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Uniconazole effect on endogenous hormones, proteins and proline contents of barley plants (*Hordeum vulgare*) under salinity stress (NaCl)

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Abstract. *Bakheta MA, Hussein MM. 2014. Uniconazole effect on endogenous hormones, proteins and proline contents of barley plants (*Hordeum vulgare*) under salinity stress (NaCl). Nusantara Bioscience 6: 39-44.* Pot experiments were carried out during two growth seasons 2010/2011 under greenhouse conditions of the National Research Centre, Dokki, Cairo, Egypt to investigate the response of barley plants (*Hordeum vulgare* L) grown under salinity stress (2500 or 5000 ppm) to spraying with solutions of uniconazole at 150 or 200 ppm. The obtained results showed that irrigation with saline solutions caused increases in the amounts of abscisic acid (ABA), crude protein, total soluble-protein, and proline contents. The results showed that spraying barley plants grown under saline solutions with uniconazole increased endogenous hormone contents of ABA, cytokinins, crude protein, total soluble protein, and proline but caused decreases in the amounts of endogenous indole acetic acid (IAA) and gibberellic acid (GA_3). High protection of abscisic acid in treating plants with uniconazole and under salt stress (interaction effect) increases proline, proteins and soluble protein which has been proposed to act as compatible solutes that adjust the osmotic potential in the cytoplasm. Thus, these biochemical characters can be used as a metabolic marker in relation to salinity stress.

Keywords: Barley, salinity, uniconazole, abscisic acid, indole acetic acid, gibberellic acid, proline

INTRODUCTION

Plants, growth and production are affected by natural stresses in the form of biotic and abiotic stresses, inversely. The abiotic stress causes loss of hundred million dollars annually, because of reduction and loss of products (Mahajan and Tuteja 2005). Salinity is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world. Plant's behavioral response to salinity is complex and different mechanisms are adopted by plants when they encounter salinity. The soil and water engineering methods increase farm production in the damaged soil by salinity, but achievement of higher purposes by these methods seems to be very difficult (Yokoi et al. 2002). The high salinity of the soil affected the soil penetration, decreased the soil water potential and finally caused physiological drought (Yusuf et al. 2008). The plants under salinity condition change their metabolism to overcome the changed environmental condition. One mechanism utilized by the plants for overcoming the salt stress effects might be via accumulation of compatible osmolytes, such as proline and endogenous hormones. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress are an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm. Thus, proline can be used as a metabolic marker in relation to stress.

Since this soil salinity considers one among the several environmental stresses causing drastic changes in the growth, physiology, and metabolism of plants and threatening physiology and metabolism of plants the cultivation of plants around the globe. Salt accumulation in irrigated soils is one of the main factors that diminish crop productivity, since most of the plants are not halophytic (Jamal et al. 2011). Salt stress induces various biochemical and physiological responses in plants and affects almost all plant processes; salinity also induces water deficit biosynthesis, even in well-watered soils by decreasing the osmotic potential 1 the inhibition of gibberellic acid (Turan et al. 2009).

Uniconazole [(E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-l-yl)-1-penten-3-ol] is a new plant growth retardant in the triazole family. It inhibits gibberellin biosynthesis within the plant (Zhou and Leul 1999), reduces the concentration of endogenous indole-3-acetic acid, and increases the concentration of zeatin, ABA and ethylene (Izumi et al. 1988, Zhou and Leul 1999). Foliar application of uniconazole has been shown to retard leaf elongation, improve tiller number and root growth. Uniconazole applied as a foliar spray at the three-leaf stage improved plant growth, including plant height, leaf size and number, leaf area per plant and increased seed and oil yields of winter rape compared to untreated plants (Leul and Zhou 1998). It was also shown to enhance the plant photosynthetic rate, soluble protein, and total sugar concentrations (Yang et al. 2005a, b). According to Gandee et al. (1997), Zhang et al. (2001), certain interactions existed between uniconazole and N fertilizer, which affected plant growth and yield formation. Our previous study also showed some interactions between uniconazole and different cultivars, sowing dates, planting densities,

and N application levels, which affected the yield formation and enhancement (Yang et al. 2004). However, the effect of uniconazole on grain proteins is uncertain and available studies have not been reported. Exogenous application uniconazole produced some benefit in alleviating the adverse effects of salt stress and they also improve germination, growth, fruit setting, fresh vegetable, and seed yields and yield quality (Bekheta 2009).

