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EEFECT OF SOME APPLIED ANTIOXIDANTS ON PHYTOHORMONES CONTENT OF TWO RICE (*Oryza sativa L*.) CULTIVARS UNDER DROUHT STRESS CONDITIONS.

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ABSTRACT

One of the main problems of rice cultivation and production is the lack of water resources. In this study the effect of applied antioxidants (ascorbic acid , humic acid and seaweed extract) on endogenous indole acetic acid (IAA), gibberellins (GA), cytokinins (cyto.) and abscisic acid (ABA) contents in two rice cultivars Sakha 101 (as a sensitive cultivar) and Giza 178 (as a tolerance cultivar) were measured under some drought treatment . Results indicated that high drought treatment decreased the promoters hormones (IAA, GA, and Cytokinin) but increased the inhibitor one (ABA). When applied antioxidants were used, the promoters (IAA, GA and cytokinin) increased but the inhibitor was decreased. Data suggested that ascorbic acid is one of the applied antioxidants which enhanced phytohormones content under drought stress in rice.

INTRODUCTION

Drought is the most important limiting factor for crop production which cause sever problem in many regions of the world (Passioura, 2007). According to statistical study drought affected land areas more than double from 1970 to early 2000 in the world Isendahl and (Schmidt 2006). Drought is a world-spread problem seriously influencing grain production and quality with increasing population Hongbo, *et al.,* (2005). Rice (*Oryza sativa* L.) as a paddy field crop is particularly susceptible to water stress (Yang, *et al.,* 2008). It is estimated that 50% of the world rice production is affected more or less by drought stress (Bouman, *et al.,* 2005).

To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought condition. Plants behavior responses to drought condition are complex and different mechanisms are adopted by plants when they encounter drought (Jones 2004). One mechanisms utilized by the plants for overcome the water stress effect might be changes in phytohormones concentration (Farooq *et al*., 2009). Plants hormones devoted to promoters such as indol acetic acid, gibberellins and cytokinins and inhibitor such as abscisic acid. Under drought condition, abscisic acid was increased (Yang, *et al*., 2007) but promoters such as IAA, GA and cytokinin were decreased (Yang *et al.,* ,2001). Water stress remarkable increased ABA accumulation, whereas substantially decreased GA contents (Qin and Tang, 1984). On the other, drought stress damages plant cells thus the accumulation of reactive oxygen species (Ros) were increased (Dat, *et al.,* 1998).

Application of applied antioxidants can improve phytohormones concentrations of plants and enhance stress tolerance (Ozaki Takuo *et al.,* 2003), ascorbic acid reduced the rate of Ros generation, improve the activity of promoters (IAA, GA and cytokinins) and decreased the rate of inhibitor (ABA).

Humic acid is a natural substance which enhanced stress tolerance and regulated hormone level (Abd-El-Kareem 2007), seaweed extract, humic acid organic compounds which generated auxins, gibberellins, indole acetic acid and cytokinins. (El-Shami 1987).

Growth substances like ascorbic acid, humic acid and seaweed extract enhance plant tolerance to drought, salinity and chilling (Zhenfi Guo *et al*., 2005). The present study was conducted to study the effect of drought stress on plant hormones in the two tested rice cultivars and evaluated the effect of the three applied antioxidants under study on rice under different water stress treatments **.**

MATERIALS AND METHODS

Two field experiments were carried out at El- Khielaleh village, Bilquas district, Dakahlia governorate, Egypt during the summer season of both 2007 and 2008 seasons to test the alternative effect of some growth substances on two cultivars (Sakha 101 and Giza 178) on rice phytohormones under certain drought schemes.

Asplit plot design with four replicates was used. The main plots were devoted to the two cultivars Sakha 101 (drought sensitive) and Giza 178 (drought tolerance). The sub plots were assigned to four irrigation treatment, continuous flooding, irrigation every 7 day, irrigation every 10 days and irrigation every 13 days. Growth substance as follows water only, ascorbic acid (100 ppm), humic acid (500 ppm) and seaweed extract (500 ppm) were located in the sub-sub plots.

