to stress is due to its compatibility with osmosis.. Recently, it was also shown that some of these compatible solutes are very efficient

in reducing the extent of K^+ loss in response to both salinity (Cuin and Shabala, 2005) and oxidative stress (Cuin and Shabala, 2007) in barley.

Table 8: Effect of different hemin concentrations on amino acid contents (g/100g dry weight) barley grains grown under saline conditions in 2011/2012 season.

Treatment		Hemin concentrations (μ mol)		
Amino acids	Control	50	75	100
Aspartic	0.51	0.56	0.57	0.54
Threonine	0.25	0.33	0.28	0.23
Serine	0.45	0.57	0.49	0.47
Glutamic A	2.73	2.88	2.76	2.70
Proline	13.84	9.35	8.18	6.93
Glycine	0.65	0.81	0.77	0.76
Alanine	0.58	0.65	0.63	0.60
*Valine	0.60	0.65	0.65	0.61
*Isoleucine	0.35	0.37	0.35	0.32
*Leucine	0.73	0.83	0.81	0.80
*Phenylalanine	0.28	0.36	0.35	0.35
*Histidine	0.21	0.24	0.21	0.20
*Lysine	0.26	0.28	0.29	0.32
Ammonia	0.55	0.84	0.71	0.66
*Arginine	6.62	6.31	6.19	5.95
*Essential	9.06	9.05	8.86	8.55
Non-Essential	19.00	15.14	13.70	12.23
Total amino acid	28.05	24.18	22.55	20.78
Ess./Non-Ess. ratio	0.48	0.60	0.65	0.70

Conclusion:

Barley plants grown under saline condition exhibited several behaviors which reflect the deleterious effect of salinity on plant growth, macro and micronutrient, yield attributes and economic yields and nutritional values of produced grains. External application of different hemin concentrations showed positive result on all studied parameters. Barley plants treated with hemin showed metigative and protective role in counteraction salinity of irrigation water. So, hemin application is a promising in alleviating salinity stress effects.

References

A.O.A.C., 1990. Association of Official Agricultural Chemists, Official methods of analysis. 12th ed., Washington, D. C.

Abo-Hamed, S.A., M.E. Younis, O.A. EL-Shahaby and A.H. Ibrahim, 1999. Osmotic adustment and water relations of three Sorghum cultivars subjected to water relations deficit at two stages of plant growth. J.Union Arab Biol. Cairo., 7(B): 191-207.

Ahmad, A.N., U.H.J. Intshar, A. Shamshad and A. Muhammad, 2003. Effects of Na, SO and NaCl salinity levels on different yield parameters of barley genotypes. Intl. J. Agric. Biol., 5(2): 157-159.

Al-Hakimi, A.M.A., 2000. Interactive effects of Ca+2 and NaCl salinity on gas exchange and growth of *Vicia faba*. J. Union Arab Biol., Cairo., (8B): 33-43.

Almansouri, M., J.M. Kinet and S. Lutts, 1999. Compared effects of sudden and progressive impositions of salt stress in three durum wheat (*Triticum durum* Desf.) cultivars. Journal of Plant Physiology, 154: 743-752.

Ashraf, I. and N. Harris, 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3-6

Babu, S., A. Sheeba, P. Yogameenakshi, J. Anbumalaramathi and P. Rangasamy, 2007. Effect of salt stress in the selection of salt tolerance hybrids in rice (*Oryza Sativa* L.) under *in vitro* and *in vivo* condition. Asian. J. Plant Sci., 6(1): 137-142.

Beale, S.I. and J.D. Weinstein, 1990. Tetrapyrrole metabolism in photosynthetic organisms. In HA Daily, ed, Biosynthesis of Heme and Chlorophyll. McGraw-Hill, New York, pp: 287-391.

Benzioni, A., A. Nerd, Y. Rosengartner and D. Mills, 1992. The effect of NaCl salinity on growth and development of Jojoba clones: I. Young plants. Plant Physiol., 139: 731-736.

