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EFFECT OF SALINITY AND PRIMING WITH PHYTOHORMONES ON GROWTH AND SOME PHYSIOLOGICAL PARAMETERS ON WHEAT PLANTS.

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It was found for decades that phytohormones have a prime role in modifying the plant growth. Especially, when these plants are exposed to biotic or abiotic stress. Verify this fact, this study examined the effect of salinity (0.0, 150 and 300 mM of NaCl) with different plant growth regulators (PGR), indole-3-acetic acid (200 ppm IAA) and/or Kinetin (200 ppm KIN). The pre-soaking effects of two plant hormones, IAA and KIN on wheat grains exposed to moderate and high NaCl levels were investigated. According to the results, an increase in salinity caused a progressive decrease in shoot and root lengths, fresh and dry matter yields and the reduction in the compatible compounds chiefly under the highest level of NaCl. While proline content and malondialdehyde (MDA) were increased. Meanwhile, salinity triggered an imbalance in endogenous phytohormones, including KIN and IAA. In our experiment, the application of IAA or KIN or even the combination between them can control the growth processes, improve the salt tolerance of the plant and promote its growth.

Keywords: compatible, endogenous, imbalance, prime and soaking.
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INTRODUCTION

Wheat is the most important cereal crop in Egypt and a staple food for the Egyptian people. Egyptian wheat production and imports are forecast to be high in 2019-20 as demand continues to increase. Therefore, increasing the agriculture sector with wheat is a national demand.

Soil salinity can be classified into two groups; natural/primary salinity and human- induced/secondary salinity. The increased salt concentration in the soil inhibits the ability of a plant to take up water. If excessive amounts of Na^+ and Cl^- were taken up in large amounts by roots and accumulated in the leaves, then both Na^+ and Cl^- ions drastically affect plant growth by damaging metabolic processes and reducing photosynthetic efficiency **(Kapale et al., 2018).**

Plants face two fundamental problems with saline conditions. Firstly, excess NaCl in the soil reduces the osmotic potential of the soil solution and leads to inhibition in water uptake therefore, water deficit in plants. This consequently leads to a disturbance in cell division and affects the integrity of metabolic reactions in plants. Secondly, stimulation in the uptake and accumulation of Na^+ and Cl^- ions which decline the absorption of essential minerals and thus toxicity to plants **(Shekafandeh et al., 2017).** In saline conditions, the unique plant response to external osmotic potential changes is the accumulation of metabolites, which does not generate any problem for normal metabolic reactions of cells and acts as consistent solutions. If these toxic ions Na^+ and Cl^- are not moved into the vacuole of the cell, organic solutes that are compatible with metabolic activity even at high concentrations must accumulate in the cytosol and organelles to balance the osmotic pressure of the cell **(Mostajeran and Gholaminejad, 2014).**

Generally, plant growth and development include a sequence of biochemical processes, which are regulated by plant hormones, proteins and enzymes where, fruit development is closely associated with plant hormones **(Sun et al., 2018).** Plant growth regulators are small chemical messengers derived from the secondary metabolism that take center stage in the combination and translation of environmental demands into physical plant responses. These phytohormones are considered to play a key role in mediating plant defense responses against abiotic stresses, such as drought, light, and salinity **(Pérez-Alonso and Pollmann, 2018).**

Indole acetic acid (IAA) was the first identified hormone that stimulates plant growth and is widely distributed in plants. The physiological effects of IAA can be detected at two levels. At the cellular level, it encourages cell division, whereas at the organ and whole-plant levels, its effects initiate from the seedling stage to grain filling stage.

Cytokinins can also enhance resistance to salinity and high temperature in plants. Kinetin acts as a direct free radical scavenger or it may involve as an antioxidative defense**.** It was found that cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and abscisic acid **(Iqbal et al., 2006).** Based on this idea, our experiment was conducted to know the strategy which the salinized plants followed and to examine the role played by the two different types of phytohormones and the combination between them as a defense regulator.

MATERIALS AND METHODS

Plant material and salt treatment: Wheat grains (*Triticum vulgaris* L.) variety "Giza 168" were obtained and procured from the local seed center, Qena, Egypt. The seeds were grown in the garden of the Agronomy Department, South Valley University. Ten grains of wheat were grown in plastic pots containing (8 kg/pot) air-dried soil (sand /clay 3:1by v/v). Four sets of plants (three biological replicates each) were treated with three different concentrations of NaCl (0.0, 150 and 300 mM) respectively. The first set of wheat plants were watered with different salinity levels only. The second set of wheat grains were presoaked in IAA (200 ppm). In the third set, wheat grains were presoaked in KIN (200 ppm). The last fourth set wheat grains were presoaked in a combination between IAA and KIN. The presoaking took place for two hours. Soils were watered with water containing respective concentration of NaCl as stated above immediately after two weeks of sowing and watering was carried out for 90 days, after which samples were collected for further experiments.

