الإستجابة الفسيولوجية و التشريحية لنباتات قمح مجهدة ملحياً للمجال المغناطيسى

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الملخص العربى

بهدف دراسة التغيرات الفسيولوجية و التشريحية لنباتات القمح (.Triticum aestivum L) صنف سخا ٩٣ المعرضة لمجال مغناطيسي تحت ظروف الإجهاد الملحي، تم إجراء تجربتي أصص في الصوبة الزجاجية في مزرعة التجارب بكلية الزراعة – جامعة المنوفية – شبين الكوم –مصر و ذلك خلال الموسم (٢٠١١/٢٠١٠)، تم أخذ العينات النباتية بعد ١٠٠ يوم من الزراعة.

و أوضحت النتائج المتحصل عليها أن معاملات المغنطة المختلفة (بذور ممغنطة – الري بماء ممغنط – بذور ممغنطة مع الري بماء ممغنط معاً) تسبب زيادة واضحة في نمو النباتات و بعض الصفات الفسيولوجية و الكيماوية مثل (العلاقات المائية و نفاذية الأغشية و البرولين الكلي و بعض الهرمونات النباتية) تحت تأثيرمستوى الملوحة (١٠ ديسيمينز/متر) مقارنة بالكنترول. الصفات التشريحية للساق مثل (سمك الساق، سمك الفجوة، عدد الحزم الوعائية خلال القطاع، سمك الحزم الوعائية و سمك الأوعية الخشبية) و بعض الصفات التشريحية للورقة مثل (سمك الورقة، سمك العرق الوسطي، سمك الأوعية الخشبية) لنباتات القمح تحسنت بوضوح بالمعاملة بمعاملات المغنطة المختلفة و تداخلهم مع مستوى الملوحة (١٠ ديسيمينز/متر)، بينما كان هناك نقص ملحوظ في نقص الماء الورقي و معدلات نتح و تركيز هرمون الأبسيسيك أسيد (المثبط) في النباتات. و كان تأثير معاملة الماء الممغنط أكثر وضوحاً في تطور و نمو النبات.

PHYSIO-ANATOMICAL RESPONSES OF SALINITY STRESSED WHEAT PLANTS TO MAGNETIC FIELD

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ABSTRACT: In order to investigate the physiological and anatomical changes of wheat plants (Triticum aestivum L. cv. Sakha 93) exposed to magnetic field under salinity conditions. Pot experiment was carried out in a greenhouse at the Experimental Farm of Faculty of Agriculture, Menofiya University, Shibin El-Kom, Egypt during the growing season of 2010/2011. Plant samples were taken 100 days of sowing. The obtained results of magnetic treatment (magnetized seeds, magnetized water and the combination of magnetized seed and water treatments) showed that plant growth and some physiological, biochemical characters i.e. (water relations, membrane integrity, total proline and endogenous phytohormone) were significantly increased at salinity level (10 dS/m) compared to the control. The anatomical stem parameters i.e. (stem diameter, stem cavity diameter, number of vascular bundle/cross section, vascular bundle diameter and vessel diameter] and the anatomical leaf parameters i.e. [lamina thickness, midrib thickness, midrib vascular bundle diameter and vessel diameter] of wheat plant were markedly enhanced by the different magnetic treatments and their combination at salinity level (10 dS/m) compared to the control, while there were a remarkable decreases in leaf water deficit, transpiration rate and the concentration of ABA in plant shoot. Generally, the effect of magnetized water treatment was more pronounced in the plant development.

Key words: Magnetic water, wheat plants, growth, water relations, chemical constituents, phytohormones, anatomical.

INTRODUCTION

Usage of magnetic field (MF) to improve the crop productivity is a recent technology which gives better plant growth and yield than chemical fertilizers (Shine et al., 2011). The changes determined at the field strength and periods of MF with the lowest frequency have also been drawn the attention of the biologists, molecular biologists, chemists and physicists (Atak et al., 2003). Magnetic treatments enhanced the biochemical processes and various functions in plants like chloroplast properties and mRNA quality (Goodman et al., 1995), which stimulate the production of proteins and activity of enzymes, and thus some studies reported that MF had a positive effect on the growth, nutrient and water uptake [Vashisth and Nagaraja (2010)].

It is common knowledge that salinity inhibits plant growth through its effect on the metabolism and biochemical changes that being occur during plant life. The direct effect of salinity may be attributed to the accumulation of levels of toxic ions; however the indirect effect is due to the high osmotic pressure of salts on many crops i.e. bean, tomato, rice and wheat (Munns, 2006).

