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Phytohormones Responses of *Ricinus communis* to Saline Soils and Regulation of Biosynthetic Hormone Pathways

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Abstract

This study has been conducted to assess and analyze the nature of saline soil characteristics and their effect on Phytohormones responses of *Ricinus communis* has been studied for four-season in four different sites. Samples were collected seasonally for the period February 2018 to January 2019, taken from four selected sites in (Najaf), Iraq. four levels of saline soils were cultivated with the halophyte species (*Ricinus communis*). The soils had the following salinity levels:

1) Nonsaline soil (S1, 0.9 dS. m^{-1})

2) Slightly saline soil (S2, 4.2 dS. m^{-1})

3) Moderately saline soil (S3, 7.2 dS m^{-1})

4) Highly saline soil (S4, 14.1 dS m^{-1})

The environmental factors of Soil were additionally evaluated for electrical conductivity, pH, and soil ion concentrations prior to planting. Hormones play crucial roles in the plant's response to saline stress. In Winter (GA) and (Za) free contents enhanced to first response then (IAA) bound contents at Spring then (IAA) free contents in Summer at last (ABA) bound contents in Autumn as a gradual response to increasing saline stress.In general, it can be used Phytohormones' contents as an indicator of saline stresses. It is also possible for species adapted to these environments to be used as salt-tolerant gene materials to enhance conventional crops during selection, breeding and improving environments.The water may change gene expression, which is reflected in a change in the nuclear gene cloning, which raising (ABA) level.

Keywords: *Ricinus communis*; Salinestress; Auxin (IAA); Cytokinin (CK); Gibberellic acid (GA); Abscisic acid (ABA)

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Introduction

Plant cells and tissues show numerous metabolic responses to Saline stress, some of which may have adaptive significance [1]. The level of ROS in plants is controlled by synchronous action of enzymatic and non-enzymatic antioxidants. Among non-enzymatic antioxidants include compounds with low molecular weight are responsible for different vital pathways during response to various abiotic stresses are "The most powerful players in intercellular regulation". Trace amount of growth regulators can change a broad vital metabolism, Phytohormones are grouped in to auxin (IAA), cytokinin (CK), gibberellic acid (GA) and abscisic acid (ABA) [2]. Plants hormones have deferent functions in growth and development. In nutrient allocation, and source/sink transitions however, based on former description relating to sensors and second messengers, and could transmitting information over large distances [3].

Auxin {Indole-3-acetic acid (IAA)}

IAA is predominantly produced in cells of the apex (bud) and young leaves of a plant [4]. Plants mainly produce IAA from tryptophan through indole-3-pyruvic acid [5]. IAA is also produced from tryptophan through indole-3-acetaldoxime in Arabidopsis [6]. IAA is the most abundant endogenous auxin.Auxin can be divided into free and conjugated forms, and there are many types of conjugated auxin including ester-conjugated auxin.

Gibberellins (GAs)

Gibberellins are plant hormones that regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence [7]. They are produced by leaves and root apex, also they are produced in fruits and young seed embryos are considered a main source of them.

Abscisic acid (ABA)

Abscisic acid was called "abscisin II" originally because it was thought to play a major role in abscission of leave and fruits. Though

ABA is generally thought to play mostly inhibitory roles, it has many promoting functions [8]. ABA is a naturally occurring hormone in plants. It is partially produced via the mevalonic pathway in chloroplasts and other plastids. In contrast to auxin, gibberellins, and cytokines, which are represented by various active derivatives, ABA is a single compound. The production of ABA is accentuated by stresses such as water loss and freezing temperatures. It is believed that biosynthesis occurs indirectly through the production of carotenoids [9], may explain an apparent role in pathogen defense. A large recent study showed that treating plants with exogenous hormones will transiently change genome large transcript profiles [10]. A complex gene regulated by hormones under abiotic stresses. The ability of plants under a wide range of saline stresses to change balanced through the interaction of hormonal plant growth regulators and the redox signaling. Plant hormones produce (ROS) as second signal cascades that convey information concerning changes in concentrations of hormone and/or mediate sensitivity to all range of adaptive responses [11]. (Castor oil plant) *Ricinus communis* is a tree belonging to the family of Acalyphoidceae, leaves and seeds are poisonous, but their seeds contain 50% of their weight oil for medical use. Studies show that *Ricinus communis* grows in soils of different pH, salinity, fertility and textures, and attains high productivity under different climatic conditions has been a most preferred unique plant system, where saline stress is the most limiting factor [12]. In the present study, a sub-acute experiment was conducted to examine the combined effects of Saline stresses on *Ricinus communis* by testing Phytohormones system of the plant to identify their potential role as biomarkers.