Plant analysis: Plant samples were collected within 90 days of sowing and prepared for determination of some growth parameters: samples were collected at harvest from each treatment, cleaned, and dried at 70 C^0 for 48 hr. and weight. Height (cm), fresh and dry weight of shoots and roots (g/plant) **(Basra et al., 2002)**. Some physical and biochemical characters were estimated: The determination of soluble carbohydrate content, the anthrone-sulfuric acid method of **Fales (1951**) was used. The soluble protein was determined according to the method of **Lowrey et al. (1951),** Free amino acids were determined according to the method of **Moore and Stein (1948**), Proline was determined according to the method of **Bates et al. (1973)** while, lipid peroxidation was determined in the term of malondialdehyde (MDA) content by the method of **Heath and Paker (1968)**.

Extraction and quantification of plant hormones Indole acetic acid (IAA) and Kinetin (KIN): Leaves from both salt- treated and control plants were collected for extraction and quantification of IAA and (KIN) after 90 days of treatment. Extraction and quantification of KIN and IAA were done according to the protocol given by **Durley et al. (1982)** and **Wurst et al. (1984).**

RESULTS AND DISCUSSION

The results given in Table **1** revealed that the rise of NaCl level led to a reduction in shoot and root lengths especially at the highest level (300 mM).

Besides, the fresh and dry matter yields of both the tested wheat organs were significantly decreased, as compared with the absolute control plants.

The little effect of salinity on the shoot and root lengths at a moderate level (150 mM) may be due to moderately salt-tolerant cultivar which able to maintain the value of growth criteria around non-treated plants. This strategy agreed with the theoretical consideration of **Pattanagul and Thitisaksaku (2008)**. The opposite trend in the case of the application of phytohormones was observed, where the shoot and root lengths beside fresh and dry matter yield of both two tested organs were increased markedly in all levels and whatever the phytohormone type when compared with those of the experimental stressed ones. It is remarkable that using IAA or the combination between IAA and KIN attained a prodigious value in previous data.

Plant hormones such as indole-3-acetic acid (IAA) and cytokinins have a vital role in plant growth regulation and plant defense against abiotic stresses e.g. salinity. Quantification of the hormone concentration can detect different plant strategies to cope with the stress, e.g. suppression of growth or mobilization of plant metabolism (**Jasim et al., 2016)**.

Table **1:** Effect of different levels of salt stress (NaCl) and soaking the grains in IAA and/or KIN on shoot and root lengths (L) (cm plants^{-1}), fresh (F. wt.) and dry (D. wt.) g plant $^{-1}$ weights in wheat plants[three replicates].

Treatments			Shoot			Root		
IAA(ppm) 0	KIN(ppm) 0	NaCl (mM) 0	L.	F. wt.	D. wt.	L.	F. wt.	D. wt.
			40 ±2.16	29.5±0.41	11.2±0.16	10.2±0.75	5.35±0.04	2.35 ±0.20
0	0	150	38 ±1.63	24.6**±0.29	8.8*±0.22	7.7±0.54	4.12**±0.17	1.70*±0.33
		300	31**±1.41	17.5**±0.46	5.3**±0.22	5.2**±0.22	3.55**±0.19	1.22**±0.21
200		0	48**±2.16	34.2**±0.14	13.7**±0.22	10.7 ±1.0	6.24*±0.04	3.03* ±0.08
		150	42 ±1.63	27.5*±0.36	9.4* ±0.65	8.6 ±0.51	4.30**±0.22	2.17±0.12
		300	38 ±0.817	19.3**±0.16	7.2**±1.02	7.4*±0.65	4.07**±0.10	1.95±0.04
	200	0	45*±0.817	27.8±0.16	11.7±0.65	11.5±0.67	5.20 ±0.34	3.02*±0.18
		150	40±1.41	24.5**±0.22	9.2* ±0.78	8.0 ±0.25	4.81 ±0.59	2.35 ±0.18
		300	36* ±2.45	18.2**±0.16	7.5**±1.02	6.5**±0.46	3.80**±0.36	2.23 ±0.13
200	200	0	44* ±1.41	29.7 ±0.22	12.5±0.99	11.0 ±0.65	6.40**±0.29	3.66**±0.05
		150	39 ±0.817	27.1 ±0.082	10.6 ±0.91	8.4 ±0.54	5.30 ±0.16	2.88*±0.24
		300	34** ±1.63	18.8**±0.71	6.9**±0.86	7.0* ±0.43	3.07**±0.28	2.08 ±0.09
L.S.D at 5%			3.8	2.7	1.8	2.66	0.76	0.51
L.S.D at 1%			5.2	3.7	2.5	3.64	1.04	0.70

