Improvement of Salt Tolerance in Durum Wheat (*Triticum Durum Desf*.) by Auxin and Kenitin Application

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Abstract

The aim of this work is to study the effect of soil salinity using different levels of NaCl (0, 10, 15 g/ NaCl) on some morphological and physiological properties of durum wheat (*Triticum durum Desf*.) var KEBIR in the vegetative stage. The results obtained allowed us to observe the negative impact of saline stress on the leaf area, Chlorophyll b, and carotenoids at moderate and higher salinity levels. The effect of salt on chlorophyll a content remains insignificant. On the other hand, proline content was highly affected by salt stress. It was excessively increased with the rise of osmotic stress levels, especially at higher salinity levels (15g/l NaCl). Phytohormones, such as auxin and cytokinin, are known to be involved in the regulation of plant responses to salinity stress. Also, it counteracts the adverse effect of stress conditions. Exogenous spraying of indole-3-acetic acid (IAA) and kinetin (K) with three various levels (10, 20, 30 p.p.m.), during the vegetative phase, indicated that an increase in the leaf area and pigments content are associated with a decrease in the proline content.

Keywords: Indole-3-Acetic Acid (IAA), foliar application, salt stress, growth regulateurs

Introduction

Consequently, wheat is a major cereal crop in many parts of the world and it is commonly known when asking about cereals. It belongs to poaceae family. Globally after maize, wheat is the second most produced food among the cereal crops, while rice ranks third (Datta et *al*., 2009). While wheat and barley are the main cereal crops in Algeria, durum wheat is by far the most cultivated grain. However, wheat crop often confront abiotic stresses such as drought and salinity, which are among the most important strength-limiting factors of wheat production particularly in arid and semi-arid regions (Fercha et *al*., 2011).

In Algeria, there is approximately 3.2 million ha currently threatened by salinity (Benmahioul et *al.,* 2009). Due to the developing area of salt-affected land, salinity has become an everlasting challenge to agriculture and food supply (Flowers, 2004). Increased incidence of salinity on arable lands suggests the need for better understanding of the plant tolerance mechanisms. This is in a bid to sustain crop productivity by modulating growth conditions to the best possible extent. Furthermore, the inhibition of growth and development, reduction in photosynthesis, respiration, and synthesis in sensitive species has been reported under salinity (Hussain et *al*., 2013; Saud et *al*., 2014). Parida and Das (2005), Tuteja (2007), and Munns and Tester (2008) showed that adaptation to all stresses is accompanied with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline, protection of cellular machinery, maintenance of ionic homeostasis, scavenging of free radicals, expression of certain proteins, and upregulation of their genes and induction of phytohormones. Therefore, a well-focused approach combining the molecular, physiological, biochemical, and the metabolic aspects of salt tolerance is essential to develop salt-tolerant crop varieties. Exploring suitable ameliorants or stress alleviant is one of the tasks of plant biologists. In recent decades, exogenous protectant such as plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, Indole acetic acid (IAA), Cytokinins (CKs) etc.), have been found to be effective in mitigating the salt induced damage in plant (Iqbal et *al*., 2012; Yusuf et *al*., 2012). Therefore, the present project was undertaken to find out whether the foliar application growth regulators (IAA and K) with different levels 10, 20 ,30 p.p.m on Durum wheat (*Triticum durum Desf*.) can alleviate the harmful effect of salinity on plant growth.

Materials and Methods

Our study was carried out on the genotype KEBIR -1, durum wheat (*Triticum durum Desf*.), whose seeds was gotten from The technological Institute of Large Cultures of Constantine, Algeria (ITGC). The durum wheat seeds were disinfected with 0.5% sodium hypochlorite solution and rinsed several times with distilled water. Then, it was grown in plastic pots containers of about 2kg of homogeneous clay soil with a high percentage of organic matter, neutral pH up to light alkaline, and which is not saline by an average of 12 seeds/pot grown towards the end of November. The soil samples were taken from a field at the level of the Constantine region (North