Materials and Methods

The used soil was collected from different sites of saline- In the province of Najaf (36 ° 52'39 "N 39 ° 02'02" E) in Iraq. Soil samples were examined after collecting from the top of the surface of the soil (10-15 cm) in the design of random squares and four times for each type, then dried with air and then sieve using a measuring mesh sieve 4 mm. EC was tested for soil samples as follows:

- Non-saline soil (S1, EC=0.8 dS. m^{-1})
- Low Salinity Soil (S2, EC=3.9 dS. m^{-1})
- Soil moderately saline (S3, EC=6.9 dS. m^{-1})
- High salinity soil (S4, EC=13.5 dS. m^{-1})

Samples were collected from the soil (where the plant grew) to determine EC, pH, and ion content. Plant materials were harvested in four different sites on each locality. Plants were harvested seasonally fourth time during the study. Sample collected in an ice bucket and in the shortest possible time brought to the laboratory. Plant samples were cleaned with tap water and distilled water, Fresh plant material (fully expanded and undamaged leaves), was separated from whole plants and kept in cool place 4℃ for studies. Content of free and bound plant hormones were determined for indole acetic acid (IAA), gibberellic acid (GA), Zeatin and abscisic acid (ABA) according to the method using by Unyayar (1996) as follow:

- For hormones determination, 1 gm of plant tissue (leaves) was squashed with 60 ml of mix solution that is consisted of methanol, chloroform, and ammonia at a ratio of (12:3:5). Then, 25 ml of dH_2O was added to the previous solution. Chloroform layer was removed.
- pH was adjusted to 2.5, then 15 ml of acetic acid were added, and hormones mix was extracted. To calculate the free content of plant hormones, optical absorbance of the mix extract was evaluated using a UV-visible spectrophotometer (EMCLab, Germany) at 3 different wave lengths (280, 254, 263) nm for indole acetic acid (IAA), gibberellic acid (GA), Zeatin and abscisic acid (ABA) respectively
- To calculate the bound content of plant hormones in the same samples above, the left aqueous layer was moved to an oven of 70℃ for an hour to extract the bound hormones, then step (2) was repeated. A content of each hormone calculated using standard curve. Note: pH level was adjusted using HCl(1N) and NaOH 1(1N).

Statistical analysis

Analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The data were presented as the means for each treatment. Means were compared using the LSD test at the 0.05 probability level.

Results

pH and EC values for soil were determined before and after the growth of saline plants. EC results were significantly reduced after saline plant growth in soil types S1=LSS, S2=SMS, and S3=SHS. The amount of EC in the SHS soil type was 3.5 dsi/mand 4.2 dsi/m after the castor cultivation, respectively, compared to 14.6 dsi/m SHS of uncultivated (Table 1). Results from soil analysis, for pH, the cultivation of castor in saline soil did not affect the pH values of the soil (Table 1). The values of soluble soil ions Na^{++}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, and SO_4^{-2} were higher within saline-infected soils prior to transplantation and control. The increase in salinity levels was reflected in ion concentrations. However, ion concentrations in all saline soils decreased after the cultivation of saline plants. Despite the reduction in ion concentrations for saline plants, the castor plant recorded a significant increase in the reduction of ions concentrations. For example, the removal of sodium ions by castor was 1.9 times and the removal of Cl^{-} ions by castor was 1.7 times. similar results were obtained for other ions.