The use of plant growth regulators is directed in general, to improve yield quality and /or quantity of many crops through regulating and adjusting the balance of endogenous hormone level in favors of normal physiological process and turn yield. The present investigation was conducted to study the role of uniconazole in ameliorating the adverse effects of salinity through its effects on the endogenous hormones and proline contents.

MATERIALS AND METHODS

Pot experiments were carried out in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during two successive growing seasons (2009/2010 and 2010/2011). Naked barley grains (*Hordeum vulgar*e L) were obtained from the Agricultural Research Center, Ministry of Agriculture. The grains were selected for uniformity by choosing those of equal size and same color. The selected grains were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min. and washed again with distilled water. Factorial experiment laid out in a randomized block design with nine replicates. Ten days after sowing, the seedlings were thinned to two seedlings per pot. In order to reduce compaction and improve drainage, the soil was mixed with yellow sand in a 1:1 proportion. Granular ammonium sulfate 20.5% N at a rate of 40 kg N ha-1 and single super-phosphate (15% P_2O_5) at a rate of 54 kg P_2O_5 ha-1 were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing.

The growth regulator used was uniconazole, a plant growth retardant manufactured by Sumitomo Chemical Company, Ltd, Japan. Fresh solutions of uniconazole (uni.) at the rate of 150 and 200 ppm were applied twice to barley plants, the 1st one spray after 30 days from sowing while the 2nd spray after two weeks later, in addition to the control group (plants sprayed with distilled water). Irrigation water consisted of three concentrations of salt (tap water as a control, 2500 and 5000 ppm) in the form of sodium chloride (NaCl) solution applied 10 days after sowing (DAS). Samples were taken after two weeks for the second application of uniconazole (60 DAS) include number of fresh plants and selected recently leaves to be used for estimation of endogenous hormones and proline contents.

Determination of endogenous hormones by using GLC and HPLC

Extraction

Extraction and separation were essentially similar to that reported by Wasfy and Orrin (1975). The plant material was immersed in cold 85% ethanol in glass Stoppard brown jars which was kept in deep freezer till extraction. The frozen material was homogenized in cold ethanol (85%) by an electric auto mix, then extracted by an electric stirrer with 85% ethanol at about 0°C. The solvent was changed 3 times during the extraction period of 6 hours. The 3 extracts after filtration, were combined together and concentrated under vacuum at 30-35°C to few mL which were kept in a deep freezer till required.

The aqueous phase was adjusted to pH 8.8 by using NaOH (1%). The alkaline aqueous residue was shaken three times with equal quantities of ethyl acetate using separating funnel. The combined ethyl acetate fraction was evaporated to dryness and held for further purification. The aqueous fraction was acidified to pH 2.8 with HCl (1%) and shaken three times with equal volumes of ethyl acetate. The remaining aqueous phase was discarded. The combined acidic ethyl acetate phase was reduced in volume (fraction I) to be used for determination of the acidic hormones (gibberellins "GA3", auxins "IAA" and abscisic "ABA") by using GLC. The aqueous phase was adjusted to pH 5.5 with 1% NaOH and extracted three times with water-saturated n-butanol. All n-butanol phases were combined (fraction II) and reduced to 5 mL volume, then stored at-20°C for cytokinins analysis using HPLC.

Methylation of endogenous hormones

Diazomethane was prepared from methylamine hydrochloride according to the method described by Vogel (1975). Identification of endogenous hormone peaks: Identification of peaks was performed by comparing the relative retention time (RT) of each peak with those of IAA, GA3 and ABA standards. The relative properties of the different individual components were therefore obtained at various retention times on samples. The retention time (RT) of the peaks of authentic samples was used in the identification and characterization of the peaks of samples under investigation (Shindy and Smith 1975).

Identification and determination of auxins, gibberellins, and abscisic acid

The retention time (RT) of the peaks of authentic samples was used in the identification and characterization of the peaks of samples under investigation (Shindy and Smith 1975). Peak identification was performed by comparing the relative retention time of each peak with those of IAA, GAs and ABA standards. The relative properties of the different individual components were therefore obtained at various retention times on samples.