Culture practices:

The nursery area was fertilized with calcium superphosphate (15.5% P_2O_5) at the rate of 100 kg/fed on dry soil before ploughing. Nitrogen in the form of urea (46% N) was added at the rate of 72 kg/fed. Zinc sulphate (24% Zn SO_4) at the rate of 24 kg/fed was applied after puddling and before seeds broad cast. Rice seeds at the rate of 60 kg/fed were soaked in each of water only, ascorbic acid (100 ppm), humic acid (500 ppm) or seaweed extract (500 ppm) for 24 hours and incubated for 48 hours. Thereafter, seeds were sown at may 15^{th} in both seasons.

Weeds were chemicaly controlled using Saturn 50% at the rate of 2 liters/ fed as recommended , seven days after sowing. Blood –worm was controlled by furadan 10% at the rate of 6 kg/fed applied before seeding. Rice varieties used were : Sakha 101 (drought sensitive) and Giza 178 (drought tolerance). These cultivars were obtained from Rice Research and Training Center, Sakha, Kafer El-Sheikh, Egypt. Description of these cultivars are presented in Table (1).

Table (1): Genotypic and phenotypic characteristics of rice cv. Giza 178 and Sakha 101.

Characters	Sakha 101	Giza 178
Crosses	G. 178/Milyang 79	G. 175/Milyang 79
Leaves type	Bright erect	Bright erect
Tillering capacity	High	High
Stature	Short	Short
Grain	Short	Short
Response to N	High	High
Blast resistance	Resistant	Resistance
Salt sensitivity	Sensitive	Tolerant
Drought resistance	Sensitive	Tolerant
Duration	140 days	130-135 days
Type	Japonica	Japonica x indica

Permanent field:

The previous crop was wheat (*Triticum aestivum* L.) in both seasons. The permanent field was well prepared through good ploughing and leveling. Phosphorus in the form of calcium superphosohate (15.5% P_2O_5) at the rate of 100 kg/fed was added on the dry soil. The sub-sub plot dimensions were five meters in length and three meters in width (15m). Nitrogen in the form of urea (46%N) at the rate of 69 kg/fed were applied in two splits; 2/3 of the rate as basal application + 1/3 of the as top-dressing at five days before panicle initiation.Weeds were controlled with Saturn 50% at five days after transplanting . The other cultural practices were followed as recommended by Rice Research and Training Center, Ministry of Agriculture and Land Reclamation , Egypt .

Each irrigation treatment was thoroughly separated from each other where it was surrounded by deep channels to prevent any lateral movement of water from irrigation treatment to another.

Plants were sprayed twice after 40 and 50 days from sowing (during the vegetative growth stage) with each of:

1- Tap water.
2- Ascorbic acid at (250 ppm).
3- Humic acid at(1000 ppm).
4- Seaweed extract at (1000 ppm).

Mechanical and chemical analysis of soil:

Representative soil samples were taken from the experimental sites at a depth of 0 to 30 cm from soil surface and prepared for both mechanical and chemical analysis according to (piper 1950). Results of the mechanical and chemical properties of the experimental sites in 2007 and 2008 seasons are presented in Table 2.

Sampling dates:

Samples were taken throughout the experimental period during booting stage(80 days after sowing).

Table 2. Mechanical and chemical analysis of soil at the experimental sites during 2007 and 2008 seasons.

Characters	2007	2008
A. Mechanical analysis:		
Soil fraction:		
Sand %	15.00	17.00
Silt %	27.10	26.30
Clay %	57.90	56.70
B. chemical analysis:		
pH	8.10	7.90
E.C. mm hos/cm	2.00	1.70
Organic matter (O.M) %	1.30	1.53
Available N ppm	29.00	30.00
Available P ppm	15.00	13.00
Available K ppm	300.00	350.00

Studied traits:

Phytohormones determination :

At booting stage(80 days from sowing) ,thirty grams fresh weight of the shoots were extracted with 80%cold methanol according to the method (Shindy and Smith 1975). The acidic ethyl acetate fraction was concentrated to dryness to determine IAA,GA and ABA. Whereas, alkaline fraction was used to determine cytokinins. Bioassay techniques of (Bently and Housley 1954) (Wheat cleoptile straight assay) was followed for IAA, lettuce hypocotyls assay (Frankland and Wareing, 1960)) for gibberellins, radish cotyledons assay (Letham,1967) for cytokinins, and wheat transpiration assay (Rademacher,1978) for ABA.