IAA free contents in leaves in S1 show highest value significantly ($p<0.05$) in Summer as contrast with other seasons and sites (Figure 1). The (IAA) content is significantly enhanced in Summer more than other seasons in S1, S2 and S4, while S3 showed the highest value in Winter (Figure 1). S1 showed a highervalue in Summer>Spring>Winter>Autumn. The same in S2 and S4. S2 showed a higher value in Winter>Spring. Significant differences in each season in different sites (Figure 1).

IAA bound contents in leaves in S1 show highest value significantly ($p<0.05$) in Summer and Spring as contrast with other seasons and sites (Figure 2). The (IAA) content is significantly enhanced in Spring more than other seasons in S1, but in S2, S3 and S4 showed

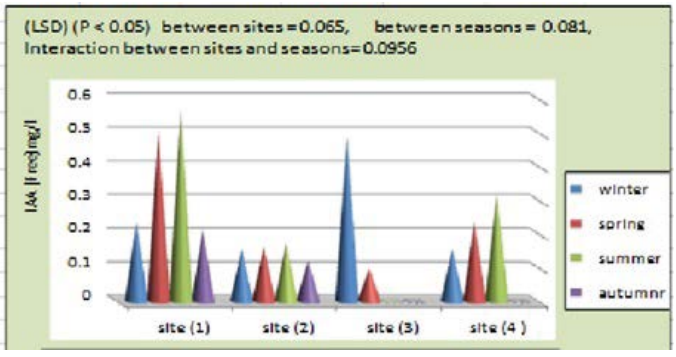

Figure 1: Variation in Indole-3-acetic acid free hormones contents (mg/I F.W.) in *Phragmites australis*in different sites and seasons.

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Table 1: pH and EC values and soluble soil ions values Na^{++}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, and SO_4^{-2} for control and breeding soil after harvesting the castor plants for the four different salinity levels.

Plants	Soil Type	EC dS /mc	pH	Na+ meq/L	K+ meq/L	Ca++ meq/L	Mg++ meq/L	Cl- meq/L	SO4-- meq/L
Control	S1	0.87a	7.3a	1.45a	0.4a	0.54a	4.32a	2.54a	1.45a
	S2	4.122a	7.4a	10.5a	0.82a	20.6a	19.5a	7.54a	4.5a
	S3	6.7a	7.6a	17.5a	1.32a	31.76a	27.5b	12.5a	4.6a
	S4	14.6a	7.7a	47.54a	1.42a	45.6a	45.7b	25.7a	6.43a
RCS	S1	0.64a	7.6a	0.78b	0.25a	0.25b	2.32a	1.26a	1.23a
	S2	1.3b	7.7a	4.54b	0.32b	10.63b	9.8b	2.56b	1.24a
	S4	2.1b	7.8a	6.45c	0.34b	17.45b	16.5b	3.6a	1.31b
	S4	3.5b	7.9a	12.4c	0.35b	25.75b	25.67a	6.8c	1.74c

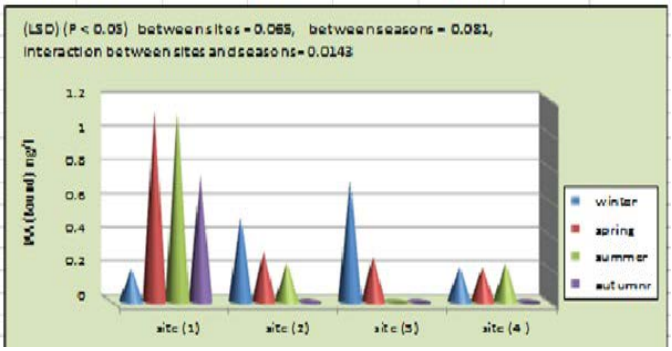

Figure 2: Variation in Indole-3-acetic acid bound hormones contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

the highest value in Winter (Figure 2). S1 showed a higher value in Spring>Summer>Autumn>Winter while, S2, S3 and S4 showed a highervalue in Winter >Spring. Significant differences in each season in different sites (Figure 2).

