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Growth Responses of Rice Seedlings to Triacontanol in Light and Dark

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Abstract. Triacontanol, a 30-carbon primary alcohol, applied in nutrient culture solutions to rice (*Oryza sativa* L.) seedlings at 2.3×10^{-8}M (10 µg/l), caused an increase in dry weight and leaf area of the whole plants. The response could be observed as early as 3 h of treatment. It was observed at relatively high and low light intensities as well as in the dark where control plants lost but triacontanol-treated plants gained in dry weight. The dry weight gain in the dark was, however, eliminated by removing CO_2 from the atmosphere. Triacontanol-treated plants also increased their content of Kjeldahl-N and contained 30% more total N per plant than controls after 6 h in the dark.

Key words: Growth stimulation – *Oryza* – Protein synthesis – Triacontanol.

Introduction

Coarsely chopped alfalfa (*Medicago sativa* L.) hay was shown to increase growth and yield when placed in a band below and to the side of crop seeds or seedlings (Ries et al., 1976). An application of 117 kg/ha of alfalfa hay increased the yield of early tomatoes by 10 metric tons per hectare. Yields of cucumber, lettuce and wheat were also increased in the field. Several other crop species including rice and corn accumulated dry weight more rapidly from small applications of alfalfa hay under various controlled environmental conditions.

A crystalline substance, isolated from the chloroform-soluble fraction of alfalfa hay, was identified as triacontanol, a straight-chain 30 carbon saturated alcohol ($CH_3(CH_2)_{28}CH_2OH$) by mass spectrometry (Ries et al., 1977), and it was shown that triacontanol at the extremely low concentration of 10 µg/l (2.3×10^{-8}M) increased the water uptake and dry weight of rice seedlings when sprayed on the foliage or when applied in nutrient cultures. Foliar applications of triacontanol also increased the growth of corn and barley grown in soil. Synthetic triacontanol produced a similar response to the isolated natural product when applied to rice at 1–100 µg/l in nutrient solutions or as sprays at the same concentrations on tomatoes grown in soil. Octocosanol, the 28-C analog of triacontanol ($CH_3(CH_2)_{26}CH_2OH$), applied in nutrient culture at similar rates, did not increase growth or water uptake of rice (Ries et al., 1977). Triacontanol was first identified by Chibnall et al. (1933) in alfalfa. It occurs in small quantities in the waxes of many plant species (Kolattukudy and Walton, 1972). In *Triticale* and wheat, triacontanol accounts for 4% and 3%, respectively, and octocosanol for 80% of the free alcohols found in the wax of leaves (Tulloch and Hoffman, 1974).

In this paper, we report on time-course studies and growth analyses which were conducted to determine the effect of triacontanol on dry weight accumulation in rice seedlings grown in nutrient cultures, and the dependence of this effect on light and atmospheric CO_2.

Material and Methods

Plant Culture and Treatment

Rice (*Oryza sativa* L.) seed cv. IR-8, (PI 312627) or Starbonnet (provided by T. Johnston, ARS, U.S. Department of Agriculture, Stuttgart, Ariz., USA) was surface treated with 0.1% (w/v) mercuric chloride, planted in 77-ml plastic cups containing vermiculite and watered with $^1/_4$-strength Hoagland's nutrient solution containing 3 mM of nitrate nitrogen (pH 5.0). The plants were grown under an 8-h night at 25° C and a 16-h day at 30° C with 21 µW cm^{-2} and 8 µW cm^{-2} in the blue and red spectral regions, respectively (IL150 Photometer, International Light, Newburyport, Mass., USA). In the test with different light intensities the light intensity

