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INFLUENCE OF THE TRIAZOLE GROWTH RETARDANT UNICONAZOLE ON PHYTOHORMONE LEVELS IN WHEAT PLANTS.

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The effect of soaking wheat grains in uniconazole, a triazole type growth retardant, was studied on the endogenous levels of IAA, ABA, cytokinins and IAA-oxidase activity. Uniconazole treated plants had lower IAA and higher ABA and cytokinins (Z, ZR, ZG) contents. IAA-oxidase activity was also increased.

Abbreviations : ABA = Abscisic Acid; IAA, IndoleAcetic Acid; Z = Zeatin, ZR = Zeatin Riboside; ZG = Zeatin Glucoside.

Keywords : Endogenous plant hormones, IAA-oxidase, wheat plant, uniconazole (S-3307).

تأثير اليونيكونازول (مثبط نمو الترايازول) على مستوى الهرمونات الداخلية لنبات القمح

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تم دراسة تأثير مثبط النمو الترايازول بنقع حبوب القمح في اليونيكونازول على مستوى الهرمونات الداخلية لأفرع نبات القمح من IAA ، ABA ، السيتوكينين ، ونشاط IAA-oxidase. وجد أن النباتات المعاملة باليونيكونازول قد انخفض محتواها من IAA وزاد محتواها من ABA والسيتوكينين (زياتين ، زياتين-ريبوسيد ، زياتين-جلوكوسيد) ، كما ازداد أيضاً نشاط IAA-oxidase في النباتات المعاملة.

INTRODUCTION

Uniconazole is a triazole type of a new growth retardant of practical use and has potent biological activity for higher plants (Oshio *et al*., 1990; Kar and Gupta, 1993 and Wang and Chen, 1997). Like many other growth retardants, the effects of traizoles may be attributed to interference with gibberellin biosynthesis and the reaction sites are three oxidative steps from ent-kaurene to kaurenoic acid (Izumi *et al*., 1985; Lenton, 1987 and Rademacher *et al*., 1987). Triazoles efficacy on other endogenous hormones was also reported by several investigators (Lenton, 1987; Ye *et al*., 1995 and Wang, *et al*., 1997). In addition to retardation properties it is known to affect reproductive growth and development of different plants (Hathout, 1995 and Khalil and Al-Abdulkreem, 1999). These biological phenomena are regulated by the balance among different endogenous hormones. The objective of this study was to investigate the effects of uniconazole on endogenous IAA, cytokinins and ABA in wheat plants at different stages of growth.

MATERIALS AND METHODS

Plant growth and treatment

Two pot experiments were conducted during two successive seasons of 1992 and 1993 in the experimental greenhouse of Girls College of Science, Dammam, Saudi Arabia.

Uniform wheat grains *Triticum durum* cv. Legaim, were soaked in aqueous solutions of uniconazole (S-3307) obtained from Sumitomo Chemical Co., Ltd., at concentrations of 0, 100, 800 mg/L active ingredient (a.i.). All grains were gently aerated and left at 25°C for a period of 12 hours, then thoroughly washed with water and sown in pots filled with 12.5 kg of soil. Mechanical analysis of the soil was 28 % sand, 40 % silt and 29 % clay. The pots were fertilized at the rate of 0.25, 0.2 and 0.1 g of N, P_20_5 and K_20 per kg of soil respectively divided in two applications. Samples were collected at random after 15, 45 and 60 days from sowing. Fresh shoots were frozen in liquid nitrogen immediately after sampling.

Estimation of endogenous hormones

Indole acetic acid and Abscisic acid (IAA & ABA) :

Endogenous IAA and ABA were determined using gas liquid chromatography (GLC). Extraction and purification for IAA were performed according to Jensen and Junttila (1982) and for ABA according to Terry *et al*. (1982). Extraction and purification were carried out in dim light.

GLC procedure

A GLC (Perkin Elemer, Sigma 3B) was used. The GLC was equipped with flame ionization detector and stainless steel column (6 feet length X 3 mm i.d.). The column was packed with 1 % OV-17. The oven temperature was programmed to increase by 10°C per minute in the range of 200°C up to 300°C. The temperatures of the injector and detector were 280°C and 300°C

respectively. Flow rates of nitrogen, hydrogen and air were adjusted to 30 ml/min., 30 ml/min and 300 ml/min., respectively.

