J Plant Growth Regul (1990) 9:207–212

Journal of
Plant
Growth
Regulation

Improvement of Uniconazole-Induced Protection in Wheat Seedlings

R. A. Fletcher and G. Hofstra

Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Received November 14, 1989; accepted January 25, 1990

Abstract. Uniconazole [(E)-(p-chlorophenyl)-4, 4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] belongs to a group of triazoles which, in addition to their fungitoxic and plant growth-regulating (PGR) properties, protect plants from various stresses. Compared to the conventional methods of treatment which include seed, soil drench, and foliar spray, the present study shows that a convenient and simple method of administering the chemical is by imbibing wheat (*Triticum aestivum* L. cv. Frederick) seeds in uniconazole solution for 20 h. The soaked seeds can be stored after air drying and germinated when desired. Addition of potassium to the uniconazole solution and exposure of the seeds to 40°C for the last 2 h during the imbibition period further enhanced the PGR effectiveness and improved the efficacy of the uniconazole-induced protection against drought and low- and high-temperature stresses. Uniconazole increased both carotenoid and chlorophyll levels and in combination with KCl, the increase in chlorophyll was more than twice that of the controls. The combination pretreatment of the imbibed seeds used in this study not only magnifies the protective and PGR effects of uniconazole but also supports the concept of acclimation and cross-tolerance.

In a comparative study using several triazoles including triadimefon and uniconazole, these compounds demonstrated both plant growth-regulating (PGR) and fungitoxic properties (Fletcher et al. 1986). The triazoles protect plants against various stresses including low and high temperature, drought, and air pollutants (see reviews by Davis et al. 1988, Fletcher 1985, Fletcher and Hofstra 1988). Fletcher and Hofstra suggested (1985) that the PGR and protective properties of the triazoles are mediated by interference with the isoprenoid pathway and subsequent shift in the balance of important plant hormones.

In addition to inhibiting gibberellin biosynthesis (Davis et al. 1988, Fletcher and Hofstra 1988), the triazoles also decrease ethylene evolution (Abbas et al. 1989, Hofstra et al. 1989, Wang and Steffens 1985) and increase cytokinin levels (Fletcher and Arnold 1986, Izumi et al. 1988). They also have been shown to increase chloroplast size in wheat leaves (Gao et al. 1988) and the chlorophyll levels in leaves of many other species (Davis et al. 1988, Fletcher and Hofstra 1988). Cytokinins accelerate chloroplast differentiation (Harvey et al. 1974) and stimulate chlorophyll production, and these effects can be enhanced with the addition of potassium (Fletcher et al. 1982). Because some of the triazole PGR effects were similar to those of cytokinins, the present study examines the influence of potassium on the triazole effects and determines if these chemicals could be administered to the seeds by imbibition. Based on the principle of acclimation (Lin et al. 1984) and the concept of cross-tolerance (Fletcher et al. 1988, Levitt 1980), this study also investigated whether an exposure of the seeds to 40°C for 2 h during the imbibition period enhances the stress-protective properties of the triazoles.

Materials and Methods

Plant Material

Wheat seeds were imbibed in distilled water, KCl (40 mM), and uniconazole (1 mg L^{-1}) solutions either singly or in various combinations for 20 h at room temperature (20 ± 2°C). One set was exposed to 40°C for the last 2 h of the imbibition period. KCl at 40 mM was chosen, as it had been found to be the most effective for stimulating chlorophyll synthesis (Fletcher et al. 1982). Uniconazole at 1 mg L^{-1} had been found to be most effective in preliminary experiments. The seeds were air dried for at least 5 days to ensure dryness. They were planted in sectioned plastic flats in Promix, a commercial potting soil (Plant Products, Ontario, Canada). Seedlings were grown in a controlled environ-

ment room maintained at 25/21°C day/night temperature with a 16-h photoperiod and a relative humidity of 70%. The light was provided by Sylvania Metalarc lamps and the photon flux at plant level was 220 $\mu E\ m^2\ s^{-1}$.

Growth Analysis

Ten days after planting, the number of germinated seeds was counted, fresh and dry weights of the shoots and roots were recorded, and the height of each measured, from the tip of the longest leaf to the soil level.