HPLC of cytokinin substances

The retention time (RT) of the peaks of authentic samples was used in the identification and characterization of the peaks of samples under investigation (Shindy and Smith 1975). The peak was performed by comparing the relative retention time of each peak with those of IAA, GAs and ABA standards. The relative properties of the different individual components were therefore obtained at various retention times on samples. Endogenous cytokinins fraction as zeatin was determined by HPLC isocratic UV analyzer ODS Hyparsil C18 column, 20 min gradient from 0.1N acetic acid. pH 2.8 to 0.1 N acetic acid in 95% aqueous ethanol, pH 4. The flow rate: 1 mL /min,

detection: UV 254 nm, standards of zeatin, was used (Muller and Hilgenberg 1986).

Determination of proline

Amino acid-free proline was determined in fresh young leaves of barley plants according to the calorimetric method described by Bates et al. (1973).

Determination of crude protein and total soluble protein

Determination of crude protein and total soluble protein were determined according to the modified method described by Reuveni et al. (1992) with some modifications

Statistical analysis

All the collected data were subjected to the proper statistical analysis as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Endogenous hormones

Indole acetic acid (IAA) and Gibberellins (GA3)

Data presented in Table 1 indicated that irrigated barley plants with solutions of NaCl (2500 or 5000 ppm) caused marked decreases in the endogenous amounts of IAA and GA_3 in comparison with that obtained from the plants that irrigated with tap water. Regarding the influence of uniconazole alone on the endogenous levels of IAA and GA_3 of barley plants, the results show that application of uniconazole at 150 or 200 ppm has the same trend of saline solutions.

Concerning the interaction effect between uniconazole and salinity, the data revealed that application of uniconazole on barley plants irrigated with saline solutions (2500 or 5000 ppm) caused a marked decrease in the endogenous amounts of IAA and GA_3 in comparison with that obtained from their corresponding control (plants sprayed with distilled water and irrigated with tap water). The decrements were directly proportional to the concentration used of uniconazole and salinity, i.e. the highest value of decrements were obtained from the application of uniconazole and salinity at 200 and 5000 ppm respectively. These results are in harmony with that recorded by Egamberdieva (2009).

Abscisic acid content (ABA)

It is clear from the data presented in Table 1 that irrigated barley plants with solutions of NaCl up to 5000 ppm caused an increase in the endogenous amounts of ABA in comparison with the amounts obtained from the plants irrigated with tap water. Regarding the influence of uniconazole, the results show that application of uniconazole at 150 or 200 ppm has the same trend of saline solutions. Concerning the interaction effect of uniconazole and salinity, the data indicated that application of uniconazole on barley plants were grown under salinity stress caused marked increases in the endogenous content of ABA in comparison with that obtained from their corresponding control.

Table 1. The effect of uniconazole on the contents of endogenous hormones of barley plants grown under salt stress (NaCl).

Salinity (NaCl) ppm	Uniconazole (UN) ppm	Gibberellic acid (GA_3) mg/g fresh weight	Indole acetic acid(IAA) mg/g fresh weight	Abscisic acid (ABA) mg/g fresh weight	Cytokinins (CK) µg/g fresh weight
Tap water	0	130.10	48.7	0.93	339
	100	117.33	41.00	1.19	347
	150	100.90	34.30	1.35	350
2500 ppm	0	120.05	40.10	1.38	314
	100	109.11	36.21	1.58	327
	150	98.71	28.90	1.79	346
5000 ppm	0	108.09	32.50	1.51	300
	100	99.22	30.11	1.72	318
	150	81.11	21.76	1.88	329
Mean values of salinity		116.11	41.33	1.15	345
		109.29	35.07	1.58	329
		96.14	28.12	1.73	316
Mean values of uniconazole		111.09	40.43	1.27	345
		102.22	35.77	110	329
		93.57	28.22	1.67	330

The obtained results are in harmony with Rademacher (2000) who concluded that enzymes, similar to the ones involved in GAs biosynthesis, are also important in the formation of abscisic acid, ethylene, sterols, flavonoids, and other plant constituents. Side activities of such enzymes can mostly explain changes in the levels of these compounds found after treatment with growth retardants. The promotion in the growth of hormone-treated plants grown under stress conditions could be attributed to its effect on hormonal balance between the values of growth promoters and inhibitors. Application of ABA on plants grown under drought stress induced biosynthesis of gene manipulation called AtNCED3 (9-cis-epoxycarotenoid diogenase), this gene is thought to be the key enzyme in ABA biosynthesis, which in turn led to accumulation of endogenous ABA, improved drought tolerance (Iuchi et al. 2001). Under stress conditions, plants tend to increase endogenous ABA, which may contribute to the maintenance of water relations between the second and the third day of water stress treatments Yang et al. (2004).