Bohnert, H.J., D.E. Nelson and R.G. Jensen, 1995. Adaptations to environmental stresses. The Plant Cell 7, 1099-1111.

Chapman, H.D. and R.F. Pratt, 1978. Methods analysis for soil, plant and water. Univ. of California Div. Agric. Sci., 16-38.

Chen, T.H.H. and N. Murata, 2002. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Current Opinion in Plant Biology, 5: 250-257.

Cottenie, A., M. Verloo, L. Kiekens, G. Velgh and R. Camerlynch, 1982. Chemical analysis of plants and soils, Lab, Anal Agrochem. State Univ. Ghent Belgium, 63.

Cuin, T.A. and S. Shabala, 2005. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley root. *Plant and Cell Physiology*, 46: 1924-1933.

Cuin, T.A. and S. Shabala, 2007. Amino acids regulate salinity-induced potassium efflux in barley root epidermis. *Planta*, 225: 753-761.

David Franzen, 2007. Salt accumulation processes. North Dakota state Univ., Fargo ND 58105.

Di Martino, C., S. Delfine, R. Pizzuto, F. Loreto and A. Fuggi, 2003. Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. *New Phytologist*, 158: 455-463.

Dolphin, D., 1994. Photomedicine and photodynamic therapy. *Can J Chem*, 72: 1005-1013.

Dumbroff, E.B. and A.W. Cooper, 1974. Effect of salt stress applied in balanced nutrient solutions a several stages during growth tomato. *Bot. Gaz.*, 135: 219-224.

Eppendorf, N. and G. Hing, 1970. Interaction manual of flame photometer B 700-E. Measuring method, Description of the apparatus and Instructions for use.

FAO., 2004. <http://apps.fao.org/faostat/default.jsp>, accessed February 2004.

Fowler, C.W. and D.C. Rasmusson, 1969. Leaf Area Relationships and Inheritance in Barley. *Crop Science*, 9(6): 729-731.

Franco, A.C., E. Ball and V. Luttge, 1991. The influence of nitrogen, light and water stress on 2 CO exchange and organic acid accumulation in 3 the tropical C -CAM tree, clusia minor. *J. Exp. Bot.*, 42: 597-603.

Gehrke, C.W., L.L. Wall, J.S. Absheer, F.E. Kaiser and R.W. Zumwolt, 1985. Sample preparation for chromatography of amino acids: acid hydrolysis of proteins. *J. Assoc. off. Anal. Chem.*, 68: 811-816.

Granick, S., 1961. Magnesium protoporphyrin monoester and protoporphyrin monomethyl ester in chlorophyll biosynthesis. *Journal of Biology and Chemistry*, 236: 1168-1172.

Hamdy, A., V. Sardo and A.F. Ghanem, 2005. Saline water in supplemental irrigation of wheat and barley shoots. *Egypt J. Agron.*, 28(2): 46-56.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology*, 51: 463-499.

Heuer, B., 2003. Influence of exogenous application of proline and glycine betaine on growth of salt-stressed tomato plants. *Plant Science*, 165: 693-699.

Hotta, Y., T. Tanaka, L. Bingshan, Y. Takeuchi and M. Konnai, 1998. Improvement of cold resistance in rice seedlings by 5-aminolevulinic acid. *Journal of Pesticide Science*, 23: 29-33.

Hotta, Y., T. Tanaka, H. Takaoka and Y. Takeuchi, 1997b. New physiological effects of 5-aminolevulinic acid in plants: the increase of photosynthesis, chlorophyll content, and plant growth. *Bioscience, Biotechnology, and Biochemistry*, 61: 2025-2028.

Hotta, Y., T. Tanaka, H. Takaoka, Y. Takeuchi and M. Konnai, 1997a. Promotive effects of 5-aminolevulinic acid on the yield of several crops. *Plant Growth Regulation*, 22: 109-114.

Jackson, M.L., 1967. Soil chemical analysis. Constable and Co. Ited., London.