GAs free contents in leaves in S1 show highest value significantly ($p<0.05$) in Winter as contrast with other seasons and sites (Figure 3). The (GAs) content is significantly enhanced in Winter more than other seasons in S1, S2 and S3, but in S4, Spring showed the highest value (Figure 3). S1, S2 and S3 showed a highervalue in Winter>Spring>Summer>Autumn while, S4 showed a higher value in Spring>Autumn>Winter. Significant differences in each season in different sites.

GAs bound contents in leaves in S4 show highest value significantly ($p<0.05$) in Winter and Spring as contrast with other seasons and sites (Figure 4). The (GAs) content is significantly enhanced in Spring more than other seasons in S1, S2 and S4 but in S3 showed the highest value in Winter. S1, S2 and S4 showed a highervalue in Spring>Summer>Autumn>Winter while, S3 showed a higher value in Winter>Spring. Significant differences in each season in different sites (Figure 4).

Za free contents in leaves in S1 show highest value significantly ($p<0.05$) in Winter and Spring as contrast with other seasons and sites (Figure 5). The (Za) content is no significantly enhanced in Winter and Spring more than other seasons in S1 and S3, but in S2, Autumn showed the highest value and in S4, Spring showed the highest value (Figure 5). Significant differences in each season in different sites.

(Za) bound contents in leaves in S2 show highest value significantly ($p<0.05$) in Spring as contrast with other seasons and sites (Figure 6). The (Za) content is significantly enhanced in Spring more than other seasons in S2, S3 and S4 but in S1 showed the highest value in Winter (Figure 6). S2 and S3 showed a higher value in Spring>Autumn>Summer>Winter while, S1 showed a higher value

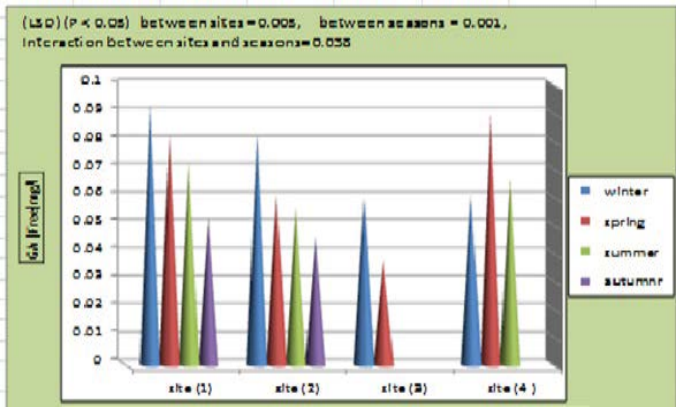

Figure 3: Variation in gibberellins free hormone contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

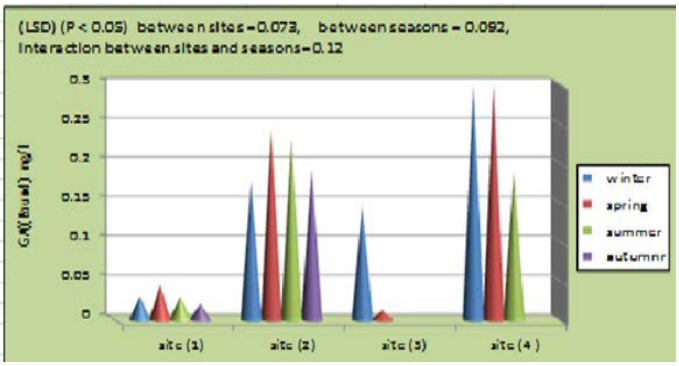

Figure 4: Variation in Gibberellins bound hormone contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

in Winter>Spring>Autumn>Summer and S4 showed a highervalue in Winter and Spring>Autumn>Summer. Significant differences in each season in different sites (Figure 6).

(ABA) free contents in leaves in S4 show highest value significantly ($p<0.05$) in Spring as contrast with other seasons and sites (Figure 7). The (ABA) content is significantly enhanced in Spring more than other seasons in S3 and S4 but in S1and S2 showed the highest value in Winter (Figure 7). S1 and S2 showed a highervalue in Winter>A utumn>Summer>Springwhile, S3and S4 showed a highervalue in Spring>Winter>Autumn. Significant differences in each season in different sites (Figure 7).