Wheat considered the first strategic food crop in Egypt. Cultivated area extended to reach 3.1 million feddan in 2011 growing season, yielded about 8.6 million ton, with an average of 2.8 ton/fed FAO (2011), while the wheat consumption are about 17.2 million ton, in turn there are a big gap between wheat consumption and production in Egypt. Since, self-sufficient of strategic cereal crop including wheat is a major target within the next ten year plan of Egyptian government. The use of modern technology to maximize the growing areas of wheat including salt soil either in old valley or new reclaimed lands is a great goal.

Therefore, the objective of this study to evaluate physiological, biochemical and anatomical changes of wheat plants exposed to magnetic field under salinity conditions.

MATERIALS AND METHODS

Pot experiment was performed in a greenhouse at the experimental Farm of Faculty of Agriculture, Menofiya University, Shibin El-Kom, Egypt during the season of 2010/2011. Salinity levels treatments [ECe 2.5 (control) and 10 dS/m], four magnetic treatments (tap water (control), magnetized seeds, magnetized water and combination of magnetized seed and water treatments) were used. The mixture of salts consists of MgSO₄, CaSO₄, NaCl, MgCl₂ and CaCO₃ in the ratio of 10: 1: 78: 2: 9, respectively. *Magnetic treatments were done as follows:*

- 1. Irrigation with normal tap water (control).
- 2. Magnetized seeds were passed through the magnetic funnel.
- 3. Irrigation with magnetized water by passing it through a magnetron.
- 4. Combination of magnetized seeds and magnetized water.

A magnetic tube used for treating the water was magnetron (model U.T.I, 1 inch diameter, output 4–6 m3/h) and a magnetic funnel for treating seeds, produced by Magnetic Technologies L.C.C., Russia, branch United Arab Emirates.

Wheat plants (*Triticum aestivum* L. cv. Sakha 93) were grown in a `greenhouse during the growing season of 2010/2011. Ten seeds were sown in each polyethylene pots. Four replicates per treatment were used of each treatment and arranged in a randomized complete block design. Plant samples were taken 100 after sowing date. Fertilization of N, P and K were added to the soil according to the Ministry of Agriculture recommendations.

The following characters were measured: 1-Growth analysis: Plant height, Leaf area using the formula of Aase (1978) and dry weights of whole plants.

2- Physiological parameters:

2.1. Water relations:

2.1.1. The total water in leaves were determined using the method described by

- Gosev (1960). Relative water content (RWC) and leaf water deficit (LWD) were calculated according to Kalapos (1994).
- 2.1.2. Osmotic pressure: calculated according to the method described by Gosev (1960).
- 2.1.3. Transpiration rate: determined using the method described by Kreeb (1990).
- 2.1.4. Membrane integrity (permeability): The percentage of electrolyte leakage was calculated according to Yan *et al.*, 1996.

3- Biochemical aspects:

- 3.1. Total concentration of proline in leaves (µg/g F.wt.) was determined according to Bates *et al.*, (1973).
- 3.2. Endogenous phytohormones: The endogenous phytohormones in the leaves of wheat plants were determined after 60 days from sowing according to the method described by Shindy and Smith (1975), while hormone analysis was performed using HPLC according to Crocier and Moritz (1999).

4-Anatomical parameters:

The effect of magnetic treatments under salinity conditions on the anatomical structure of leaf (lamina), and stem of wheat plants were studied 80 days of sowing before aging. Specimens 1cm long was taken from the fourth upper internode and the fourth upper leaf. Specimens were fixed in Formalin Alcohol Acetic acid mixture (FAA,1:18:1v/v), washed and dehydrated in alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52-54 1C m.p). The embedded specimens were sectioned on a rotary microtome at a thickness of 10-12 mm. Sections were mounted on slides. Staining was accomplished with safranine and lightgreen, clearedin xylol and mounted in Canada balsam (Culter et al., 2007). Slides were microscopically examined and the measurements and counts of wheat were taken and averages of 10 readings from 3 slides were calculated.

Statistical analysis: All data collected were subjected to the standard statistical

analysis following the proceeding described by Gomez and Gomez (1984) using the version 2.0 of (GENESTAT) program.