Jung, H.J., Y. Lee, K. Kang, Y.S. Kim, B. Grimm and K. Back, 2008. Toxic tetrapyrrole accumulation in protoporphyrinogen IX oxidase-overexpressing transgenic rice plants. *Plant Mol Biol*, 67: 535-546.

Jung, Y., K. Yang, S.B. Lee, S.M. Jang, S.B. Ha and K. Back, 2004. Dual targeting of *Myxococcus xanthus* protoporphyrinogen oxidase into chloroplasts and mitochondria and high-level oxyfluorfen resistance. *Plant Cell Environ.*, 27: 1436-1446.

Kariola, T., G. Brader, J. Li and E.T. Palva, 2005. Chlorophyllase 1, a damage control enzyme, affects the balance between defense pathways in plants. *Plant Cell*, 17: 282-294.

Laisk, A. and F. Loreto, 1996. Determining 2 photosynthetic parameters from leaf CO exchange and chlorophyll fluorescence. Rubisco specificity factor, dark respiration in the light, excitation distribution between photosystems, alternative electron transport rate and mesophyll diffusion resistance. *Plant Physiol.*, 110: 903-912.

Lauteri, M., A. Scartazza, M.C. Guido and E. Brugnoli, 1997. Genetic variation in photosynthetic capacity, carbon isotope discrimination and mesophyll conductance in provenances of *Castanea sativa* adapted to different environments. *Function Ecology*, 11: 657-683.

Marschner, H., 1995. Mineral nutrition of higher plants. London Academic Press, pp: 889.

Mass, E.V. and G.J. Hoffman, 1977. Crop salt tolerance of tolerance current assessment. ASCEJ. Irr. Drainage Div., 103: 115-135.

Meloni, D.A., M.A. Oliva, H.A. Ruiz and C.A. Martinez, 2001. Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. *Journal of Plant Nutrition*, 24: 599-612.

MSTAT-C, 1988. A microcomputer program for the design, management and analysis of agronomic research experiments. Michigan State University

Phung, T.H., H. Jung, J.H. Park, J.G. Kim, K. Back and S. Jung, 2011. Porphyrin Biosynthesis Control under Water Stress: Sustained Porphyrin Status Correlates with Drought Tolerance in Transgenic Rice. *Plant Physiol.*, 157(4): 1746-1764.

Poljakoff-Mayber, A., 1982. Biochemical and physiological responses of higher plants to salinity stress. In Biosaline research. A look to the future (A. San Prieto, ed.). PP. 245-270. Plenum press New York, N.Y. ISBN O-306-40892-9.

Rahdari, P. and S.M. Hoseini, 2012. Drought Stress: A Review International journal of Agronomy and Plant Production., 3(10): 443-446.

Reinbothe, S., C. Reinbothe, K. Apel and N. Lebedev, 1996. Evolution of chlorophyll biosynthesis: the challenge to survive photooxidation. *Cell* 86: 703-705.

Rhodes, D. and A.D. Hanson, 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology*, 44: 357-384.

Rontein, D., G. Bassett and A.D. Hanson, 2002. Metabolic engineering of osmoprotectant accumulation in plants. *Metabolic Engineering*, 4: 49-56.

Saffan, S.E., 2008. Effect of Salinity and Osmotic Stresses on Some Economic Plants Research Journal of Agriculture and Biological Sciences, 4(2): 159-166.

Sairam, R.K. and A. Tyagi, 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.*, 86: 407-412.

Sakamoto, A. and N. Murata, 2000. Genetic engineering of glycine betaine synthesis in plants: current status and implications for enhancement of stress tolerance. *Journal Experimental Botany*, 51: 81-88.

Senge, M.O., 1993. Recent advances in the biosynthesis and chemistry of chlorophylls. *Photochemistry and Phytobiology*, 57: 189-206.

Shabala, S and T.A. Cuin, 2006. Osmoregulation versus osmoprotection: re-evaluating the role of compatible solutes. In: Teixeira da Silva J, ed. *Floriculture, ornamental and plant biotechnology –advances and topical issues*. Tokyo, Japan: Global Science Books, pp: 405-416.