- East Algeria). Therefore, the pots were placed in the greenhouse at a temperature between 17-40 ° C with an estimated relative humidity between 65-90%. Irrigation is done at a rate of 1/3 capacity in the field (0.1L). Through the use of tap water for two weeks, 7 plants were selected in each pot. From the third week, irrigation was started every week by 0.3L for each salinization levels (0, 10, 15 g / l). From the third leaf, after 15 days of salinity treatment, the phytohormones solutions were sprayed. This was done by spraying the shoot system of the growing plants (each pot with 10 Cm^3 of phytohormones solutions). Also, the control plants were sprayed with distilled water a week after the plants were used for analysis.

The Studied Parameters

Physico-chemical Soil Parameters and Leaf Area (cm^2)

The physicochemical analysis of the soil were carried out according to the methods of Bonneau and Souchier (1994). However, the leaf area (LA) was measured using an area meter (LI-COR, model LI-3000).

Chlorophyll (A , B and Carotenoids) Content

The content of the chlorophyll a, b, and the total carotenoids were determined by following the method of Vernon and Seely (1966) improved by HEGAZI et *al.* (1998). The plant sample was treated with a mixture of 75% acetone and 25% ethanol. The Absorbance was measured using spectrophotometer (UV-visible type JENWAY 6300) at 480, 649, and 665 nm wavelengths. The chlorophyll a, b, and carotenoids concentrations were expressed in mg/g FW.

Proline Content

Quantitative determination of free proline content was performed according to Monneveux and Nemmar (1986).

The Statistical Test

The statistical test was done by the analysis of three-factor variance. This is followed by a Comparison of Newman-Keuls means (NSK) with a 95% threshold Confidence by the Excel Stat version 2016 software.

Results and Discussion

Physico-chemical Soil Parameters

In view of the results obtained (Table I), the soil analyzed appears to be non-saline of clayey texture with an alkaline pH of the order of 7.8 High organic matter (MO) and total limestone. In addition, the amounts of carbonates (CO3-) and bicarbonates (HCO3) were very low. They are of the order of 0 meq / l, 2 meq / l, respectively. Thus, this confirms the results of the electrical conductivity CE.

Table 1. Analysis of soil physico-chemical parameters

HCO3(méq/l)	CO3- (méq/l)	MO (%)	pH	CEC (méq/g)	CE 25°C (MS/cm)	Total limestone (%)	Active limestone (%)
2	-	2,38	7,8	0,135	1,38	17	9,5