RESULTS AND DISCUSSION 1-Growth analysis:

It is clear from Table (1) that salinity level (10 dS/m) significantly decreased the plant height, leaf area and dry weight of wheat compared to the control. the deleterious effect with salinity stress on growth analysis may be due to increasing the osmotic pressure of the soil solution to a point with prevented the uptake of water (Abo-kassem, 2005), resulting in water stress and decreasing cell division and cell elongation of plant tissue as well as decreasing the intensity of photosynthesis (Munns et al., 2006) or may be attributed to salinity suppression of cell enlargement and cell division of leaves as well as an insufficient ion accumulation resulting from water deficit, which led to a decrease in leaf area (Nieman, 1965).

Magnetic treatments at salinity levels resulted in marked increase of plant height, leaf area and dry weight of wheat plants were increased if compared with the control. The increment was generally clearer in the plants treated with the combination of magnetized seeds and water in the most of obvious characters. Similar results were recorded by Radhakrishnan and Kumari (2012) stated that the growth characters of soybean plants were enhanced by magnetic treatments application as compared to untreated plants. Magnetized treatment showed the highest increase in the most of growth characters at salinity level (10 dS/m). The increases in growth of wheat caused by magnetic treatments may be due to the increasing of IAA and GA syntheses beside the decreases of the ABA synthesis as indicated in Table (4) and this may bring about promoting cell division and enlargement (Turker et al., 2007) or may be to increasing mineral uptake and this led to the earlier production in plants than that the untreated ones (Shalaby, 2008).

1- Water relations:

Data shown in Table (2) indicated that, salinity level (10 dS/m) led to a significant

decrease in total water content, relative water content and transpiration rate while leaf water deficit (LWD), osmotic pressure and membrane integrity were significantly increased. The same Findings recorded by Selim et al., (1996) on wheat. The harmful effect of salinity on the water relations in wheat plants may be due to the accumulation of toxic ions (Na⁺ and CI) (Hasegawa et al., 2000), salt in soil solution and excessive amounts of salt accumulate in leaves and reach toxic levels led to reduce plant capacity to take up water and lower the external water potential, leading to slower growth (Munns et al., 2006). The absorption of sufficient amounts of ions increased the internal osmotic pressure (Pasternak, 1987) or to the accumulation of organic substances such as sugars and amino acids especially proline (Wynjones, 1981). The adverse effect of salinity on the membrane integrity (permeability) might be due to the reduction in the concentration of Ca²⁺ where, high NaCl induces calcium deficiencies in tomato (Navarro et al., 2000).

Table (2) provided clear Data in evedence that, magnetized treatments caused a significant increase in total water content, relative water content, osmotic pressure and membrane integrity, while leaf water deficit LWD and transpiration rate were significantly decreased. The highest effect was occurred by combination of magnetized seeds and water followed by magnetized water compared each treatment alone. Moreover, it was obvious that, magnetic treatments reduced the harmful effect of salinity on water parameters in leaves of wheat plants. Generally, the interaction between salt level dS/m) and the combination Λf magnetized seeds and water recorded the maximum values. The results of the magnetic treatments effects on wheat plants may be due to increasing in water uptake and thus moderates heat in the plant. Magnetized water also characterized by their lower surface tension and which may lead to increase the minerals and nutrients inside the plant due to their greater water solubility (Eşitken and Turan, 2004), increased the concentration of proline which led to increase osmotic pressure in cells and

thus increases water uptake also, increased the enzymatic activity and plant metabolism so called "ponderomotive effects" Souza-Torres *et al.*, (2006). Water uptake is maximized by increasing vessels number, reducing the vessels size in stems (Sobrado, 2007). The reduction in level of Ca⁺² content according to the effects of

magnetic fields (MF) increased the membrane integrity by the direct effect on the calcium channels and pumps, and thus the excess Ca⁺² removal from cytosol to extracellular medium by Ca⁺², ATPase and/or Ca⁺²/H might be a reason to lower level of calcium in MF treated plants (Hajnorouzia *et al.*, 2011).

Table (1): The effect of magnetic treatments under salinity stress on some growth characters of wheat plants at 100 DAS during the season (2010/2011).

		plants at 100 B/10	y	
Characters		Plant height (cm)	Leaf area (cm2/plant)	Dry weight (g/plant)
Salinity (ECe dS/m)	Magnetic T.		(CITIZ/Plant)	Whole plant
	Control	49.00	164.68	0.89
2.5 (Control)	M. Seed	63.33	376.00	2.32
2.5 (Control)	M. Water	57.33	335.16	2.09
	M. S.×W.	74.00	383.88	2.37
	Control	28.00	131.72	0.30
10	M. Seed	37.00	187.30	0.79
	M. Water	39.67	204.35	0.89
	M. S.×W.	37.67	200.14	0.90
	Salinity	0.82	10.55	0.17
LSD 5%	Magnetic	0.73	9.44	0.15
	Sal. × Mag.	1.63	21.10	0.34

Table (2): The effect of magnetic treatments under salinity stress on water relations in leaves of wheat plants at 100 DAS during the season (2010/2011).

