J Plant Growth Regul (1993) 12:1–3

Journal of
Plant
Growth
Regulation

Uniconazole (S-3307) Induced Cadmium Tolerance in Wheat

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Received December 4, 1991; Accepted February 8, 1992

Abstract. Uniconazole, a triazole, was applied to seed at a concentration of 0.1 g kg^{-1} seed to protect wheat plants from the toxic metal cadmium (Cd). The degree of protection afforded by uniconazole against Cd toxicity was assessed by measuring fresh and dry weights of shoots and roots and by estimating the chlorophyll and solute leakage level in the leaves. Fresh weights and dry weights of roots and shoots were higher in Cd + uniconazole treated plants compared to uniconazole and cadmium treatment alone. Uniconazole + cadmium treated plants were darker in color, having more chlorophyll. Solute leakage was increased with the increasing concentrations of Cd and loss of membrane permeability was alleviated by the use of uniconazole.

Uniconazole (S-3307)-(E)-(p-chlorophenyl)-4,4-dimethyle-2-(12,4-triazol-1-yl)-1-penten-3-ol, is a triazole derivative and has been shown to have potent fungitoxic and growth regulator properties in plants (Fletcher et al. 1986). In addition to these properties, uniconazole has been referred to as a multiprotectant (Fletcher and Hofstra 1985). It protects plants from several unrelated stresses, including injury from drought, heat, chilling, and air pollutants. Its potential use as a plant protectant has been reviewed by Fletcher and Hofstra (1985, 1988). Protection against these stresses may in part be due to increases in tocopherol and ascorbic acid which act as antioxidants. It has been suggested that the plant growth regulating and protective properties of the triazole are primarily mediated by interference with the isoprenoid pathway through inhibition of C-14 demethylation reactions and the consequent block of sterol and gibberellin biosynthesis (Fletcher and Hofstra 1988).

Since the triazoles protect plants from several unrelated stresses, the objective of this work was to assess the role of a triazole (uniconazole) in protecting plants from metal toxicity. It is known that toxic metals, to a large extent, are dispersed in the biosphere through sewage irrigation and industrial effluents. Metals affect morphological, physiological, biochemical, embryological, and cytological activities in plants. We have examined the protective properties of uniconazole against cadmium in wheat, and we now present those results.

Materials and Methods

Wheat (*Triticum aestivum* L. cv. Fredrick) seeds were obtained from Stokes Seeds, Ltd. (St. Catharines, Ontario, Canada). Uniconazole was applied as a seed treatment at a concentration of 0.1 g kg-1 seed. Seeds were kept 6 h in uniconazole solution and dried for further experimental work. Treated and nontreated seeds were planted in vermiculite in styrofoam cups (336 ml) with perforated bottoms, and each cup was placed in a second nonperforated cup that served as an irrigation reservoir.

Both uniconazole-treated seed and nontreated seed were treated with different concentrations (25, 50, 100, and 200 ppm) of cadmium solution. Plants were watered regularly with distilled water and kept in a controlled-environment room under 16 h of light and 8 h of darkness, with day/night, temperature of 25/20°C, respectively. Light was provided from Sylvania metarc lamps with 75 W m^{-2} irradiance at plant level.

Fresh and dry weight of roots and shoots were determined 45 days after sowing the seeds.

Chlorophyll Determination

Chlorophyll content was extracted with 80% acetone and then centrifuged at 2500 *g* for 10 min. The volume of the supernatant was brought to 15 ml and the absorbance at 663 and 645 nm, and recorded with a Beckman Acta III Spectrophotometer. Total chlorophyll level (a + b) was estimated following Kirk (1968).

Solute leakage from leaves was determined by taking two leaves from each treatment, putting them in 16 ml of double-distilled water, and gently swirling by hand. After the initial determinations, the conductivity of the incubation medium was measured again after 2 h. The solute leakage was expressed as the difference between the initial and final conductivity of the solution.

Table 1. Effect of different concentrations of cadmium in the presence and absence of uniconazole on growth of wheat seedlings.

	Root growth[a]		Shoot growth[a]	
Treatments	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	24.68 ± 2.31	3.28 ± 0.14	21.72 ± 4.16	4.27 ± 0.63
Uniconazole	25.01 ± 1.61	4.55 ± 0.17	23.14 ± 2.30	4.02 ± 0.73
Cadmium				
25 ppm	16.76 ± 1.43	2.10 ± 0.14	14.53 ± 1.13	2.40 ± 0.17
50 ppm	10.36 ± 0.38	1.71 ± 0.12	9.70 ± 1.11	1.75 ± 0.03
100 ppm	3.91 ± 0.13	0.45 ± 0.01	4.17 ± 0.93	1.03 ± 0.02
200 ppm	0.95 ± 0.03	0.22 ± 0.07	1.70 ± 0.06	0.45 ± 0.01
Cadmium + uniconazole				
25 ppm + uni	22.34 ± 1.61	4.32 ± 0.19	12.18 ± 1.63	2.48 ± 0.73
50 ppm + uni	18.51 ± 2.13	4.05 ± 0.63	9.50 ± 1.01	2.49 ± 0.09
100 ppm + uni	9.32 ± 0.13	0.58 ± 0.06	6.50 ± 0.86	1.24 ± 0.08
200 ppm + uni	1.89 ± 0.01	0.58 ± 0.05	2.79 ± 0.43	1.00 ± 0.01

[a] ± SD.