MO organic matter, CEC: cationic exchange capacity, CE: electrical conductivity

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture
7,37	5,33	20	67	Clay

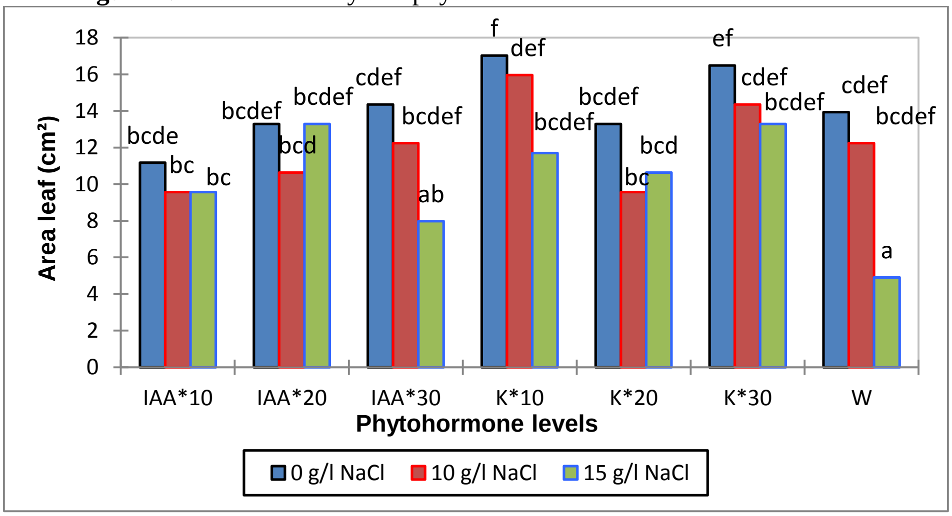
Leaf Area

The values of leaf area of the tested plants through either salinization or salinization with phytohormones treatment (IAA or kinetin) were represented in Table 2 and Figure 1. The leaf area of the unsprayed plants was excessively decreased with the rise of salinization levels especially at higher salinity levels compared to unstressed plants. The means of leaf area at 15g/l NaCl achieved 4, 90 cm²**.** Similarly, Laaziza Ben Khaled et *al*. (2007**)** has shown, in plants grown in presence of 100 mM NaCl, that the number of Leaves has been reduced by 40% compared to control. Also, the leaf area is no more than 60% compared to that of the control**.** Ali et *al*. (2004) and Hamdia et *al*. (2014) reported that the reduction in leaf area of maize, wheat, cotton, broad bean and parsley tested plants, under saline conditions were also due to reduced growth. This is as a result of decreased water uptake and the toxicity of sodium and chloride in the shoot cell. The leaf area was generally lowered by increasing osmotic stress. This inhibitory effect may be attributed to the effects of salinity on several facets of plant activities such as enzyme activity (Seckin et *al*., 2009), DNA, RNA, protein synthesis (Anuradha & Rao, 2001), mitosis (Kriedemann, 1986), osmotic adjustment (Hamdia & El-Komy, 1998), hormonal balance (Jackson, 1997; Debez et *al*., 2001; Iqbal & Ashraf, 2013; Zholkevich & Pustovoytova, 1993), and photosynthesis (Amuthavalli & Sivasankaramoorthy, 2012).

Spraying these salinized plants with any of the phytohormones (IAA or kinetin) mostly resulted in a marked increase in leaf area. Therefore, the inhibitory effect of salinity stress was completely ameliorated especially at the high salinization levels of 15g/l NaCl at all concentrations of the growth regulators. It is worthy to mention that the values of leaf area were higher than control untreated plants. The highest mean values in this salinity levels resulted from the treatments 20 p.p.m. IAA and 30 p.p.m. Kinetin with means of 13,29 cm². In the relatively low and moderate salinity levels of 0g/l and 10 g/l NaCl , the highest mean values achieved 17,01 and 15,95 cm² respectively after the treatments by 10 p.p.m. Kinetin.

Exogenous application of IAA 10 p.p.m., 20 p.p.m., and 30 p.p.m. at 15 g/l NaCl and Kinetin 10p.p.m. and 30 p.p.m at 0g/l or 10g/l NaCl were promoted. Generally, the leaf area of tests plants alleviated to some extent the suppressive effect of salinity. Similar increase in leaf area by the application of growth regulators have been observed by Sritharan et al. (2005) and Vamil et al. (2010). This observed increase in the leaf area of salt stressed plants after hormonal treatments may indicate that the phytohormonal applications increased the plant efficiency of water uptake, conservation, and utilization (Javid et al., 2011). Consequently, there was a rapid increase in cell division, cell enlargement, and accumulation of building units.

Figure 1. Effect of salinity and phytohormones levels on the leaf area



IAA: Indole-3-Acetic Acid (Auxin), K: Kenitin

Chlorophyll (A , B) and Carotenoids Content

Chlorophyll (Chl) is one of the most important pigments, and is responsible for green colour in plants. Changes in photosynthetic parameters could potentially be used as a screening method for salinity tolerance in plants. This is because more tolerant cultivars are expected to exhibit less disturbances in photosynthetic processes (Belkhodja et ***al*.,** 1999).