Chlorophyll Synthesis

For chlorophyll determination, the seedlings were germinated in the dark for 7 days. The etiolated seedlings were exposed to a photon flux of 40 $\mu E\ m^2\ s^{-1}$ for varying lengths of time. The leaves, 0.1 g, from each replicate were homogenized in 10 ml of 80% acetone and centrifuged at 2500 *g* for 10 min. The volume of the supernatant solution was brought to 15 ml and absorbency read at 663, 645, and 480 nm. Chlorophylls a and b and carotenoids were estimated according to methods described previously (Asare-Boamah et al. 1986).

Imposition of Stresses

Water stress was imposed by withholding water when the seedlings were 6 days old. Transpiration rates were recorded daily by weighing the plants in their containers.

For heat stress, the seedlings were exposed to 50°C for 4 h and then returned to the controlled environment room described earlier.

For cold stress, the seedlings were cut 1 cm above the soil surface and placed in a programmed freezer at 0°C for 16 h, followed by a cooling rate of 2°C/h. After removal from the freezer at desired temperatures, the seedlings were transferred to 4°C for 2 days and then returned to the controlled environment room.

Assessment of Injury

The rise in chlorophyll fluorescence (15 readings per replication) was recorded following the method described by Smillie and Hetherington (1983) using a portable fluorometer (Model SF 10, Richard Brancker Research Ltd., Ottawa, Canada). The parameter of determination was the ratio of the initial to peak fluorescence (O:P) with an increase in the ratio demonstrating a decrease in the activity of the energy-transducing systems.

Leakage of electrolytes, which is an indication of disorganization of membrane integrity, was measured 24 h after a given stress. Ten leaf segments (4 cm long) from each replicate were immersed in distilled water, the conductivity measured with a YSI Model 31 conductivity cell, and the percent leakage was calculated using the following formula: percentage leakage = (conductivity before boiling) × 100/(conductivity after boiling).

For acute symptoms, 2 weeks after a given stress, the assessment was based on percent plant survival.

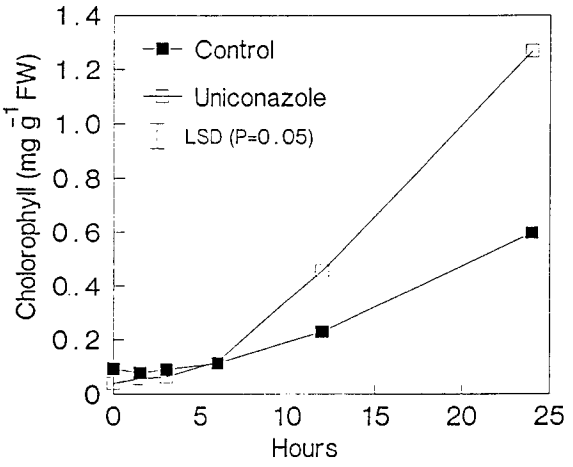

Fig. 1. Chlorophyll levels in shoots of 7-day-old wheat seedlings germinated in the dark and then exposed to light for the time indicated. Seeds were pretreated by imbibition either in water (Control) or uniconazole (1 mg L^{-1}) for 20 h. There were 10 seedlings per container and six replications per treatment.

Results and Discussion

Photosynthetic Pigments

One of the striking responses of plants to triazoles is the "greening effect," where the treated plants appear darker green with more chlorophyll than the controls (Davis et al. 1988, Fletcher and Hofstra 1988). The efficacy of applying uniconazole by imbibition was tested by determining its effects on chlorophyll synthesis. When wheat seedlings grown in the dark for 7 days were exposed to light, the chlorophyll content per unit weight of fresh leaves in those seedlings germinated from seeds imbibed in uniconazole was considerably higher than the controls (Fig. 1). After 24 h, the chlorophyll levels in the leaves of uniconazole-pretreated plants were 110% higher than the controls. Stimulation of chlorophyll levels in cucumber plants after application of triadimefon has been noted earlier (Fletcher and Arnold 1986). Cytokinins stimulate chlorophyll synthesis (Fletcher and McCullagh 1971) and both triadimefon and uniconazole have been reported to increase cytokinin levels in cucumbers (Fletcher and Arnold 1986) and rice (Izumi et al. 1988), respectively. Therefore, it is likely that the stimulation of chlorophyll synthesis in triazole-treated plants is mediated through an effect on cytokinins. If this assumption is correct, then the addition of KCl to uniconazole should further increase chlorophyll production, because the effect of cytokinins is enhanced by KCl (Fletcher et al. 1982). The early

Table 1. Growth analysis of wheat seedlings 10 days after planting.