Results and Discussion

There were marked visual differences between the cadmium-treated plants and cadmium + uniconazole-treated plants. The cadmium + uniconazole treated plants were bigger in size and darker green in color in comparison to cadmium-treated plants. Inhibition of plant growth by cadmium is well documented in cereals such as corn, *Zea mays* (Cutler and Rains 1974), and in *Sorghum halepense* and *Cicer arientinum* (Shrotriya et al. 1982). Chlorophyll loss and associated visual symptoms such as chlorosis and necrosis have also been reported in many systems (Root et al. 1975, Haghiri 1973, Singh 1988). However, in this case, inhibition of plant growth by cadmium in the presence of uniconazole is less than the plant treated with cadmium alone. The toxic symptoms (chlorosis and necrosis), which develop after 15–20 days in the case of cadmium-treated plants, are prevented by uniconazole. The data presented in Tables 1 and 2 indicate that Cd inhibited root and shoot growth and total chlorophyll content in wheat seedlings, and that it accelerated the leakage of the solutes as indicated by the conductivity of the medium (Table 2). The effect was increased with increasing concentrations of toxic metals. Treatment of seeds with uniconazole ameliorated the toxic effects of the cadmium to some extent, although at the concentrations used it did not negate the cadmium toxicity completely. The values for percentage protection by uniconazole expressed as the fresh weight and dry weight of roots and shoots, solute leakage, and chlorophyll content by uniconazole are given in Table 3. However, antagonistic effects of uniconazole on these parameters and also on solute leakage are reported for the first time.

Table 2. Effect of cadmium and cadmium + uniconazole on solute leakage from leaves as percentage conductivity (ds m^{-1}) and total chlorophyll (mg^{-1} g fresh weight).

Treatments	Conductivity[a] (ds m^{-1})	Total chlorophyll[a] (mg^{-1} g fresh weight)
Control	61.90 ± 4.63	1.65 ± 0.38
Uniconazole	54.85 ± 2.78	1.85 ± 0.26
Cadmium		
25 ppm	65.98 ± 4.38	1.40 ± 0.26
50 ppm	68.08 ± 6.19	1.35 ± 0.13
100 ppm	69.44 ± 4.27	1.31 ± 0.11
200 ppm	71.23 ± 3.61	1.26 ± 0.02
Cadmium + uniconazole		
25 ppm + uni	51.20 ± 3.17	1.57 ± 0.26
50 ppm + uni	60.50 ± 2.79	1.38 ± 0.13
100 ppm + uni	43.57 ± 3.83	1.57 ± 0.03
200 ppm + uni	59.40 ± 4.03	1.45 ± 0.07

[a] ± SD.

In drought protection of bean seedlings by triadimefon, a transient increase in ABA content has been observed (Asare-Boamah et al. 1986). Involvement of ABA and cytokinins in protection against heat and chilling injury has also been suggested (Asare-Boamah and Fletcher 1986).

The growth regulatory and protective properties of triazoles are mediated by interfering with the isoprenoid pathway and thus shifting the composition of sterol and the balance of important plant hormones in the pathway, including gibberellins, ABA, and cytokinins. The ultimate effect of exogenous application of triazoles would therefore be dependent on the dynamic equilibrium attained by the sterols and hormones at a specific stage of plant

Table 3. Percentage protection of fresh weight, dry weights of roots and shoots, conductivity, and chlorophyll by uniconazole.

	Percentage protection in					
Treatments	Fresh weight root	Dry weight root	Fresh weight shoot	Dry weight shoot	Solute leakage	Chlorophyll
Control	100	100	100	100	100	100
Cadmium						
25 ppm	25.2	31.1	14.3[a]	5.4	14.7	0.02
50 ppm	32.4	35.5	3.6[a]	20.9	7.5	3.59
100 ppm	21.4	0.7	8.8	6.7	25.8	5.47
200 ppm	3.6	6.0	4.2	14.3	11.8	2.01

[a] Values are negative.

growth and development (Fletcher and Hofstra 1985, Asare-Boamah et al. 1986, Asare-Boamah and Fletcher 1986). It is possible that a similar mechanism operated in protection against cadmium toxicity as well.

Acknowledgments. This research was supported by the Natural Science and Engineering Research Council of Canada and the Canadian International Development Agency.

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