Statistical analysis:

The data were statistically analyzed as the technique of analysis of variance (Anova) of the split split plot design according to Gomez and Gomez (1984). The treatment means were compared using the least significant difference (LSD).

RESULTS

Data in the table (3) show the effect of some applied antioxidants (ascorbic acid, humic acid and seaweed extract) on endogenous phytohormones content (IAA, GA , cytokinins and ABA) (mg/g F.Wt) in the two rice cultivar Sakha 101 (drought sensitive) and Giza 178 (drought tolerance) under different drought treatment.

Data showed that all water stress treatment decreased the content of promoters (IAA, GA and Cytokinin) but the content of ABA (inhibitor) was increased. Data also indicated that irrigation every 10 and 13 days increased the content of ABA.

Data in the same table revealed that applied antioxidants (ascorbic acid, humic acid and seaweed extract) gave positive effect on phytohormones content.

The estimated phytohormones (IAA, GA, and cytokinin) content were increased under any of applied antioxidants treatments while ABA (inhibitor) was decreased. Ascorbic acid treatment produced the highest values of promoters hormones (IAA, GA and cytokinin) followed by the other growth substances (humic acid and seaweed extract) while ABA (inhibitor) gave the least.

Table (3): Phytohormones content (IAA, GA , Cytokinin and ABA) (mg/g F.Wt) of rice cultivars Sakha 101 and Giza 178 as affected by ascorbic acid, humic acid and seaweed extract under drought stress treatment.

Characters / Treatments	indole acetic acid(LAA)			Gibberellin acid (GA)			Cytokinin(cyto)			Abscisic acid (ABA)		
	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average
A.cultivars:												
Cv1	100.3	100.5	100.4	63.5	63.5	63.5	216.3	216.5	216.4	21.00	21.44	21.22
Cv2	106.3	106.4	106.4	65.5	65.3	65.4	221.5	221.7	221.6	19.04	19.14	19.09
F.test	**	**		*	**		*	**		**	*	
B.Irrigation teratment:												
I	114.1	113.7	113.9	73.5	73.6	73.6	238.0	238.5	238.3	17.43	17.57	17.50
I_2	103.6	103.5	103.6	64.1	63.8	64.0	219.7	220.2	220.0	19.90	20.55	20.23
I_3	98.1	98.8	98.5	61.5	61.5	61.5	211.3	211.3	211.3	20.95	21.08	21.02
I_4	97.2	96.8	97.0	59.1	58.6	58.9	206.6	206.5	206.6	21.80	21.96	21.88
f.test	**	**		**	**		**	**		**	**	
LSD at 5%	0.9	0.7		1.2	1.1		1.4	1.3		0.12	0.70	
LSD at 1%	1.3	1.0		1.7	1.5		2.0	1.8		0.17	0.99	
C.growth substances:												
Control	96.5	96.0	96.5	59.5	59.3	59.4	214	214.3	214.2	21.6	17.57	19.59
A.	108.7	109.5	109.1	69.5	69.6	69.6	224.6	224.3	224.2	18.95	20.55	19.75
H.	105.1	104.6	104.9	65.8	65.5	65.7	219.8	220.1	220.0	19.51	21.08	20.30
S.	102.7	102.3	102.5	63.3	63.1	63.2	217.2	217.7	217.5	20.02	21.96	20.99
F.TEST	**	**		**	**		**	**		**	**	
LSD at 5%	1.0	0.9		1.1	0.8		1.3	1.0		0.13	0.61	
LSD at 1%	1.3	1.2		1.5	1.1		1.7	1.3		0.18	0.81	
D.interactions:												
A x B	**	**		NS	NS		NS	NS		**	**	
A X c	**	**		**	**		NS	NS		**	**	
B x C	**	**		**	**		NS	**		**	*	
A x B x C	NS	NS		NS	*		NS	*		**	NS	

Data in table (4) show that the interactions between rice cultivars and water stress treatments had significant effect on IAA content in both seasons .The highest values of IAA content was obtained by the cultivar of Giza 178 and irrigation level of continous flooding , but the lowest values of IAA content was obtained by the cultivar of Sakha 101 and water stress treatment of irrigation every 13 days .