Treatment	Germination (%)	Height (cm)	Shoot (g dry wt)	Root (g dry wt)	Root/shoot
Control	97a	20.1a	0.20a	0.22a	1.1a
H	90a	17.5b	0.21a	0.21a	1.0a
KCl	97a	21.0a	0.22a	0.24a	1.1a
KCl + H	90a	20.7a	0.19b	0.26b	1.4b
U	97a	10.9d	0.16c	0.21a	1.3b
U + H	97a	10.5d	0.15c	0.23a	1.5b
U + KCl	97a	12.1c	0.19a	0.30c	1.6b
U + KCl + H	97a	9.7d	0.15c	0.31c	2.1c

Seedlings were grown from seeds imbibed for 20 h in various combinations of water (Control); uniconazole, 1 mg L^{-1} (U); and KCl, 40 mM. One set was exposed to heat (H) at 40°C for the last 2 h of imbibition. There were 10 seedlings per container and six replications per treatment. Means within columns followed by the same letter are not significantly different at $p = 0.05$, according to Duncan's multiple-range test.

effects of KCl on chlorophyll synthesis could not be compared with those of uniconazole and the control (Fig. 1), because the addition of KCl to uniconazole delayed growth of the dark-grown seedlings, and the leaves were still partially enclosed in the coleoptiles. However, after 30 h of exposure to light, the leaves emerged and the chlorophyll values in the controls, uniconazole, and uniconazole + KCl treatments were 0.8, 1.2, and 2.1 mg g^{-1} fresh wt, respectively. This observation that KCl enhances the effect of uniconazole in increasing chlorophyll levels is similar to the effects with cytokinins (Fletcher et al. 1982), which suggests that the triazole effects may partially be regulated through cytokinins. Furthermore, these results indicate that the application of both these chemicals to the seeds by imbibition is a simple and effective method of treatment.

The 7-day-old seedlings germinated in the dark from seeds imbibed in uniconazole appeared more yellow due to higher carotenoid levels than the controls, with the values being 0.35 and 0.10 mg g^{-1} fresh wt, respectively. In addition to a light-harvesting role in photosynthesis, the carotenoids are also essential for protecting the cells against harmful effects of light and O_2 (Sieferman-Harms 1987). The higher levels of carotenoids in the uniconazole-treated seedling could play a role in the protective action of triazoles against various environmental stresses some of which occur by oxidative damage (MacKay et al. 1987, Senaratna et al. 1988).

Growth Analysis

The chemicals, either singly or in combination with heat, did not affect percent germination (Table 1), although in combined treatments (uniconazole + KCl + heat) emergence of the seedlings was delayed by 1 day (data not shown). The height of the plants was not altered by KCl, whereas those exposed to heat shock were shorter than the controls. Uniconazole either alone or in combination with KCl or heat significantly reduced the height of the plants (Table 1 and Fig. 2A). Reduction of height by uniconazole was accompanied by a reduction in shoot dry weight leading to an increase in the root to shoot ratio (Table 1). This ratio was further increased in combination with KCl and was the highest in the combination pretreatments of uniconazole + KCl + heat. Increases in the root to shoot ratio after triazole treatment has been observed in several species (Davis et al. 1988, Fletcher and Hofstra 1988), and similar observations in the present study further confirm that imbibition is an effective method of administering the chemicals.