Plant adaptive responses to drought are coordinated by adjusting growth and developmental processes as well as molecular and cellular activities. The root system is the primary site that perceives drought stress signals, and its development is profoundly affected by soil water content. Various growth hormones, particularly abscisic acid (ABA) and auxin, play a critical role in root growth under drought through complex signaling networks. Here, we report that a R2R3-type MYB transcription factor, MYB96, regulates the drought stress response by integrating ABA and auxin signals. The *MYB96*-mediated ABA signals are integrated into an auxin signaling pathway that involves a subset of *GH3* genes encoding auxin-conjugating enzymes. A *MYB96*-overexpressing Arabidopsis (*Arabidopsis thaliana*)

mutant exhibited enhanced drought resistance with reduced lateral roots. In the mutant, while lateral root primordia were normally developed, meristem activation and lateral root elongation were suppressed. In contrast, a T-DNA insertional knockout mutant was more susceptible to drought. Auxin also induces *MYB96* primarily in the roots, which in turn induces the *GH3* genes and modulates endogenous auxin levels during lateral root development. We propose that *MYB96* is a molecular link that mediates ABA-auxin cross talk in drought stress response and lateral root growth, providing an adaptive strategy under drought stress conditions Seo et al. (2009).

Cytokinins

Data presented in Table 1 indicated that irrigated barley plants with solutions of NaCl caused a decrease in the endogenous levels of cytokinins in comparison with that obtained from the plants that irrigated with tap water. On the other hand, application of uniconazole caused a marked increase in the endogenous levels of cytokinins as compared with the amount obtained from the untreated plants.

Concerning the interaction of uniconazole and salinity on the endogenous contents of cytokinins extracted from the plants treated with uniconazole and grown under salt stress, the obtained data indicated that application of uniconazole on barley plants grown under salinity stress caused marked increases in the endogenous contents of cytokinins in comparison with that obtained from their corresponding control. Similar results were obtained by Bekheta (2000); Zaky (2000) and Upreti and Murti (2004) and Bekheta and Ramadan (2005) on wheat, Vicia faba, beans and cotton plants respectively. In addition, SA reduced the damaging action of salinity on plant growth and accelerates reparation of the growth processes mediated by maintaining a high level of IAA, CKs and ABA, which in turn induce a wide spectrum of anti-stress reactions in plants.

The high concentrations of cytokinins were observed in the leaves of cotton plants grown under drought stress could be related to delay in leaf senescence. Meanwhile, the high levels of ABA observed in plants contribute to the acceleration of leaf senescence (Efetova et al. 2007).

Influence on proline content

Table 2 shows that irrigation of barley plants with salinity of 2500 or 5000 ppm caused significant increases in the endogenous content of amino acid "*proline*" in comparison with that obtained from their corresponding control (plants irrigated with tap water). Many investigators (Bekheta (2009); Khosravinejad et al. (2009) and Heidari and Sarani 2012) work in different plants indicated that salinity stress significantly increased proline content. Sanaullah (2000) pointed out that proline contents were significantly increased ($p<0.001$) in the resistant lines of wheat viz. cv. Pak-81, Lyllpur-73 and Capelle and barley viz. Jau-87 and Haider-93 with increasing concentrations of NaCl. Accumulation of proline was failed in spikes and shoots and therefore a non-significant increase in proline content, even at the highest salinity level was observed. Recently, Heidari and Sarani (2012) recorded that salinity stress significantly increased proline and soluble carbohydrate in the leaves of chamomile *Matricaria chamomilla* L.

Table 2. The influence of uniconazole on amino acid (proline) of barley plants grown salt stress (calculated as µ mol/g F. wt.)

Uniconazole treat.	0.0 ppm	150 ppm	200 ppm
Salinity ppm (NaCl)			
Control (tap water)	11.03	13.56	15.76
2500 ppm	13.90	15.66	18.09
5000 ppm	16.23	17.78	20.00
LSD at 1%	2.56		

It is clear from the same Table 2 that application of uniconazole at two used treatments (150 or 200 ppm) caused considerable increases in proline of barley plants in comparison with that obtained from the untreated plants.