Table (4) : Indole acetic acid content (LAA) As affected by the interaction between cultivars and water stress tretment (Ax B) during first and second seasons.

Irrigation regimes / Cultivars	2007				2008			
	I_1	I_2	I_3	I_4	I_1	I_2	I_3	I_4
Cv_1	110.0	100.5	96.7	93.7	109.2	100.2	97.2	93.5
Cv_2	118.2	106.7	99.5	100.0	118.2	106.7	100.5	100.5
F. test	**				**			
LSD at 5%	1.27				1.04			
LSD at 1%	1.78				1.46			

Data in table (5) clearly showed that the interactions between applied antioxidants and water stress treatment had significant effect on IAA content in both seasons . Ascorbic acid treatment gave the highest values of IAA content under continous flooding treatment compared with control , humic acid and seaweed extract treatment .

Table (5): Indole acetic acid content (IAA)as affected by the interaction between irrigation treatment and growth substances (B X C) during first and second seasons.

Growth substances	2007				2008			
	control	A.	H.	S.	control	A.	H.	S.
Irrigation treatment	108.5	119.0	116.0	113.0	108.5	119.5	115.0	112.0
I1	97.0	110.0	105.0	102.5	95.5	110.5	105.0	103.0
I2	92.5	102.5	100.0	97.5	93.5	104.0	100.5	97.5
I3	88.0	103.5	99.5	98.5	88.5	104.0	98.5	97.5
I4	**				**			
LSD at 5%	1.94				1.78			
LSD at 1%	2.59				2.38			

Results in table (6) recorded that the highest values of GA content was obtained by rice cultivar of Giza 178 and growth substance of ascorbic acid in both seasons . the lowest values of GA content was obtained by rice cultivar of Sakha 101 and control treatment of water only in both seasons .

Table(6): Gibberellic acid content (GA) as affected by the interaction between cultivars and growth substances (A x C)during 2007 and 2008 seasons.

Growth substances / Cultivars	2007				2008			
	control	A.	H.	S.	control	A.	H.	S.
Cv_1	57.5	69.5	65.2	62.0	57.5	69.0	65.2	62.2
Cv_2	61.5	69.5	66.5	64.7	61.2	70.2	65.7	64.0
F. test	**				**			
LSD at 5%	1.62				1.19			
LSD at 1%	2.16				1.59			

Results in table (7) observed that GA content was 69.5(mg/g F.Wt) when ascorbic acid treatment was used under water stress treatment of irrigation every 7 days also , the content of GA was 70 (mg/g F.Wt) under control treatment in 2007 season . In the second season GA content was70(mg/g F.Wt) when ascorbic acid treatment was used under water stress treatment of irrigation every 7 days also , GA content was 70.5 (mg/g F.Wt) under control treatment .

Table (7): Gibberellic acid content (GA) as affected by the interaction between irrigation treatment and growth substances (B x C) during FIRST and SEASONS seasons.

Growth substances / Irrigation treatment	2007				2008			
	control	A.	H.	S.	control	A.	H.	S.
I1	70.5	76.5	74.5	73.0	70.5	76.5	74.5	73.0
I2	58.5	69.5	65.5	63.0	58.5	70.0	64.5	62.5
I3	56.0	67.5	63.0	59.5	55.5	68.0	63.0	59.5
I4	53.0	64.5	61.0	58.5	53.0	64.0	60.0	57.5
F. test	*				**			
LSD at 5%	1.59				1.69			
LSD at 1%	_				2.26			

Data in table (8) show that ABA content gave the highest value when Sakha 101 was used under water stress of irrigation every 13days in both seasons .The lowest value of ABA content was obtained when Giza 178 was used under continous flooding condition .