Protection Against Heat Stress

After 4 h of exposure to 50°C, the control plants appeared flaccid and 24 h later they were desiccated and most of the leaves drooped (Fig. 2B). These deteriorative effects were prevented by uniconazole, except for desiccation of the mature leaf tips. These minor symptoms were eliminated in those seedlings pretreated with a combination of uniconazole + KCL + heat which offered total protection, hereafter referred to as the combination treatment. These visual observations that the combination treatment offered the best protection was further confirmed (Table 2) 1 week later by other tests routinely used in our laboratory for assessment of stress injury.

Measurements of chlorophyll fluorescence in vivo can be used to rapidly and nondestructively monitor cellular injury caused by environmental stresses (Smillie and Hetherington 1983). This tech-

Fig. 2. From left to right, wheat seedlings germinated from seeds imbibed for 20 h in water (Control); uniconazole, 1 mg L^{-1} (U); U + KCl, 40 mM; and U + KCl + H, 40°C for the last 2 h of imbibition. Appearance of 8-day-old seedlings: **(A)** before stress; **(B)** 1 day after exposure to heat stress (50° for 4 h); **(C)** drought stress, 7 days after withholding water; and **(D)** cold stress, 2 weeks after exposure to −8°C.

nique has been satisfactorily used previously to assess the protective effects of triazoles against damage caused by ozone (MacKay et al. 1987), chilling (Senaratna et al. 1988), and heat stress (Fletcher et al. 1988). The parameter of determination was the ratio of initial to peak fluorescence (O:P), which is inversely proportional to the photosynthetic productivity of the plant. Values reaching close to 1 are lethal and in the control plants exposed to 50°C for 6 h, the values were 0.8 indicating severe damage, whereas in the combination treatment the value was 0.5, a reading often associated with healthy leaves.

Solute leakage was also found to increase after the heat treatment (Table 2) indicating a loss of membrane integrity. The conductivity in the control plants was 66% compared to 35% in the combination treatment. The ultimate and absolute measurement of protection against a drastic stress is the ability of the plants to survive. In the control plants after exposure to heat at 50°C for 4 h, the percent survival was 50 compared to 91 in uniconazole and 100 in the combination treatment. Based on fluorescence, leakiness, and survival, plants treated with uniconazole were able to withstand heat stress better than the controls, and maximum protection was obtained with the combination treatment.

Table 2. Protection of wheat seedlings from exposure to a heat stress of 50°C for 4 h.

Treatment	Fluorescence (O:P ratio)	Leakiness (% conductivity)	Survival (%)
Control	0.8a	66a	50a
H	0.7b	66a	57a
KCl	0.6c	69a	68b
KCl + H	0.7b	68a	81c
U	0.6c	42bc	91d
U + H	0.5d	37cd	93d
U + KCl	0.5d	50b	94d
U + KCl + H	0.5d	35c	100d

Seedlings were grown from seeds imbibed for 20 h in various combinations of water (Control); uniconazole, 1 mg L^{-1} (U); and KCl, 40 mM. One set was exposed to heat (H) at 40°C for the last 2 h of imbibition. There were 10 seedlings per container and six replications per treatment. Fluorescence and leakiness were measured 1 day, and survival 2 weeks after exposure to heat stress (50°C for 4 h). Means within columns followed by the same letter are not significantly different at p = 0.05, according to Duncan's multiple-range test.

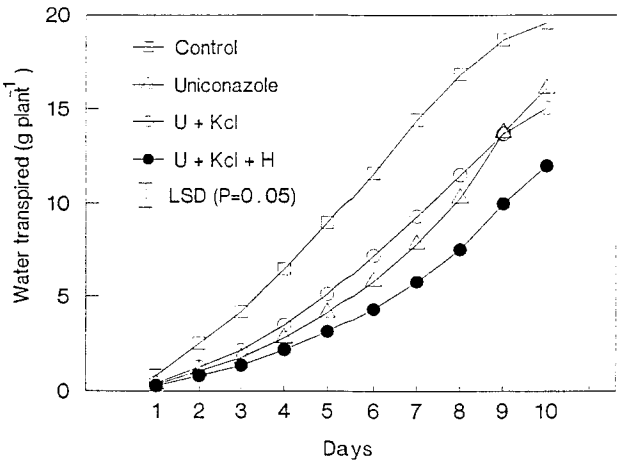

Fig. 3. Transpiration losses of 6-day-old wheat seedlings after water was withheld. Seedlings were germinated from seeds which were imbibed for 20 h in water (Control); uniconazole, 1 mg L^{-1} (U); U + KCl, 40 mM; and U + KCl + heat (H), 40°C for the last 2 h of imbibition. There were 10 seedlings per container and six replications per treatment.