The results show that application of uniconazole under salt stress led to a significant increase in the amounts of proline as compared with that obtained from their respective control. The maximum value of increments was obtained from the plants treated with uniconazole at 200 ppm and irrigated with saline water at 5000 ppm.

One of the most important mechanisms of higher plants under salt-stress is the accumulation of compatible solutes such as proline. Proline accumulation in salt-stressed plants is a primary defense response to maintain the osmotic pressure in a cell. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in salt-tolerant and salt-sensitive cultivars of many crops (Desingh and Kanagaraj 2007; Turan et al. 2007). In addition, a positive correlation was determined between proline and tissue-Na concentrations under salt stress (Bajji et al. 2001). The present study shows that uniconazole treatments induced an increase in proline concentrations in barley plants under salt stress. A similar result has been reported by Cha-Um and Kirdmanee (2009) and Turan et al. (2009).

Total crude protein and soluble protein

Data recorded in Table 3 show that irrigated barley plants with saline solutions at 2500 or 5000 ppm caused significant increases in both total-N and total soluble-nitrogen of barley plants in comparison with that obtained from the plants irrigated with tap water. Demiral and Turkan (2005) detected that total soluble protein content of salt tolerant *Oryza sativa* cv Pokkali plants increased with salinity while sensitive (*Oryza sativa* cv. IR-28) rice cultivars showed a decrease under salt stress. Similar results were reported in salt tolerant cultivars of barley, sunflower, finger millet and rice plants, These different results of salt stress showed that the responses to salt stress depend on plant species, even in varieties of same plant species plant developmental stage, duration and severity of the salt application (Parvaiz and Satyavati 2008).

Table 3. The effect of uniconazole on the total - N and total soluble-N of the leaves of barley plants grown under salt stress (NaCl)

Salinity (S) ppm	Uniconazole (UN) ppm	Total-protein as mg/100 g F. wt.	Total soluble protein as mg/100 g F. wt.
Tap water	0	52.50	13.00
	150	56.88	14.22
	200	59.67	15.33
2500	0	54.09	13.99
	150	58.12	14.74
	200	61.99	16.17
5000	0	60.00	15.00
	150	64.07	15.67
	200	68.55	16.98
LSD at 1 %		3.35	0.81

The obtained results indicate that application of uniconazole of all the used treatments (150 or 200 ppm) caused considerable increases in the total protein and total soluble protein of barley plants in comparison with that obtained from the untreated plants. These results are in harmony with Al-Rumaih and Al-Rumaih (2007) reported that using uniconazole on *Datura* significantly increased protein levels and stimulated nitrate reductase activity, particularly at lower NaCl concentrations.

Application of uniconazole under salinity stress led to increase in the amounts of total proteins and total soluble protein as compared to their respective control. Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later (Singh et al. 1987) and may play a role in osmotic adjustment. They may be synthesized *de novo* in response to salt stress or may be present constitutively at low (Pareek-Singla and Grover 1997). It has been concluded that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells (Hasegawa et al. 2000). A higher content of soluble proteins has been observed in salt tolerant cultivars of barley, sunflower, finger millet, and rice (Ashraf and Harris 2004). In higher plants, osmotic stress induces several proteins in vegetative tissues, which are related to late-embryogenesis-abundant (LEA) proteins. The correlation between LEA protein accumulation in vegetative tissues and stress tolerance indicates its protective role under dehydration stress (Ingram and Bartels 1996). Engineered rice plants overexpressing a barley *LEA* gene, *HVA1*, under the control of rice actin 1 promoter showed better stress tolerance than did the wild type (Xu et al. 1996).

CONCLUSION

It is clear from this study that the ability of plants to tolerate salt stress is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions and maintain ion homeostasis. In our study application of uniconazole at (150 or 200 ppm) on barley plants grown under salinity stress at 2500 and 5000 ppm led to increases in the synthesis of osmotically active metabolites, total proteins, total soluble nitrogen, specific proteins, amino acid proline as well as the endogenous hormones GA_3, IAA, cytokinins" and especially ABA. Proteins that accumulate in plants due to application of uniconazole on the barley plant under salinity stress may provide a storage form of nitrogen that is re-utilized later and may play a role in osmotic adjustment. Such all these compounds might be used to protect the plants against stress conditions.

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