Cytokinins zeaitn, zeatin riboside and zeatin glycoside (Z, ZR, ZG)

Endogenous cytokinins were determined using high performance liquid chromatography (HPLC). Extraction was carried according to Van Staden (1973) then purified as given by Izumi *et al*. (1988).

HPLC Procedure

Analysis of samples by HPLC was carried out on a Perkin Elmer Unit., Series (2) fitted with UV detector, delivery system and analytical C18 reversed phase column. The solvent program was a linear gradient (40 % - 60 %) methanol and 0.05 M phosphoric acid, the flow rate was 1.2 ml/min.

IAA oxidase activity

The activity of this enzyme was assayed following the method of Katsumi and Sano (1968). Enzyme activity per hour was expressed as µg of IAA oxidized by the enzyme extracted from fresh tissue.

Statistical analysis

Obtainable data were statistically analyzed using Duncan Multiple Range test (Duncan, 1955). Combined analysis of the two experiments was calculated since the same trend of the results was obtained (Snedecor and Cochran, 1980).

RESULTS

Endogenous levels of IAA

Endogenous IAA decreased significantly due to uniconazole treatments over all ages studied. Maximum reduction was attained by 800 mg/l treatment and reached 37.5 %, 37.0 % and 42.9 % for 15, 45 and 60 days age respectively (Table 1).

Endogenous levels of ABA

Table (1) shows an increase in ABA concentration of wheat plant due to uniconazole treatments. The highest increase was recorded by 800 mg/l uniconazole treatment. Percentage of increments amounted to 30.7%, 41.9 % and 55.6 % as the plants reached 15, 45 and 60 day old, respectively.

Table 1: Effect of uniconazole on IAA and ABA concentrations of wheat shoots (ng/g Fresh Wt.).

Uniconazole mg/l	Plant height % of control	Concentrations of IAA (ng/g Fresh Wt.)	Concentrations of ABA (ng/g Fresh Wt.)
15 days age			
Control	100(22.11cm)	15.2 c	10.1 a
100	70.65	13.7 b	11.0 a
800	61.20	9.5 a	12.2 b
45 days age			

Control	100 (35.09 cm)	30.2 c	17.9 a
100	78.05	27.0 b	18.5 a
800	68.34	19.0 a	25.4 b
60 days age			
Control	100 (52.69 cm)	31.2 c	18.4 a
100	76.82	25.0 b	19.0 a
800	61.17	17.8 a	28.0 b

Mean values followed by the same alphabetical letter within each column are not significant at 5 % level of probability.

Endogenous levels of cytokinins

Table (2) shows the effect of uniconazole treatments on cytokinin fractions namely Z, ZG and ZR. Marked and significant increases in cytokinin fractions were obtained by uniconazole treatments over all the stages studied. At 15 days age increments of Z reached 118 % and 168% for 100 and 800 mg/l uniconazole treatments respectively. However, ZR increased to 74 % and 84 % level and ZG to 40 % and 70.0 % %. At 45 days age cytokinin levels increased compared with those of 15 days. Uniconazole treatments at 100 and 800 mg/l increased Z content 93 % and 110 % and ZR to 57 % and 72 % respectively. With respect to ZG, 800 mg/l uniconazole treatment increased this fraction to 64.3 %.

The same trend of enhancing cytokinin levels, was observed as the plants reached 60 days old, Z increased to 123.5 % and 113.3 % and ZR to 77 % and 89 % for 100 and 800 mg/l uniconazole treatments. Limited increase in the ZG fraction resulted by 100 mg/l. However, 800 mg/l uniconazole showed a maximum increase in ZG which amounted to 74.0 %.

Table 2: Effect of uniconazole on the cytokinin concentrations (Z, ZR, ZG) of wheat shoots (ng/g Fresh Wt.).