Protection Against Drought

One week after withholding water, the control plants lost their turgor and wilted, whereas those treated with uniconazole alone or in combination with KCl or heat were turgid (Fig. 2C). Compared to the controls, the transpiration rates were decreased by uniconazole and this may be due to decreased leaf area. However, in the combination treatment the water lost after 8 days was significantly less than that of the uniconazole treatment (Fig. 3), although the seedlings were of the same size (Fig. 2C) and the dry weight of the leaves was not different (Table 1). Transpiration reduction by triazoles has been reported previously (Asare-Boamah et al 1986, Fletcher and Nath 1984); however, the present study demonstrated that the efficacy of the triazoles as an antitranspirant could be increased in combination with KCl and heat shock.

Table 3. Percent survival of wheat seedlings 2 weeks after exposure to low temperatures.

Treatment	Temperature (C)		
	−6	−8	−10
Control	53a	18a	0a
H	65b	46c	0a
KCl	87c	30b	0a
KCl + H	47a	15a	0a
U	89c	32b	0a
U + H	100d	44c	6b
U + KCl	86c	64d	0a
U + KCl + H	100d	100e	20b

Seedlings were grown from seeds imbibed for 20 h in various combinations of water (Control); uniconazole, 1 mg L^{-1} (U); and KCl, 40 mM. One set was exposed to heat (H) at 40°C for the last 2 h of imbibition. The pretreated seedlings were placed in a programmed freezer and cooled at a rate of 2°C h^{-1}. There were 10 seedlings per container and six replications per treatment. Means within columns followed by the same letter are not significantly different at p = 0.05, according to Duncan's multiple-range test.

Protection Against Chilling

At −6 and −8°C, the percent survival of the control plants was 53 and 18, respectively (Table 3). At equivalent temperatures, the percent survival of those plants treated with uniconazole was higher than that for controls. Protection of plants from cold injury by triazoles has been reported previously (Fletcher and Hofstra 1985, Senaratna et al. 1988). However, the present study demonstrated that the efficacy of uniconazole-induced protection could be improved. For example, at −8°C, the percent survival in uniconazole alone was 32, whereas in combination with KCl and heat shock it was 100 (Table 3). The appearance of plants is illustrated in Fig. 2D. Triazoles have been reported to increase cytokinins (Fletcher and Arnold 1986, Izumi et al. 1988) and, in certain systems, KCl magnifies the cytokinin effect (Fletcher et al. 1982). The increase in cold tolerance after heat shock noted in the present study, and our previous observations

(Fletcher et al. 1988) that uniconazole-induced protection of plants from heat shock can be enhanced by pretreating the plants with water stress, supports the concept of cross-tolerance proposed by earlier workers (Bonham-Smith et al. 1988, Fletcher et al. 1988, Levitt 1980).

In addition to supporting previous observations that triazoles protect plants from several unrelated stresses (Davis et al. 1988, Fletcher 1985, Fletcher and Hofstra 1988), the present study (a) demonstrates a simple procedure of imbibition for administering triazole, (b) illustrates that pretreatment with KCl and heat shock (40°C for 2 h) could improve the efficacy of triazole-induced protection, and (c) supports the concept of cross-tolerance.

Acknowledgments. The research was supported by the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Agriculture and Food. We thank V. Kallidumbil for technical assistance, D. Hamilton for photography, and Chevron Chemical Company for a generous suppy of uniconazole.