Table (8) : Abscisic acid content (ABA) As affected by the interaction between cultivars and irrigation treatment (A x B) during first and second seasons .

Irrigation treatment / Cultivars	2007				2008			
	I_1	I_2	I_3	I_4	I_1	I_2	I_3	I_4
Cv_1	17.60	21.12	22.17	23.10	17.92	22.25	22.30	23.30
Cv_2	17.60	18.67	19.72	23.10	17.22	18.85	19.87	20.63
F. test	**				**			
LSD at 5%	0.17				0.40			
LSD at 1%	0,24				0.47			

Results in table (9) showed that the interactions between growth substance and water stress treatment had significant effect on ABA content in both seasons. The highest value of ABA content was obtained by the treatment of control (water only) and water stress treatment of irrigation every 13 days. The lowest value of ABA content was obtained by growth substance of ascorbic acid and continous flooding .

Table (9) : Abscisic acid content (ABA) As affected by the interaction between irrigation treatment and growth substances (B x C) during first and second seasons .

Growth substances / treatment Irrigation	2007				2008			
	control	A.	H.	S.	control	A.	H.	S.
I1	18.05	17.00	17.30	17.40	18.50	17.10	17.35	17.55
I2	21.30	18.70	19.40	20.20	21.60	20.61	19.60	20.40
I3	22.90	19.55	20.35	21.00	23.05	19.80	20.45	21.05
I4	24.15	20.55	21.00	21.50	24.46	20.55	21.20	21.65
F. test	**				*			
LSD at 5%	0.27				0.31			
LSD at 1%	0.36				_			

DISCUSSION

The decreases in the content of promoters (IAA, GA and cytokinin) in two rice cultivars leaves were found to be remarkable during drought stress the reduction in level of cytokinin in excaudate of plants recovering from water and salinity stress as compared of control plants. Itai and Vaadia (1965), according to them, metabolic shifts and enhanced aging in shoots of stressed plants may be attributed in partly to reduced supply of root cytokinins.

Gibberellins also were found to be decreased in plants grown under drought condition. Yang et al., (2007) considered ABA as the primary drought, thus its endogenous level was increased with water stress . Qin and Tang (1984) reported that with increasing drought stresses, the tryptophan synthesis ά- monomers were gradually dissociated from the oligomers producing less action isoenzymes. This reduced the biosynthesis of L-tryptophane and consequently decreased the concentration of IAA.

Similarly, El-Desouky and Atwa (1998) stated that the biological activities of cytokinins, gibberellins and auxins were significantly reduced under drought stress . It has recently been shown that such dehydration induced ABA accumulation in rice plants is triggered by the perception of a plasma lemma-tension (Jian, *et al.,* 2001). Leaf tissue may produce ABA in response to salt, water and chilling stress. But it triggered by the dehydration mechanism, i.e. the perception of plasma lemma-tension, with much less sensitivity. The rapidity of ABA accumulation in response to drought points to more direct role of ABA in promoting growth increases in ABA were considerable and fast. This implies that ABA was either donova synthesized or released at high rates from glucose-ester conjugates of the hormone (Saker 1996).

Many earlier studies showed that leaf can accumulate much ABA in response to drought stress Zhao and Liu (1995). Sultana *et al.,* (2000). Stresses including water, salt and cold temperatures, induce ABA synthesis, ABA may be considered a plant stress hormone. It regulates several important aspects of plant growth and development. Nevertheless, ABA acts as a mediator in controlling adaptive plant response to environmental stresses. During vegetative growth stage, roots of many angiosperms synthesize ABA and transport it into the shoots under water stress conditions, ABA is an essential mediator in triggering plant responses to adverse environmental stress. This is known to occur in a number of crop plants which include rice, barley, soybean, tomato, cotton, and alfalfa. Leaf ABA content in wild plants increase with water stress. Upon rehydration, the ABA level caused to increase and returned to pre-stressed levels. Substantial evidences suggests that increased ABA levels limit water loss by reducing stomatal aperture. ABA regulates the process of adaptation into two interacting steps. First, ABA acts via differential signal transduction pathways on cells which are the least and most affected by the imposed stress. Second, ABA may regulate through some genes/gene products, which control the expression of stress or adaptative specific genes.