Stobart, A.K. and J. Ameen-Bukhari, 1984. Regulation of δ -aminolevulinic acid synthesis and protochlorophyllide regeneration in the leaves of dark-grown barley (*Hordeum vulgare*) seedlings. *Biochemical Journal*, 222: 419-426.

Storey, R. and R.G. Wyn Jones, 1978. Salt stress and comparative physiology in the Gramineae. III. The effect of salinity upon the ion relations, and glycinebetaine and proline levels in *Spartina x townsendii*. *Aust. J. Physiol.*, 5: 831-838.

Taghipour, F. and M. Salehi, 2008. The Study of Salt Tolerance of Iranian Barley (*Hordeum vulgare* L.) Genotypes in Seedling Growth Stages American-Eurasian J. Agric. & Environ. Sci., 4(5): 525-529.

Tavakkoli, E., P. Rengasamy and G.K. McDonald, 2010a. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *Journal of Experimental Botany*, 61: 4449-4459.

Tavakkoli, E., P. Rengasamy and G.K. McDonald, 2010b. The response of barley to salinity stress differs between hydroponics and soil systems. *Functional Plant Biology*, 37: 621-633.

Teakle, N.L. and S.D. Tyerman, 2010. Mechanisms of Cl^- transport contributing to salt tolerance. *Plant, Cell and Environment*, 33: 566-589.

Tiku, G.L., 1975. Ecophysiological aspects of halophyte zonation *Plant and Soil*, 43: 355.

Turki, N., M. Moncef Harrabi and K. Kazutoshi Okuno, 2012. Effect of Salinity on Grain Yield and Quality of Wheat and Genetic Relationships among Durum and Common Wheat, *Journal of Arid Land Studies*, 22-1: 311-314.

Valenzano, D.P., 1987. Photomodification of biological membranes with emphasis on singlet oxygen mechanisms. *Photochem Photobiol.*, 46: 147-160.

Watanabe, K., E. Nishihara, S. Watanabe, T. Tanaka, K. Takahashi and Y. Takeuchi, 2006. Enhancement of growth and fruit maturity in 2-year-old grapevines cv. Delaware by 5-aminolevulinic acid. *Plant Growth Regulation*, 49: 35-42.

Wu, D., S. Cai, M. Chen, L. Ye, Z. Chen, 2013. Tissue Metabolic Responses to Salt Stress in Wild and Cultivated Barley. *PLoS ONE* 8(1): e55431. doi:10.1371/journal.pone.0055431.

Xu, F., J. Zhu, S. Cheng, W. Zhang and Y. Wang, 2010. Effect of 5-aminolevulinic acid on photosynthesis, yield, nutrition and medicinal values of kudzu (*Pueraria phaseoloides*) Tropical Grasslands., 44: 260-265 260.

Yao, N. and J.T. Greenberg, 2006. *Arabidopsis* ACCELERATED CELL DEATH2 modulates programmed cell death. *Plant Cell*, 18: 397-411.

Yordanov, I., V. Velikova and T. Tsonev, 2003. Plant responses to drought and stress tolerance. *Bulg. J. Plant Physiol.*, pp: 187-206.

Yousufinia, M., A. Ghasemian, O. Safalian, A. Asadi, 2013. The effect of NaCl on the growth and Na⁺ and K⁺ content of barley (*Hordeum vulgare*, L.) cultivars Annals of Biological Research, 4(1): 80-85.

Zhang, Z.J., H.Z. Li, W.J. Zhou, Y. Takeuchi and K. Yoneyama, 2006. Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers in vitro. Plant Growth Regulation, 49: 27-34.

Zhu, J.K., 2001. Cell signalling under salt, water and cold stresses. Current Opinion in Plant Biology, 4: 401-406.

Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. Annu Rev Plant Physiol, 53: 247-273.

Zhu, J.K., 2003 Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol., 6: 441-445.

Tiku, G.L., 1975. Ecophysiological aspects of halophyte zonation Plant and Soil, 43: 355.

Jackson, M.L., 1967. Soil chemical analysis. Constable and Co. Ited., London.