References

Abbas S, Fletcher RA, Murr DP (1989) Alteration of ethylene synthesis in cucumber seedlings by triadimefon. Can J Bot 67:278–280

Asare-Boamah NK, Fletcher RA (1986) Protection of bean seedlings against heat and chilling injury by triadimefon. Physiol Plant 67:353–358

Asare-Boamah NK, Hofstra G, Fletcher RA, Dumbroff EB (1986) Triadimefon protects bean plants from water stress through its effects on abscisic acid. Plant Cell Physiol 27:383–390

Bonham-Smith PC, Kapoor M, Bewley JD (1988) Exogenous application of abscisic acid or triadimefon affects the recovery of *Zea mays* seedlings from heat shock. Physiol Plant 73:27–30

Davis TD, Steffens GL, Sankhla N (1988) Triazole plant growth regulators. In: Janick J (ed) Horticultural reviews, vol. 10. Timber Press, Portland, Oregon, pp 63–105

Fletcher RA (1985) Plant growth regulating properties of sterol-inhibiting fungicides. In: Purohit SS (ed) Hormonal regulation of plant growth and development, vol. 2. Bot. Publ., India, pp 103–113

Fletcher RA, Arnold V (1986) Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol Plant 66:197–201

Fletcher RA, Hofstra G (1985) Triadimefon a plant multiprotectant. Plant Cell Physiol 26:775–780

Fletcher RA, Hofstra G (1988) Triazoles as potential plant protectants. In: Berg D, Plempel M (eds) Sterol biosynthesis inhibitors. Ellis Horwood Ltd., Cambridge, pp 321–331

Fletcher RA, Kallidumbil V, Steele P (1982) An improved bioassay for cytokinins using cucumber cotyledons. Plant Physiol 69:675–677

Fletcher RA, McCullagh D (1971) Cytokinin-induced chlorophyll formation in cucumber cotyledons. Planta 101:88–90

Fletcher RA, Nath V (1984) Triadimefon reduces transpiration and increases yield in water stressed plants. Physiol Plant 62:422–426

Fletcher RA, Hofstra G, Gao J (1986) Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant Cell Physiol 27:367–371

Fletcher RA, Santakumari M, Murr DP (1988) Imposition of water stress in wheat seedlings improves the efficacy of uniconazole-induced thermal resistance. Physiol Plant 74: 360–364

Gao J, Hofstra G, Fletcher RA (1988) Anatomical changes induced by triazoles in wheat seedlings. Can J Bot 66:1178–1185

Harvey BMR, Lu BC, Fletcher RA (1974) Benzyladenine accelerates chloroplast differentiation and stimulates photosynthetic enzyme activity in cucumber cotyledons. Can J Bot 52:2581–2586

Hofstra G, Krieg LC, Fletcher RA (1989) Uniconazole reduces ethylene and 1-aminocyclopropane-1-carboxylic acid and increases spermine levels in mung bean seedlings. J Plant Growth Regul 8:45–51

Izumi K, Nakagawa S, Kobayashi M, Oshio H, Sakurai A, Takahashi N (1988) Levels of IAA, cytokinins, ABA and ethylene in rice plants as affected by a gibberellin biosynthesis inhibitor, uniconazole-P. Plant Cell Physiol 29:97–104

Levitt J (1980) Comparative stress responses. In: Levitt J (ed) Responses of plants to environmental stresses. Academic Press, New York, pp 533–570

Lin CY, Roberts JK, Key JL (1984) Acquisition of thermotolerance in soybean seedlings. Synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol 74:152–160

MacKay CE, Senaratna T, McKersie BD, Fletcher RA (1987) Ozone induced injury to cellular membranes in *Triticum aestivum* and protection by triazole S-3307. Plant Cell Physiol 28:1271–1278

Senaratna T, MacKay CE, McKersie BD, Fletcher RA (1988) Triazole-induced chilling tolerance in tomato and its relationship to antioxidant content. J Plant Physiol 133:56–61

Siefermann-Harms D (1987) The light harvesting and protective functions of carotenoids in photosynthetic membranes. Physiol Plant 69:561–568

Smillie RM, Hetherington SE (1983) Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence in vivo. Plant Physiol 72:1043–1050

Wang SY, Steffens GL (1985) Effect of paclobutrazol on water stress-induced ethylene biosynthesis and polyamine accumulation in apple seedling leaves. Phytochemistry 24: 2185–2190