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Treatments Salinity (ECe dS/m)			Leaf water def. (%)	Relative water content (%)	Osmotic Pressure C.S. (bar)	Transpiration rate mg/cm2.h	M.I. %
	Control	69.61	55.51	44.49	7.10	1.21	12.71
2.5 (2	M. Seed	78.08	34.49	65.51	7.17	0.82	16.24
2.5 (Control)	M. Water	80.67	34.91	65.09	8.18	1.01	36.93
	M. S.×W.	83.01	28.53	71.47	8.11	1.06	22.48
	Control	57.84	60.28	39.72	10.36	0.76	28.36
10	M. Seed	74.69	40.34	59.66	10.43	0.74	35.48
	M. Water	76.81	39.11	60.89	10.51	0.74	29.80
	M. S.×W.	77.18	37.17	62.83	10.43	0.50	37.90
	Salinity	1.05	1.43	1.89	0.12	0.11	2.38
LSD 5%	Magnetic	1.72	2.97	2.97	0.13	0.07	2.13
	Sal. × Mag.	3.84	6.65	6.65	0.28	0.15	4.76

3- Biochemical aspects:

3.1. Proline concentration:

Data presented in Table (3) also revealed clearly that, increasing salinity to level (10 dS/m) resulted in pronounced increase of proline concentration. Similar results were obtained by Sakr et al., (2008) on wheat plants. Magnetic treatments application caused marked increment of proline concentration. Furthermore, magnetized seed showed the clearest effect on the total proline concentration under salinity stress. It would be suggested that the proline accumulation is due primarily to the stimulation of proline biosynthesis (Bull and Stewart, 1983) by an increase pyrroline-5 carboxylate reductase activity (Delauney and Verma, 1993). So for, it is not well proline reason understood the of accumulation under the effect of MF but it can be attributed to the MF influence on the permeability of the biological membranes (Goldsworthy, 2007), foster the proline synthesis protect the membranes from damage, thus improving the water status and growth of seedlings and minimizing the injury to membranes and slightly speeding the plant maturity.

3.2. Endogenous phytohormones:

The concentration of IAA, kinetin and GA_3 in shoot of wheat plants were significantly decreased, while a marked increase of ABA concentration occurred at salinity level (10 dS/m) Table (3). Similar

results were found by Seo *et al.*, (2005) on rice. This reduction in concentration of endogenous phytohormones under salinity stress might be attributed to the biosynthesis reduction or degradation enhancement under stress conditions, as suggested by Neumann *et al.*, (1990). Meanwhile, the ABA concentrations have been found to be correlated with leaf water status (Kannangara *et al.*, 1982) or the osmotic potential of the root (Ribaut and Pilet, 1991).

It is obvious in Table (3) that all magnetic applications bring a marked increase in the concentration of IAA, kinetin and GA3 in shoot of wheat plants, while incredible decrease ABA concentration were occurred compared to the control. The combination of magnetized seeds and water showed the highest effect. These results are in accordance with those reported by Turker et al., (2007) on sunflower plants. Results obtained too in Table (3) indicated that, magnetic treatments under salinity condition increased the concentration of IAA, kinetin and GA₃ in shoot plants, while the concentration of ABA was decreased as much as 65-75% of the control. The promotion effect of magnetic field to IAA content has beneficial increase consequences on (a) Stimulating RNA synthesis which encodes for enzymes responsible for increasing cell wall plasticity. (b) Increasing oxygen uptake. (c) Raising energy supply in the form of ATP. (d)

Table (3): The effect of magnetic treatments under salinity stress on the concentration of proline and plant phytohormones in shoot of wheat plants.