The data values in Table 2 clearly demonstrates that chlorophyll b decreased under the high levels of salinity 15 g/l NaCl. Thus, the mean values reached 260,00 mg/g FW (Figure 2). Also, the lowest values for the content of the carotenoids were marked in the levels 10g/l with 0,354 mg/g FW and at 15 g/l NaCl with 0,267 mg/g FW (Figure 3) compared to unstressed plants. Chlorophyll a contents showed a non-significant alteration at all salinity stress levels (Figure 4). Our results are in agreement with those reported by Iqbal et al. (2006) and Ashraf et *al*. (2005). However, they stated that chlorophyll content was decreased under saline conditions in *triticum aestivum*. Content reduction of chlorophyll in plants such as *Poulownia imperialis* (Astorga et *al.*, 2010)**,** Bean (Beinsan et *al.*, 2003), **and** *Carthamus tinctorius* (Siddiqi et al., 2009) were reported.

Reduction in Chlorophyll concentrations is probably because of the inhibitory effect of the accumulated ions of various salts on the biosynthesis of different Chlorophyll fractions. According to Feigin et *al*. (1991) and Grattan and Grieve (1994), NaCl has an antagonistic effect on the absorption of nitrogen (N), which is an essential component of the structure of the chlorophyll molecule. Furthermore, it may be related to the activation of chlorophyllase, which catalyses the catabolism of chlorophyll (Majumdar et *al.,* 1991; Levent et *al*., 2008) and/or disorder of chloroplast structure and related proteins (Sabir et *al*., 2009). In addition, there is strong evidence that salt affects photosynthetic enzymes, chlorophylls, and carotenoids (Stepien & Klobus, 2006).

As accessory pigments, carotenoids participate in photoinduced electron transfer processes and protect chlorophyll photoxidative damage (Rodriguez et *al.*, 1997)**.** The decrease of total carotenoids content (TCC) may be explained as a result of either a low synthesis rate or enhanced degradation induced by reactive oxygen species (ROS). Thus, this has been proposed by many authors.

Treatment with phytohormones causes an increase in chlorophyll content. The maximum values for the chlorophyll b are obtained after treatments with K 20 p.p.m and IAA 30 p.p.m. with the means 282,00 mg/g FW and 276,00 mg/g FW respectively at 15 g/l NaCl. Also, the content of the carotenoids increases under the effect of treatments with K 20 p.p.m., IAA 30 p.p.m.at 15g/l NaCl , K 10 p.p.m.at 10 g/l NaCl, and K 30p.p.m.at 0 g/l NaCl with means of 0,340, 0,316, 0,429, and 0,473 mg/g FW respectively . Also, we noticed an increase in the chlorophyll a content with the application of the IAA 30 p.p.m. in media of 10 g / l with 0,89mg/g FW and 15 g / l NaCl with 0,90 mg/g FW. Similarly, increase in chlorophyll and carotenoid content by the application of growth regulators have been observed by various workers. Iqbal et al. (2006) showed that there was a general increase in pigments contents with phytohormones treatments in maize, wheat, cotton, broad bean, and parsley plants, especially in cotton and parsley plants. This may be due to the inhibition of pigment degradation or stimulation of protochlorophyll (ide) synthesis by phytohormones. In sum, phytohormones play a significant role in moderating the effects of salinity on photosynthetic traits and membrane stability (Pazuki et *al*., 2013).