(ABA) bound contents in leaves in S1 show highest value significantly ($p<0.05$) in Autumn as contrast with other seasons and sites (Figure 8). The (ABA) content is significantly enhanced in Autumn more than other seasons in S1 and S2 but in S3and S4 showed the highest value in Spring and Summer, respectively (Figure 8). S1

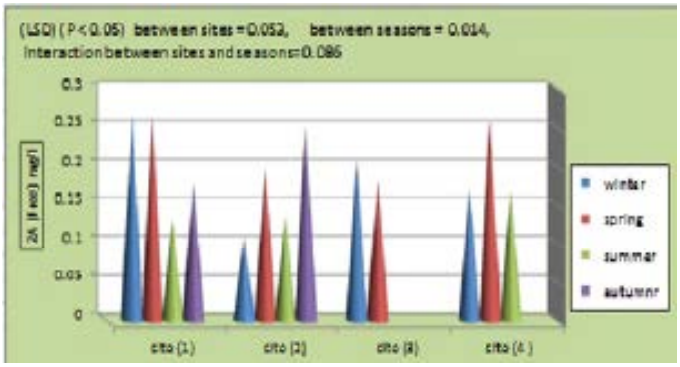

Figure 5: Variation in Zeatin free hormone contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

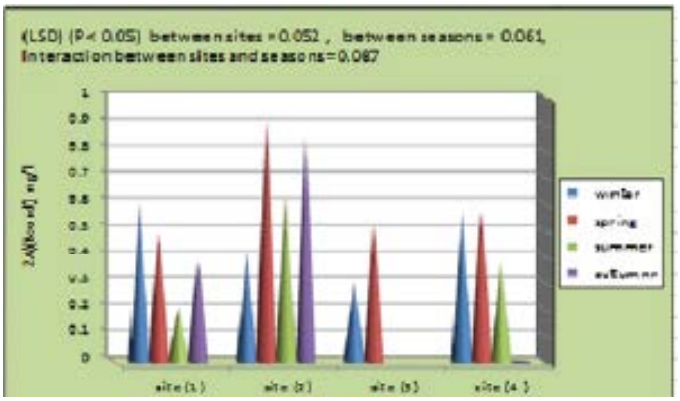

Figure 6: Variation in Zeatin bound hormone contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

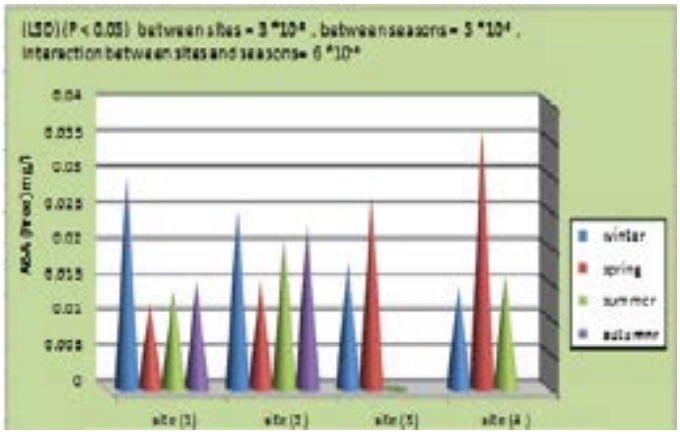

Figure 7: Variation in Abscisic acid free hormone contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

and S2 showed a highervalue in Autumn>Summer>Spring>Winter while, S3and S4 showed a higher value in Autumn>Spring>Winter. Significant differences in each season in different sites (Figure 8).