Ascorbic acid is an integral weapon in the defence a gains Ros generated by salt, water, chilling and ozone stress (Conklin & Barth 2004). (Arrigoni & De Tullio 2002) suggested that an involvement of AsA as a co-factor in synthesis of ABA, Gibberellin and ethylene under high levels of stress condition the availability co factor of AsA was decreased and result in increased ABA biosynthesis (Pastori et al., 2003). Ascorbic acid is also strictly required by some enzymes that are involved in GA biosynthesis (Arrigoni & Detullio 2000)

Humic acid (HA): Wang. *et al.,* (1999)humic acid had been hypothenized that increases in photo-chemical efficiency, antioxidant level and endogenous cytokinin and auxin levels (Zhang *et al.,* 2003). (Zhang and Schmidt 2000) found that humic acid and seaweed extract alone or in combination resulted in greater leaf -tecopherol content subject to drought . Fryer (1992) indicated that there is asignificant correlation between the concentration of - ά tocopherol in chloroplasts and drought tolerance but increasing leaf antioxidant activity may improve drought tolerance by quenching reactive oxygen species and protecting membrane intergrity (Smirnoff. 1955).

Seaweed extract have been shown to exhibit various beneficial effects on plant growth and development application of SWE increased resistance to environmental stresses such as drought and salinity. (Xun Zhang and E. H. Ervin 2004) suggested that seaweed extract is a natural products which contain cytokinins and their application resulted in increased endogenous cytokinin levels, possibly leading to improved creeping bent grass drought resistance.

Seaweed extract enhancing leaf water status and stimulate biosynthesis of endogenous cytokinins for root (T. Butler *et al.,* 2006), also it altering hormonal balances and favor cytokinin and auxin.

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تأثير بعض منظمات النمو على المحتوى الهرمونى لصنفين من أصناف الأرز تحت ظروف الجفاف

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**** مركز بحوث الأرز واليدريب بسخا – مركز البحوث الزراعية**

أقيمت تجربتان حقليتان بقرية الخلالة – مركز بلقاس – محافظة الدقهلية بمصر خلال موسمى ٢٠٠٧ , ٢٠٠٨ لدراسة محتوى بعض الهرمونات (أندول حمض الخليك , الجبريللين ,السيتوكينين , حامض الأبسيسك) لصنفين من أصناف الارز (سخا ١٠١ , جيزة ١٧٨) عند المعاملة ببعض مواد النمو (حمض أسكوربك, حمض هيومك, مستخلص أعشاب البحر) تحت معاملات رى مختلفة (رى مستمر , رى كل ٧ , ١٠ , ١٣ يوم)

أشارت النتائج إلى تأثر كلا الصنفين بظروف الجفاف حيث أن أفضل محتوى لهرمونات الاندول حمض الخليك, الجبريللين , السيتوكينين كان تحت ظروف الغمر المستمر وكان أقل محتوى لحامض الابسيسك تحت هذة الظروف .

أظهرت النتائج أن النقص فى محتوى هذة الهرمونات يتوالى بزيادة تعرض النباتات للجفاف ماعدا حامض الأبسيسك فقد زاد محتواة تحت هذة الظروف .

أوضحت النتائج أن المعاملة ببعض منظمات النمو (حمض أسكوربك, حمض هيومك, مستخلص أعشاب البحر) زاد المحتوى الهرمونى من أندول حمض الخليك, الجبريللين , السيتوكينين وقل المحتوى الهرمونى من حامض الابسيسك تحت مستويات الجفاف المختلفة بالمقارنة بالكنترول .أعطى حمض الاسكوربك أفضل النتائج تحت هذة الدراسة .

أظهرت النتائج أن محتوى صنف الارز جيزة ١٧٨ من الهرمونات المنشطة (أندول حمض الخليك ,الجبريللين ' السيتوكينين) كان أعلى من الصنف سخا ١٠١ فيما عدا حامض الابسيسك فكان محتواة فى الصنف سخا ١٠١ أعلى من الصنف جيزة ١٧٨ .

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