was 30 μW cm^{-2} and 13 μW cm^{-2}, and 15 μW cm^{-2} and 8 μW cm^{-2} for the blue and red spectral region, respectively. After 8–10 days, seedlings were transplanted to 220-ml cups wrapped in aluminum foil and containing 180 ml of the same nutrient solution. Four seedlings were suspended in the solution with a sponge-rubber disc. The nutrient solution was renewed every 2 or 3 days. Four days and again 2 days prior to the initiation of an experiment, the plants were sorted for size and similar-sized plants assigned to the same replicate for the experiment. A randomized complete block design was used to remove the variance based on plant size. Prior to the initiation of a test the cups in each replicate were assigned treatment numbers by use of a random number table. This procedure resulted in very low coefficients of variation of between 2% and 7% for these tests. Prior to a test the cups were tared, and filled with $^1/_2$-strength Hoagland's solution containing 6 mM nitrate nitrogen. Eighteen μl of chloroform for controls or of chloroform containing 1.8 μg triacontanol (Analabs, North Haven, Ct., USA), were placed on 2 cm^2 of Whatman No. 1 filter paper, air dried, and placed in the cups. This concentration of 10 μg/l or 2.3×10^{-8}M triacontanol was used for all tests described in the paper because it was previously found to be optimum for increasing rice seedling growth in nutrient cultures (Ries et al., 1977). Immediately prior to the test the cups were all brought up to 180 ml, including a set of cups without plants for measurement of evaporation. All tests except those conducted in the dark were started at the initiation of the light period. The dark tests were initiated after the 16-h light period and maintained at 30° C.

Analyses

Water uptake was measured by weighing the cups after removal of the plants and subtracting the tare and the water lost by evaporation from the control cups without plants. For tests continued for more than 1 day, the solution used was measured every 3 days and fresh nutrient solution was added and the pH maintained at 5.0 with 0.4 N H_2SO_4.

Shoots and roots were harvested separately. The expanded leaves were cut at the ligule, and the youngest visible leaf at the point where it emerged from the sheath of the next oldest leaf. The surface area of expanded leaves was measured using a Lambda (Lincoln, Neb., USA) Model LI-3000 planimeter. The plants were dried to constant weight in an oven at 110° C and the roots, expanded leaves, and sheaths were weighed separately, except where nitrogen determinations were made. The plants were dried at 43° C for 3 days in this case.

In the tests designed for comparing dry-weight accumulation in plants grown either in the absence of CO_2 or in normal air, the plants were placed in 20 × 32 cm glass jars fitted with gas inlet and outlet ports. Three jars containing two cups each were used for a total of 24 plants for each treatment. The plants were ventilated with laboratory air or air freed of CO_2 by passing it through Ascarite (sodium-hydroxide-coated asbestos) and then humidified by passing through water. The flow rate was ca. 300 ml min^{-1}. CO_2-free air was also used to purge the appropriate jars during a 2-min period required to place the plants in the jars.

Nitrogen analyses were done by the automated micro-Kjeldahl procedure of Ferrari (1960). This procedure will determine all reduced forms of nitrogen including free amino acids and ammonia.

Growth analyses was conducted according to Evans (1972). The Net Assimilation Rate (NAR) is the increase in plant weight per unit of leaf area over time interval where W=total weight per plant in mg, T=time in days, and L=leaf area in cm^2:

$$\mathrm{NAR} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\mathrm{Log_e} L_2 - \mathrm{Log_e} L_1}{L_2 - L_1}.$$

The relative growth rate (RGR) is the increase in plant weight per unit or original weight over a time interval and is obtained according to the equation:

$$\mathrm{RGR} = \frac{\mathrm{Log_e} W_2 - \mathrm{Log_e} W_1}{T_2 - T_1}.$$

The leaf area ratio is the ratio of leaf area to dry weight of leaves over a time interval:

$$\mathrm{LAR} = \frac{L_1 + L_2}{W_1 + W_2}.$$

Four to 6 replicates were used in each experiment in a randomized complete block design. The data were subjected to an analysis of variance. Means were compared by use of the least significant difference except where there was only one degree of freedom for treatment. In these instances the F value from the analysis of variance was used for comparison of means (Snedecor, 1946).

Results and Discussion

Time Course of the Triacontanol Effect

Triacontanol applications of 10 μg/l in the nutrient solution significantly increased the leaf area of rice seedlings within 8 h and the dry weight of the entire plant within 3 days (Table 1). Although separate weights were taken of expanded leaves, the remainder of the shoot, and the roots, all plant parts increased similarly in weight and only the total dry weight is shown.