Figure 2. Effect of salinity and phytohormones levels on chlorophyll b content

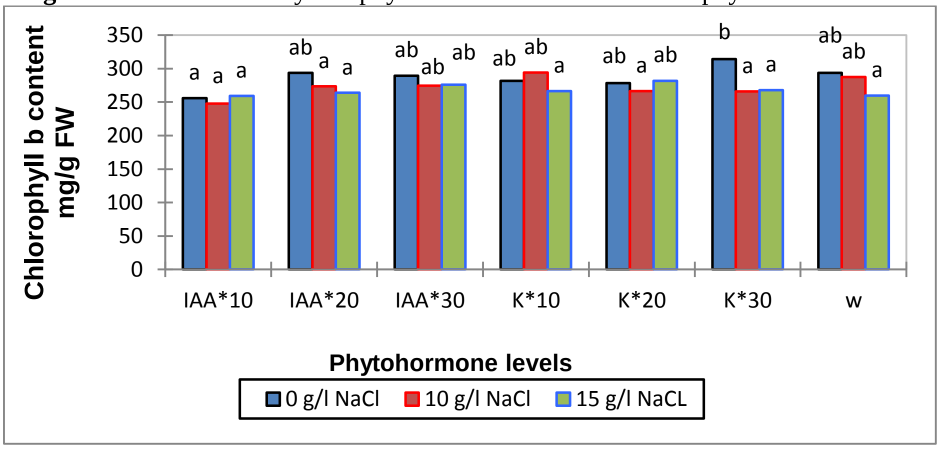


Figure 3. Effect of salinity and phytohormones levels on the carothenoids content

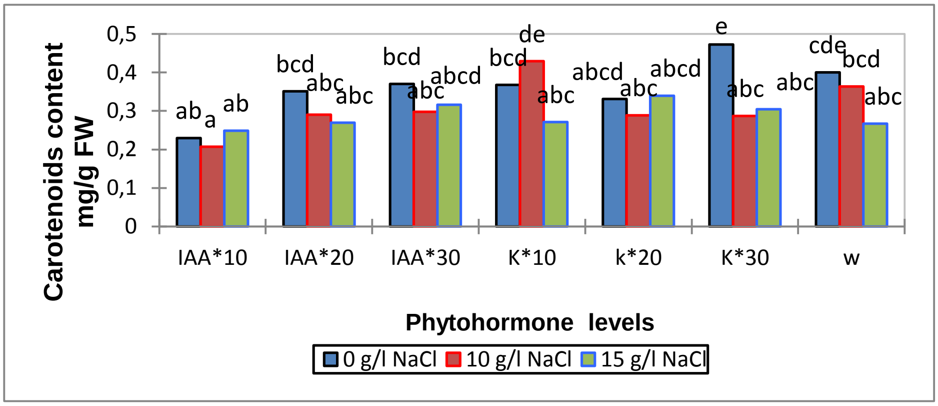
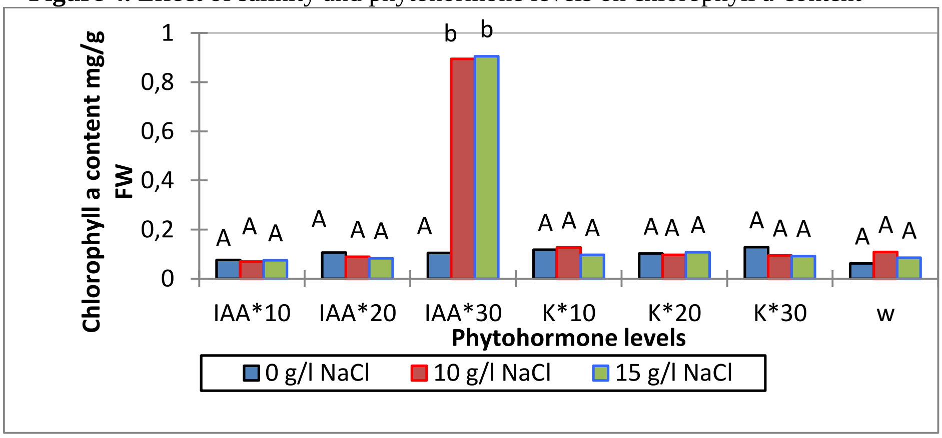


Figure 4. Effect of salinity and phytohormone levels on chlorophyll a content



Proline Content

When exposed to stress conditions such as salinity, the biochemical defense system includes proline accumulation. This amino acid has been considered as constituent of proteins. Also, it plays an important role in plant metabolism and development. It plays a highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte (Ashraf et *al.*, 1998), proline plays three major roles during stress as a metal chelator, an antioxidative defense molecule, and as a signaling molecule (Hayat et al., 2012).