*refers to significant differences

**refers to highly significant differences

The data in Table **2** postulated that soluble carbohydrate contents in both shoot and root increased slightly at 150 mM of NaCl, while it dropped markedly at the highest salinity level (300 mM). The variation in the soluble carbohydrate content related to the physiological activities of plants such as photosynthesis, respiration and the dry matter yields. So, the increase in soluble carbohydrates under moderate level may be due to the metabolites adjustment for cell adaption via stabilization of osmotic balance **(Handa et al., 1983 and Pattangul and Thitisaks, 2008).** While the reduction in soluble carbohydrate at the highest level may be attributed to the decline in photosynthesis as a result of the accumulation of toxic ions in the plant.

On the other hand, the application of IAA, KIN or even the combination between them (IAA and KIN) overcame this drop completely or partially by increasing the soluble sugars content whatever the organ tested. This increase may cause by the enhancement of the plant photosynthesis, pigments, and the protection against toxic salinity ions. Also, **Balbaa (2002)** revealed that

phytohormones treatments increased CO_2 fixation leading to more sugar synthesis as well as protecting the chloroplast from stress condition.

Concerning soluble protein contents in the two tested organs, it decreased progressively at the highest salinity level.

In contrast, the application of phytohormones provoked a sustainable enhancement in the soluble protein contents irrespective the shoot or root system and the type of phytohormone used as it compared with the salinized stressed plant.

The results concerning total free amino acids in the shoot or root decreased smoothly at a moderate salinity level (150 mM), but decreased significantly at the highest level (300 mM), as compared with the untreated absolute control plant. The negative effect of salinity on protein synthesis was aggravated by increasing salinity level. Where, salinity led to a decrease in protein synthesis, a decline in the availability of amino acids, and/or denaturation of the enzymes involved in the synthesis of amino acids and proteins **(Dubey and Rani, 1990 and Astorga and Meléndez, 2010).**

The pre–soaking of wheat grains in phytohormones regardless of the type of used or the organ tested imparts an amazing increase in total free amino acid content whatever the stress used.

The increase in soluble protein content and total free amino acids with IAA or KIN may be related to its effect on protein synthesis, they might directly affect protein synthesis as well as RNA synthesis **(Ahmed, et al., 2001)**.

The proline content accumulated highly significantly under various levels of salinity whatever the organ analysed (shoot or root). Moreover, the accumulation recorded more than 2 fold of the absolute control plants at the highest level. These results are in harmony with the result of **Mostajeran and Gholaminejad (2014).**

One of the reasons for proline accumulation under salt stress conditions may be related to its ability to act as an osmotic regulator due to its bipolar and hydrophilic properties between the cytoplasm and vacuoles. Besides, it is also responsible for protecting many cytoplasmic enzymes. These confirmations reveal that proline accumulation could be a useful feature for adaption of the plant to biotic and abiotic stresses **(Khalil et al., 2016)**.

Exogenous applications of the IAA, KIN or the combination between them exhibited in most cases showed retardation in proline accumulation in both the aboveground and underground parts when compared with the unfertilized counterparts. Also, we can observe that the accumulation of proline was more

prominent in the combination of IAA and KIN. The retardation of proline content as a result of phytohormones presoaking may be attributed to the ability of these phytohormones in alleviating the adverse effect of salinity stress. Similar results of IAA treatments were obtained by **Sadak et al. (2013)** on faba bean.

Table **2**: Effect of different levels of salt stress (NaCl) and soaking the grains in IAA and/or KIN on shoots (Sh) and roots (R) soluble carbohydrates [mg g^{-1} dry weight], soluble proteins [mg g^{-1} dry weight], total free amino acids [mg g^{-1} dry weight] and proline [mg g^{-1} dry weight] contents in wheat plants[three replicates].