Uniconazole mg/l	Zeatin Z	Zeatin Riboside ZR	Zeatin Glucoside ZG
15 days age			
Control	0.82	1.15 a	0.30
100	1.79 b	2.01 b	0.42 b
800	2.20 c	2.12 b	0.51 c
45 days age			
Control	1.45 a	2.35	0.42 a
100	2.81 b	3.70 b	0.41 a
800	3.20 c	4.05 b	0.69 b
60 days age			
Control	1.67 a	2.20 a	0.35 a
100	3.72 b	3.89 b	0.36 a
800	3.55 b	4.15 b	0.61 b

Mean values followed by the same alphabetical letter within each column are not significant at 5 % level of probability.

IAA oxidase activity

Uniconazole treatments increased significantly IAA oxidase activity over all the stages studied. Enzyme activity at 15 days age due to 800 mg/l uniconazole treatment. As the plants reached 45 and 60 days old pronounced and significant increase was observed by using uniconazole at the rate of 100 and 800 mg/l (Table 3).

Table 3: Effect of uniconazole on IAA-oxidase activity of wheat shoots (µg of IAA destroyed/hr/g Fresh Wt.).

Uniconazole mg/l	Plant age (days)		
	15	45	60
0	9.65 a	19.8 a	16.5 a
100	10.80 a	23.9 b	27.2 b
800	16.80 b	31.85 c	36.2 c

Mean values followed by the same alphabetical letter within each column are not significant at 5 % level of probability.

DISCUSSION

Reduction of endogenous levels of gibberellins by treatments with uniconazole has been previously reported in wheat plants by Al-Abdulkreem (1993). Similar responses were obtained by different triazole treated plants (Yamaji *et al*., 1991; Kim *et al*., 1994; Imam, 1995 and Wang and Chen, 1997).

The outcome of this study indicates significant decrease in the endogenous levels of IAA concomitant with reduction of shoot length. These results are correlated with those in uniconazole treated Peas cv. Alaska, rice and *Pilea cardierei* plants (Law and Hamilton, 1989; Tang *et al*., 1990 and Wang *et al*., 1994). Tang *et al*. (1992) reported decrease in IAA content of rape cv. Nigyou accompanied by increase of ABA amounted to 43.4 % and 88.7 % respectively. The same behaviour was recorded in *Vicia faba* and Satsna cv. Hyashi plants (Imam and Bekheta, 1996 and Kojima *et al*., 1996). The findings in this study showed that uniconazole treatments increased ABA level of wheat plants. A similar increase has been reported in rape, wheat, and rice (Hauser *et al*., 1990; Liao *et al*., 1990 and Tang *et al*., 1992). On the other hand, reduction of ABA level was found in treated soybean plants (Grossman *et al*., 1987 and Buta and Spaulding, 1991). However, no change in ABA content was noticed in treated *Pennisetum purpureum* (Rajasekaran, *et al*., 1987).

Reduction of IAA in this study is attributed to enhancement of IAA-oxidase activity as similar conclusion was reported by Wang *et al*. (1997) who found a decrease in IAA accompanied with high activity of IAA oxidase and reduction of the rate of transformation of 3H tryptophan into 3H IAA.

The most obvious effect of uniconazole on the endogenous hormones level was the marked and significant increase of cytokinins (Z, ZR, ZG) in wheat plants. Zeatin is the more active natural occurring cytokinins and ZR is an important translocation form (Letham and Palni, 1983). Increase in cytokinin fractions was reported in rice seedling, sunflower suspension and rape plants (Oshio *et al*., 1990; Grossman *et al*., 1993; Grossman *et al*., 1994 and Ye *et al*.,

1995). The high level of endogenous cytokinins in the present study led to significant increase of number of tillers and leaves concomitant with high level of photosynthetic pigments and protein (Khalil and Al-Abdulkreem, 1999). It has been suggested that growth regulating effects of triazoles are mediated with the isoprenoid pathway and thus shifting the balance of important plant hormones in that pathway, including gibberellins, ABA and cytokinins (Fletcher and Hofstra, 1985). From these observations it can be inferred that the physiological effects induced by uniconazole may be regulated by the balance of endogenous hormones.

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