Statistical analysis presented in Table 2 and Figure 5 showed that free proline content was highly affected by salt stress at high levels salt (15g/l NaCl). The increase achieved 1035,00 µg/g FW compared to the level of salts 0 g / l and 10 g / l NaCl with the means of 59,84 µg/g FW and 159,00 µg/g FW respectively. Rapid accumulation of free proline is a typical response to salt stress and similar responses have been observed by earlier workers in rice (Lin et *al.*, 2002; Buhl et *al.*, 1983). Singh et *al*. (1973) and Tall et *al*. (1979) demonstrated that free proline accumulated in different plant species as a result of water stress is induced either by the addition of salts or by the decrease of moisture content. In other research on Barley (*Hordeum vulgare L.*), it was observed that proline is a reducer component of osmosis pressure in response to increase of salinity (Ueda et *al.*, 2007). Also, increasing of proline content in cotton (Desingh et *al.*, 2007) and wheat (Khan and *al.*, 2004) was synchronised with the increasing of salinity level which was reported. Boggess et *al*. (1976) and Morris et *al*. (1969) reported that, within the leaves of many plants subjected to moderate or severe water stress, one striking change in nitrogen metabolism is the accumulation of free proline as a result of de novo synthesis from glutamic acid. Thus, the accumulated proline could be considered as a storage nitrogen compound (Barnett et *al*., 1966) and/or a metabolic adaptation product (Singh et *al*., 1973). Recent studies proposed that phytohormones play a major role in regulating and controlling proline metabolism during salinity tolerance (Iqbal et *al*., 2014).

After spraying with phytohormones, the accumulation of proline was considerably retarded, whatever the level of phytohormone used. The accumulation of proline has been considerably decreased by applying the three doses of phytohormone used for the 15 g / l NaCl level compared with the stressed plant (media 15 g / l NaCl). Also, they were not treated with either of the two hormones. The minimum proline content is 258,00 µg/g FW for treatment with K 10 p.p.m. and 610,50 µg/g FW for treatment with IAA 20 p.p.m. always for the saline medium 15 g / l NaCl. The effect of three doses of both hormones for 0g / l and 10 g / l NaCl media remains insignificant. Therefore, these results are in agreement with those of Immamul and Larher (1983) and Ali mahrokh et al. (2016) who reported that the use of cytokinine hormone in concentration of 50 mg/lit increased 37.73% chlorophyll a and decreased 16.58% proline amino acid. Also, auxine hormone in concentration of 10 and 20 mg/lit increased 22.78% chlorophyll a and decreased 18.02% proline of maize cultivar KSC 704. This retardation may lead to the conclusion that each of the two phytohormones (IAA or kinetin) used could alleviate the adverse effects of salt stress. If proline accumulation is considered as an indication of stress injury, thus it can be said that, the exogenously applied growth hormones seem either to protect the plant against salt stress injury. Consequently, the synthesis of proline is retarded and/or it plays a specific role in proline transformations to other growth constituent.

Figure 3. Effect of salinity and phytohormones levels on the proline content

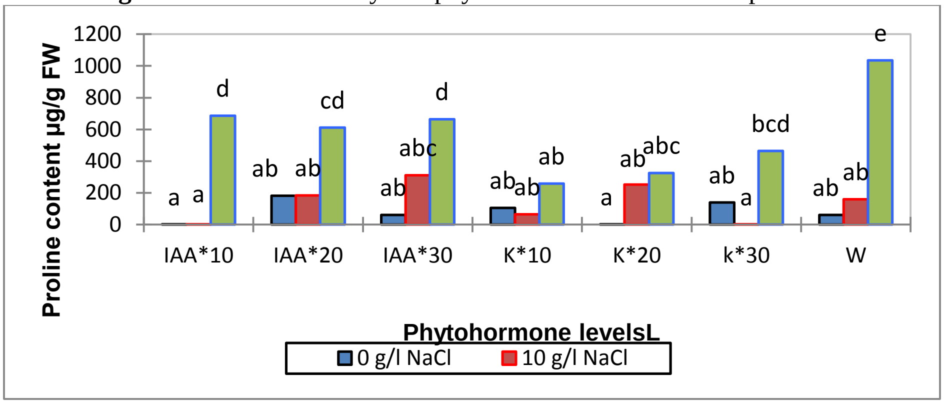


Table 2. Synthesis (Estimated Means) – interaction between Phytohormones (Water (w), Auxin (IAA)and Kinetin (K)) /Levels phytohormone(10,20 and 30 p.p.m.)/Stress levels(0, 10, and 15 g/l NaCl)