Treatments	Characters	Proline (µmol/g	Indole acetic acid (IAA)	Kinetin	Gibberellic acid (GA3)	Abscisic acid (ABA)	
Salinity(ECe dS/m)	Magnetic T.	"F.W.)	μg/100	g FW	mg/100g FW		
	Control	1.17	897.38	264.07	27.88	2.55	
2.5	M. Seed	2.62	967.29	303.43	45.43	1.20	
(Control)	M. Water	2.18	907.34	417.90	48.07	1.55	
	M. S.×W.	1.74	958.20	297.05	64.78	1.70	
	Control	4.23	421.78	193.12	19.75	7.12	
10	M. Seed	7.79	533.04	198.81	30.43	1.72	
	M. Water	4.67	781.26	261.19	43.69	2.01	

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M. S.×W.	6.55	473.12	203.16	42.89	2.46

Stimulates ethylene production. (e) Inducing root development and cell lengthening, as reported by (Guanglin and yaling, 1995). Moreover, Gibberellic acid had the ability to reduce the accumulation of Na⁺¹ in flag leaves of wheat (Tuna *et al.*, 2008), it had been accepted that there was a competition between sodium and potassium leading to reduced level of internal K⁺ at high Na⁺ concentration (Botella *et al.*, 1997), high concentration of magnesium, calcium or both elements inside the plant decreased the sodium absorption ratio in plants (Merhaut, 2007).

4-Anatomical structures:

The anatomical structures of stem in wheat plants i.e. [stem diameter, stem cavity diameter, number of vascular bundle/cross section, vascular bundle diameter and vessel diameter] and the anatomical characteristics of leaf in wheat plants i.e. [lamina thickness, midrib thickness, midrib vascular bundle and vessel diameter] were decreased at salinity level (10 dS/m) [Table, 4] and [fig. 1, 2, 3 and 4]. The same Findings were obtained by Farouk (2011) on wheat. A reduction in stem diameter due to reduction in thickness of cortex, vascular (phloem and xylem) tissue and the decrease in mesophyll tissue size and/or reduction

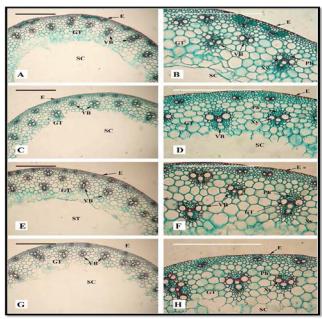
DNA content led to cell division and expansion.

All magnetic treatments bring a marked increase in the anatomical characteristics of stem and leaf in wheat plants. The highest increase in the stem anatomical characteristics occurred by magnetized water treatment, while magnetized seed showed the highest increase in leaf anatomical characteristics. Marked increases in the anatomical characteristics of stem and leaf in wheat plants were occurred under salinity level (10 dS/m). The magnetized water showed the treatment in the anatomical characteristics of stem, while the best increase on leaf occurred when the combination treatment were applied. The enhancing in anatomical structures of stem and leaf of wheat plant due to the magnetic treatments under or salinity condition may be normal attributed to the high level of cytokinins and IAA in those plants (Table, 3). MF may induce the cambium differentiation to vield xylem and phloem tissues, improve the absorption and conducting water to the growing organs, in addition improve the translocation of photoassimilate which led to increment in plant growth (Akram et al., 2002).

Table (4): Internal stem and leaves structure parameters of wheat plants as affected by magnetic treatments under salinity level during the growing season (2010/2011).

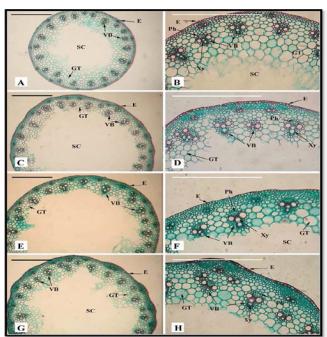
Treatments	Characters		Stem				Leaves			
Salinity (ECe dS/m)	Magnetic T.	Stem diameter (mm)	Stem cavity diameter (mm)	No. of vascular bundle / cross section	Vascular bundle diameter (µm)	Vessel diameter (µm)	Lamina Thickness (µm)	Midrib thickness (µm)	Midrib vascular bundle diameter (µm)	Vessel diameter (µm)
	Control	2.42	1.62	54	83.0	18.5	180	290	116.2	33.2
2.5	M. Seed	3.22	2.40	66	166.0	41.5	240	380	157.7	66.4
(Control)	M. Water	3.28	2.48	68	199.2	49.8	200	340	149.4	49.8
	M. S.×W.	2.80	2.02	58	149.4	49.8	190	320	124.5	37.4
10	Control	1.36	0.76	32	79.6	13.9	140	240	99.6	16.6
	M. Seed	1.84	1.28	52	132.8	26.6	200	280	108.4	19.9

M. Water	2.04	1.34	46	132.8	36.5	140	250	103.6	33.2
M. S.×W.	1.88	1.36	48	149.4	31.5	160	320	116.2	26.6



Salinity level 2.5 dS/m (Control)

Fig. (1): Stem cross sections of wheat plants (*Triticum aestivum* L. cv. Sakha 93) as affected by different magnetic treatments at salinity level (2.5 dS/m, control).