In previous work (Ries et al., 1977), differences in water uptake had been observed within a few days of application, ostensibly indicating an effect on transpiration. The data in Table 1 show, however, that although the triacontanol-treated seedlings took up more total nutrient solution, the amount taken up expressed in ml cm^{-2} leaf area was similar for

Table 1. Effect of triacontanol on growth and water uptake of 18-day-old "IR-8" rice seedlings harvested at different times after treatment at the beginning of the light period

0 = no triacontanol, + = 10 μg/l triacontanol
Values are quantity per plant

Time after treatment (h)	Leaf area (cm^2)		Dry wt. (mg)		Water uptake (total)			
					ml		ml cm^{-2} leaf area	
	0	+	0	+	0	+	0	+
0	7.2		44.8					
8	7.6	8.2*	50.8	53.4	1.8	2.0	0.24	0.25
24	7.8	9.0*	52.5	58.6	3.0	3.2	0.39	0.35
72	12.1	13.7	70.9	81.7*	16.2	18.4	1.33	1.34
216	20.3	22.4	174.5	204.0**	55.5	65.5**	2.73	2.92

* ** F value significantly different between triacontanol and control for same parameter within columns at the 0.05 and 0.01 level, respectively

Table 2. Growth analysis of 18-day-old "IR-8" rice seedlings treated with triacontanol at the beginning of the light period and harvested at different times

0 = no triacontanol, + = 10 µg/l triacontanol

Time after treatment (h)	RGR (mg mg^{-1} day^{-1})		LAR (mg cm^{-2})		NAR (mg cm^{-2} day^{-1})	
	0	+	0	+	0	+
0–8	0.38	0.52	0.15	0.16	2.46	3.39
9–24	0.05	0.14	0.15	0.15	0.33	0.90
25–72	0.15	0.17	0.16	0.16	0.94	1.03
73–216	0.15	0.15	0.13	0.13	1.09	1.15

treated and non-treated plants. The increased water uptake from triacontanol-treated plants was thus based on the increase in leaf area.

Growth analysis based on the RGR data showed that the direct effect of triacontanol occurred within the first 24-h period (Table 2). After 24 h, there was no direct effect of triacontanol on relative growth rate. The treated plants grew more, but this was because they were larger from the effect prior to 24 h. Further analysis of RGR into the components of NAR and LAR indicated that the effect was not based on any difference in distribution of dry matter between leaves and the rest of the plant (LAR), but on a difference in the net assimilation rate.

Triacontanol Effect under Two Different Light Intensities

Another time-course study was conducted to determine the effect of triacontanol at two light intensities. Since the previous experiment had shown that the direct effect of triacontanol occurred within the first 24 h, the time period in this experiment was limited to one 16-h light period. Triacontanol increased the leaf area and dry weight at both intensities, although the response was greater in the lower light intensity (Fig. 1). The dry weight of triacontanol-treated plants had significantly increased compared to controls 4 h after treatment, and the leaf area 8 h after treatment. There was no significant difference in dry weight for plants harvested after 16 h, but there was a difference in leaf area at both light intensities. Water uptake per plant was significantly different after 16 h for both light intensities; however, when this was expressed on a leaf area basis, it appeared that the plants took up more water because the triacontanol-treated plants had more leaf area and not because of an effect on transpiration (Fig. 1).

Growth analysis of these data indicated that the RGR was greater for plants receiving triacontanol than control plants for the first 8 h for low light intensity, but only for the first 4-h period under high light intensity (Table 3). Triacontanol caused the rice seedlings to accumulate as much dry weight cm^{-2} of leaf area (NAR) at low light intensities as the control plants did at the higher light intensity. Leaf area in-