Treatments			**Soluble carbohydrates**		**Soluble proteins**		**Total free amino acids**		**Proline**	
IAA(ppm)	KIN(ppm)	NaCl (mM)	Sh	R	Sh	R	Sh	R	Sh	R
0	0	0	69.7±0.57	63.3±1.12	27.7±0.90	17.5±0.57	11.07±0.79	6.9±0.74	1.8±0.29	0.93±0.06
0	0	150	70.9±1.00	70.7*±1.12	29.3±1.2	18.9±0.70	12.2±0.65	8.3±0.62	2.9**±0.29	1.4*±0.25
0	0	300	33.4**±1.14	42.3**±1.37	20.2**±1.69	13.1*±0.94	6.2**±0.37	5.2 ±0.75	3.8**±0.59	2.9**±0.29
200	0	0	79.8**±0.96	91.2**±1.56	31.3**±1.12	28.1**±0.70	14.7**±0.65	13.4**±0.49	1.3 ±0.22	0.53 ±0.07
200	0	150	82.1**±0.78	93.1**±0.78	35.2**±0.82	29.5**±0.46	17.2**±0.75	15.6**±0.33	1.9 ± 0.37	0.87 ±0.06
200	0	300	50.2**±1.35	64.0±1.47	28.4 ±1.14	24.1**±0.86	20.5**±0.70	18.4**±0.94	2.5*±0.33	1.8**±0.22
0	200	0	76.0**±1.56	86.1**±0.99	30.1 ±0.94	27.8**±0.75	13.7*±0.73	12.3**±0.73	1.9 ± 0.29	0.81 ±0.04
0	200	150	78.0**±1.47	87.2**±0.99	33.5**±1.59	28.4**±1.00	15.2**±0.62	14.2**±0.67	2.3 ± 0.29	1.1 ±0.16
0	200	300	45.3**±1.44	57.3±0.83	26.8 ±0.91	22.3**±1.00	18.3**±0.96	16.3**±0.78	3.1**±0.43	2.1**±0.16
200	200	0	71.3±1.04	68.0±1.02	29.7±1.39	26.3**±0.88	14.1**±0.65	11.2**±0.70	1.7 ± 0.25	0.91 ±0.05
200	200	150	73.1±1.11	79.04**±1.62	31.8**±1.5	28.1**±0.70	16.0**±1.2	13.9**±0.54	2.5*±0.29	1.2 ±0.16
200	200	300	38.0**±0.99	44.1**±0.86	25.3 ±0.71	23.2**±1.3	19.3**±0.59	17.2**±0.80	3.5**±0.29	2.0**±0.16
L.S.D at 5%			3.8	7.4	2.6	3.30	2.2	2.0	0.55	0.41
L.S.D at 1%			5.2	10.12	3.6	4.52	3.01	2.74	0.75	0.56

*refers to significant differences

**refers to highly significant differences

Different salinity levels resulted in a marked accumulation in MDA content in the shoot of wheat plants and the increment was steady by increasing the salinity level in Table **3**.

Table **3:** Effect of different levels of salt stress (NaCl) and soaking the grains in IAA and/or KIN on shoot MDA content (n mol gm^{-1} fresh weight), endogenous IAA and KIN contents (µg g^{-1} dry weight) in wheat plants [three replicates].

Treatments			MDA	IAA	KIN
IAA(ppm)	KIN(ppm)	NaCl (mM)			
0	0	0	1.37 ±0.16	22.9±1.34	44.6±0.83
0	0	150	3.42**±0.16	32.4±1.8	31.2±0.74
		300	5.51**±0.20	40.2*±1.4	26.0**±1.1
200		0	1.32±0.16	29.4±1.01	52.1±1.5
		150	2.38±0.06	45.4*±1.31	44.2±0.75
		300	3.07**±0.09	56.14**±1.58	32.7±0.85
	200	0	1.35±0.11	30.2±0.86	65.6**±0.33
		150	2.63*±0.14	51.3**±0.96	45.3±0.78
		300	3.45**±0.11	63.8**±0.65	36.2±0.75
200	200	0	1.40±0.11	25.6±0.37	55.4±0.37
		150	2.57*±0.14	39.1±0.78	42.4±0.46
		300	4.30**±0.22	48.3**±1.2	31.7±0.57
L.S.D at 5%			1.10	17.6	13.8
L.S.D at 1%			1.51	24.1	16.2

*refers to significant differences

**refers to highly significant differences

Generally, MDA is the decomposition product of polyunsaturated fatty acids of membranes under different types of stress. The rate of lipid peroxidation level in terms of MDA can, subsequently, be used as an indicator to assess the tolerance of plants to oxidative stress as well as the sensitivity of plants to salt stress **(Saedipour, 2016).** The accumulation of MDA in the tested wheat cultivar may be related to the production of highly toxic reactive oxygen species (ROS) which in turn, led to an increase in the membrane injury. This postulation was agreed with **Azuma et al., (2010).** Exogenously applied IAA or KIN was shown to be effective in the reduction of MDA content.