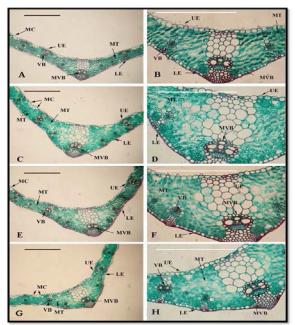
Salinity level 10 dS/m

Fig. (2): Stem cross sections of wheat plants (*Triticum aestivum* L. cv. Sakha 93) plants as affected by different magnetic treatments at salinity level (10 dS/m).

Where: A & B= Control

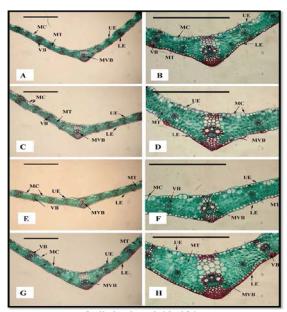
C & D= Magnetized seeds treatment

E & F= Magnetized water treatment G & H= Magnetized seeds and water Left panels were magnified by 40X while the right panels were magnified by 100X. Letters are Details: E= Epidermis layer, PH= Phloem, XY= Xylem, GT= Ground Tissue, VB= Vascular bundle, SC= Stem Cavity.



Salinity level 2.5 dS/m (Control)

Fig (3): leaves cross sections of wheat plants (Triticum aestivum L. cv. Sakha 93) as affected by different magnetic treatments at salinity level (2.5 dS/m, control).



Salinity level 10 dS/m

Fig (4): Leaves cross sections of wheat plants (Triticum aestivum L. cv. Sakha 93) as affected by different magnetic treatments at salinity level (10 dS/m).

Where: A & B= Control C & D= Magnetized seeds treatment E & F= Magnetized water treatment G & H= Magnetized seeds and water

Left panels were magnified by 40X while the right panels were magnified by 100X. Details: UE= Upper Epidermis layer, LE= Lower Epidermis layer, MC= Motor cell, VB= Vascular bundle, MVB= Midrib vascular bundle, PH= Phloem, XY= Xylem, BS= Bundle Sheath, MX= Metaxylem, PX= Protoxylem, PR= Parenchyma Tissue, MT= Mesophyll Tissue.

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الإستجابة الفسيولوجية و التشريحية لنباتات قمح مجهدة ملحياً للمجال المغناطيسى

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الملخص العربي

بهدف دراسة التغيرات الفسيولوجية و التشريحية لنباتات القمح (Triticum aestivum L.) صنف سخا ٩٣ المعرضة لمجال مغناطيسي تحت ظروف الإجهاد الملحي، تم إجراء تجربتي أصص في الصوبة الزجاجية في مزرعة التجارب بكلية الزراعة – جامعة المنوفية – شبين الكوم –مصر و ذلك خلال الموسم (٢٠١١/٢٠١٠)، تم أخذ العينات النباتية بعد ١٠٠ يوم من الزراعة.

و أوضحت النتائج المتحصل عليها أن معاملات المغنطة المختلفة (بذور ممغنطة – الري بماء ممغنط – بذور ممغنطة مع الري بماء ممغنط معاً) تسبب زيادة واضحة في نمو النباتات و بعض الصفات الفسيولوجية و الكيماوية مثل (العلاقات المائية و نفاذية الأغشية و البرولين الكلي و بعض الهرمونات النباتية) تحت تأثيرمستوى الملوحة (١٠ ديسيمينز/متر) مقارنة بالكنترول. الصفات التشريحية للساق مثل (سمك الساق، سمك الفجوة، عدد الحزم الوعائية خلال القطاع، سمك الحزم الوعائية و سمك الأوعية الخشبية) و بعض الصفات التشريحية للورقة مثل (سمك الورقة، سمك العرق الوسطي، سمك الأوعية الخشبية) لنباتات القمح تحسنت بوضوح بالمعاملة بمعاملات المغنطة المختلفة و تداخلهم مع مستوى الملوحة (١٠ ديسيمينز/متر)، بينما كان هناك نقص ملحوظ في نقص الماء الورقي و معدلال نتح و تركيز هرمون الأبسيسيك أسيد (المثبط) في النباتات. و كان تأثير معاملة الماء الممغنط أكثر وضوحاً في تطور و نمو النبات.