H /LH/LS	ChA (mg/g FW)	ChB (mg/g FW)	Ca (mg/g FW)	P µg / g FW	AL(cm²)
K*30*0	0.128 a	314.500 b	0.473 e	138.500 ab	16.485 ef
K*10*10	0.127 a	294.000 ab	0.429 de	63.965 ab	15.950 def
IAA*30*0	0.106 a	289.500 ab	0.370 bcd	60.450 ab	14.355 cdef
K*10*0	0.118 a	282.000 ab	0.368 bcd	104.500 ab	17.015 f
W*10	0.110 a	287.500 ab	0.364 bcd	159.000 ab	12.230 bcdef
IAA*20*0	0.106 a	293.500 ab	0.351 bcd	181.500 ab	13.290 bcdef
K*30*15	0.093 a	268.000 a	0.305 abcd	463.000 bcd	13.290 bcdef
K*20*15	0.108 a	282.000 ab	0.340 abcd	325.500 abc	10.630 bcd
K*20*0	0.103 a	278.500 ab	0.331 abcd	0.510 a	13.290 bcdef
IAA*30*10	0.895 b	274.500 ab	0.298 abc	311.500 abc	12.230 bcdef
IAA*30*15	0.905 b	276.000 ab	0.316 abcd	664.000 d	7.970 ab
IAA*20*15	0.083 a	264.000 a	0.270 abc	610.500 cd	13.290 bcdef
W*0	0.062 a	293.500 ab	0.401 cde	59.840 ab	13.925 cdef
K*20*10	0.097 a	266.500 a	0.289 abc	253.000 ab	9.565 bc
K*10*15	0.097 a	266.500 a	0.271 abc	258.000 ab	11.695 bcdef
K*30*10	0.095 a	266.000 a	0.287 abc	0.201 a	14.355 cdef
IAA*20*10	0.090 a	273.500 ab	0.291 abc	184.000 ab	10.635 bcd
W*15	0.086 a	260.000 a	0.267 abc	1035.000 e	4.905 a
IAA*10*15	0.075 a	259.500 a	0.249 ab	685.500 d	9.565 bc
IAA*10*0	0.076 a	256.000 a	0.230 ab	0.195 a	11.165 bcde
IAA*10*10	0.069 a	248.000 a	0.208 a	0.360 a	9.565 bc
Pr > F	< 0.0001	0.002	< 0.0001	< 0.0001	< 0.0001
Significant	yes	yes	yes	yes	yes

Data represent mean values of three replicates. Within columns, mean values followed by different letters are statistically significantly different based on Newman-Keuls (SNK) test at P = 0.05.

Conclusion

Our result show that salt is one of the major abiotic stressor that limits plant growth and productivity in many areas of the world due to the increasing use of poor quality of water for irrigation. In Algeria, the aridity which is linked to a water loss by evapotranspiration which is greater than precipitation covers about 95 percent of the areas (from 100 to 400 mm / year) (Halitim, 1985). In these areas, the supply of water by irrigation resulted in an increase and extension of the salinity of the soil (Daoud, 1993). Plant adaptation or tolerance to salinity stress involves complex physiological and metabolic pathways.

Therefore, an alternative strategy for improving salt stress could be by exogenous application of plant growth regulators. Thus, this focuses on the use of phytohormones such as IAA or kinetin, which has important effects on the regulation of plant response to the environment and the control of certain metabolic changes. It has been reported that treatment with IAA or kinetin reduces the adverse effects of salt stress in wheat. The data support the hypothesis that the beneficial effects of hormones may result from an increase in leaf area, chlorophyll a, b, and carotenoids. In contrast, lower proline has been caused under saline condition levels. This improves plant growth even under high salt concentrations.

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