Probably, plant growth regulators inhibited the lipid peroxide generation as seen from the low MDA content and diminished lipid peroxidation through the stimulation of antioxidants tightly regulating the ROS homeostasis. Our results are supported by data obtained by **Niczyporuk and Bajguz (2013).**

IAA controls almost all aspects of plant life, from seed germination to vegetative growth and flowering. In the present study, the endogenous IAA content increased markedly with increasing the level of NaCl. Our results were fortified by the results of **Wang et al. (2001**), who postulated that IAA

generally increase in plants in response to elevated salt stress. There is evidence that more salt- tolerant plant increases the level of IAA. The accumulation of IAA in shoot which might involve in the cell wall extensibility. Our findings also by the results of **Yurekli et al. (2004)**.

The reason why cytokinin and kinetin are retarded under salt stress may be attributed to the reduction in cytokinin biosynthesis and also its decomposition. These findings are keep pace with **Bano et al. (1994) and Shashidhar et al. (1996).**

Also, the reduction of cytokinin and kinetin levels probably correlated with a low transpiration of salinized plants. These results are in good accordance with those reported by **Llanes et al., (2016).**

It was found that the exogenous application of cytokinins could reestablish the imbalance that takes place by salinity stress, allowing plants to grow better at high NaCl levels. These results coincide with the findings of **Yurekli et al. (2004).**

Finally, based on the data reported in the present study, it is suggested that soaking of wheat grains in IAA or KIN or even the combination between them enhanced the ability of these grains to grow successfully under salinity conditions. For some literature, KIN and IAA in combination could indicate an antagonistic effect like some nutrients do with one another and perhaps cause a toxic effect on some tested parameters. Hence, it is possible to recommend the soaking of Giza 168 wheat grains in IAA or KIN to achieve sensible plant growth and grain yield.

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بعض التأثيرات الفسيولوجية لنمو نبات القمح المعامل بالملوحة والهرمونات النباتية

نورا حسن يوسف و أحمد محمد اسماعيل و نجلاء عدلى حسين
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استهدف هذا البحث تأثير مستويات مختلفة من محلول كلوريد الصوديوم (٠.٠ – ١٥٠ – ٣٠٠) ملى مول على نبات القمح (جيزة ١٦٨) كما اشتملت الدراسة تأثير بعض الهرمونات النباتية على النباتات المروية بالملوحة عن طريق المعاملة بنقع حبوب القمح فى محلول لهرمون اندول حمض الخليك (٢٠٠ جزء فى المليون) وهرمون الكينتين (٢٠٠ جزء فى المليون) كل على حدة ونقع هذه الحبوب فى خليط من الاندول والكينتين معا (٢٠٠ جزء فى المليون) وتأثيرها على النمو وبعض العمليات الايضية المختلفة والمحتوى الداخلى للنباتات من تلك الهرمونات.

وقد أظهرت الدراسة نقص ملحوظ فى كل من طول المجموع الجذرى او المجموع الخضرى او الوزن الطازج والجاف خصوصا تحت التركيز المرتفع من كلوريد الصوديوم (٣٠٠ ملى مول).

وقد أدى الاجهاد الملحى الى تناقص ملحوظ فى كل من المحتوى السكر الذائب والبروتين الذائب والاحماض الامينية فى حين ارتفعت كمية الحمض الامينى (البرولين) فى كل من المجموع الجذرى والمجموع الخضرى وخاصة تحت التركيز المرتفع من كلوريد الصوديوم .

كذلك ارتفعت معدلات اتلاف نفاذ الاغشية (ممثلة فى كمية المالون الدهيد) وارتفع المحتوى الداخلى للمجموع الخضرى من اندول حمض الخليك بينما تناقص المحتوى الداخلى من هرمون الكينتين .

وحينما تم نقع حبوب القمح فى الهرمونات النباتية المختلفة (اندول حمض الخليك او الكينيتين او الاثنين معا) بتركيز ٢٠٠ جزء فى المليون تحسن النمو بشكل ملحوظ والذى صاحبه تحسن ملحوظ فى معظم القياسات سالفة الذكر عند المقارنة بقريناتها التى تم معالجتها بالملوحة فقط .