Discussion

The higher concentration of saline in the contaminated sites stimulates the oxidative stress in the plants (Figures 1-8). The synergistic or antagonistic hormone action and the coordinated regulation of hormone biosynthetic pathways play crucial roles in the adaptation of plants to saline stress. The auxin synthesis associated with, perception, and action has been shown to be regulated by ethylene [13]. Auxin was

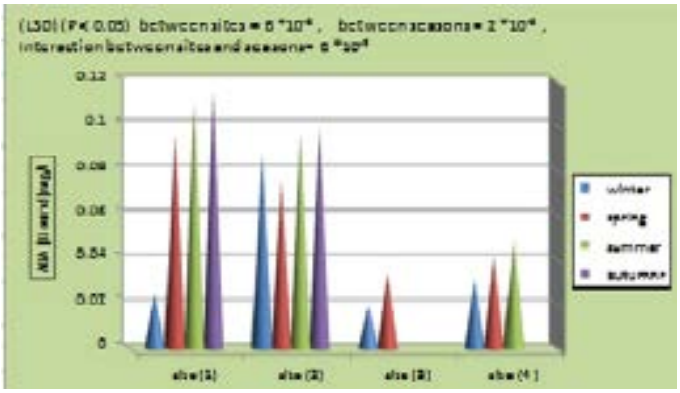

Figure 8: Variation in Abscisic acid bound hormone contents (mg/I F.W.) in *Phragmites australis* in different sites and seasons.

found to affect ethylene biosynthesis. Recently, (Za) was also shown to be a positive regulator of auxin biosynthesis, and it was signaling acts to maintain appropriate (Za) and (IAA) concentrations in developing root and shoot tissues [12]. (GA) regulate many common physiological processes. A member of the GA3 gene family, showed improved tolerance under saline stress [11] (ABA) regulates stomatal opening during stress. Recent studies suggest that other hormones such as (CK) affect stomatal function [12]. While, (ABA) induce stomatal closure, (Za) and (IAA) promote stomatal opening. Results showed the highest value of (IAA) free contents in summerand bound contents in spring, (GA) free contents in winter, (Za) free contents in winter and (ABA) bound contents in autumnin leaves in S1 show the highest value significantly in contrast with other sites and seasons, that means in winter (GA) and (Za) free contents are enhanced to first response then (IAA) bound contents in spring then (IAA) free contents in summerat last (ABA) bound contents in autumnas a gradualresponse to increase environmentalfactors like salinity anddrought, that explain change in Phragmites growth. The same in S2 and S4 while, S3 show the highest value in Winter that mean salinity of S3 in winter was diluted to be Phragmites growth.The (ABA)content is significantly enhanced in Autumn more than other seasons in S1 and S2 but in S3 and S4 show the highest value in Springand summer, respectively. (IAA), (GA) and (Za) levels have decreased with increased salt concentration, while a rise in abscisic content with increasing salt concentration may be due to the decline in the construction of amino acids such as histidine and tryptophan, which is apparent to build auxin as well as the young leaves are the synthesis of IAA Center and as growth declined due to the salinity of this reflected in the construction of IAA in leave [13]. This decline may be a result of the crash enzymes for building these hormones or may be the result of increasing the effectiveness of enzymes that work to break down and the demolition of hormones such as increased enzyme activity (IAA-Oxidase) which works to break down the hormone auxin with increasing plant agealso found reduced both the hormone (IAA), (GA) and (Za) content when a corn plant to salinity stress [14,15]. Abscisic acid play greats role in (GA) reduce concentration of the fact that (ABA) and (GA) have the same vital building trails path [16]. Za reduce concentration because of changes in water potential or may be the result of a decline in the Biosynthesis of (Za) or for changes in the molecule of (Za) which lead to stop building or because of the accumulation of inhibitors such (ABA) [17]. Abscisic acid increase with changes in the salt concentration, since it was found that the hormone increases the tensile under saline conditions and drought [18]. Since the initial response to decrease in water potential because of the water deficit may be a result of the release compound restricted in chloroplasts and the study confirmed that the water may

change gene expression, which is reflected in a change in the nuclear gene cloning, which raising (ABA) level [19,20]. According to them, the present study indicates that phytohormonescontents changedue to higher concentration of saline (Figures 1-8). This clearly indicates that the enhanced production of superoxide radicals creates an oxidative stress to the *Ricinus communis* as saline indicator.

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