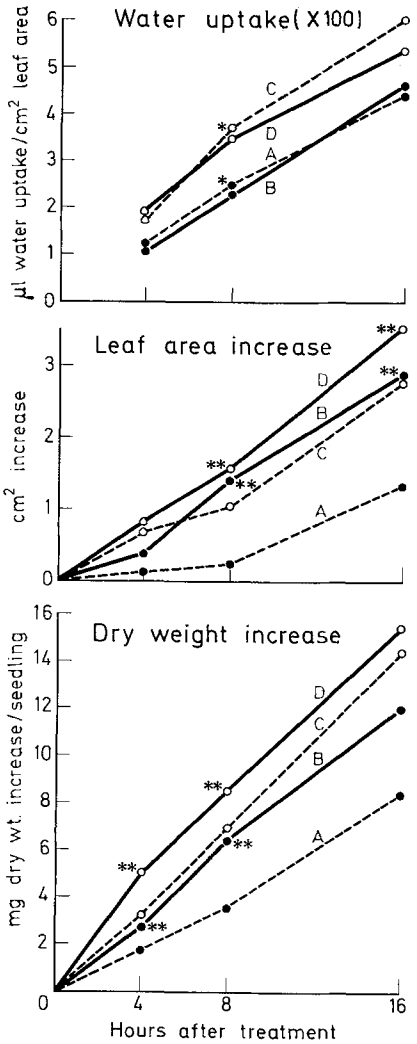

Fig. 1. Increase in dry weight and leaf area and the water uptake of 14-day-old "Starbonnet" rice seedlings treated with triacontanol (10 µg/l) under two different light intensities at the beginning of the light period. High light was 30 and 13 µW cm^{-2} and low light 15 and 8 µW cm^{-2} in the blue and red spectral range, respectively. The plants were exposed to these light conditions 36 h prior to treatment. At zero time the plants under low light intensity weighed 26.0 mg with 4.41 cm^2 leaf area. The high-light plants weighed 29.5 mg per plant with 4.01 cm^2 leaf area per plant. * and ** indicate F values for differences between treatments significant at the 0.05 and 0.01 level respectively between comparisons at individual times for each light intensity. *A* low-light control; *B* low = light + triacontanol; *C* high-light control; *D* high = light + triacontanol

Table 3. Growth analysis of 14-day-old "Starbonnet" rice seedlings treated with triacontanol (10 μg/l) under different light intensities. Treatments started at beginning of light period

0=no triacontanol, +=10 μg/l triacontanol; RGR=mg mg^{-1} day^{-1}, LAR=mg cm^{-2}, NAR=mg cm^{-2} day^{-1}

Growth parameter	Light intensity	Time after treatment (h)					
		0–4		5–8		9–16	
		0	+	0	+	0	+
RGR	Low	0.36	0.59	0.36	0.67	0.46	0.49
	High	0.56	0.89	0.66	0.57	0.56	0.50
LAR	Low	3.72	3.76	3.54	3.90	3.65	4.20
	High	3.36	3.31	3.37	3.45	3.49	3.74
NAR	Low	2.30	3.77	2.43	4.14	3.00	2.81
	High	4.02	6.42	4.73	3.96	3.90	3.19

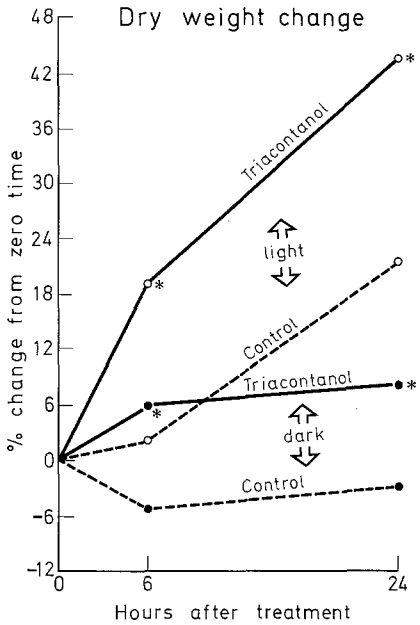

Fig. 2. Percent change in dry weight of whole 18-day-old "IR-8" rice seedlings treated with triacontanol (10 μg/l) in the light and dark over a 24-h period, expressed as % change from zero time. The dry weight at zero time was 50.8 mg per seedling. * indicates that the F value for the difference between controls and triacontanol treatments was significant at the 0.05 level

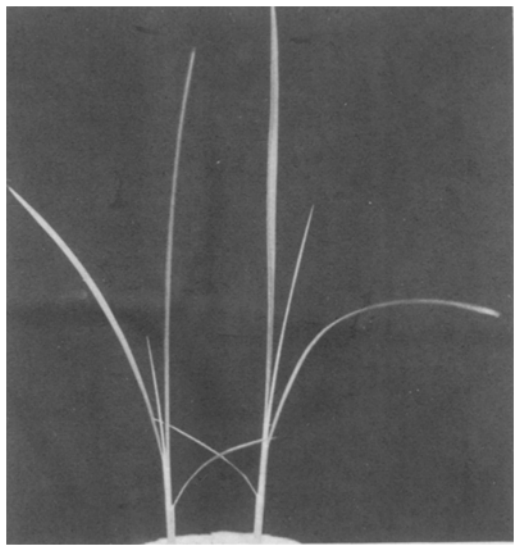

Fig. 3. Difference in growth between rice plants treated with triacontanol (10 μg/l) and controls after 6 h in the dark. A control plant is on the left and a treated plant on the right

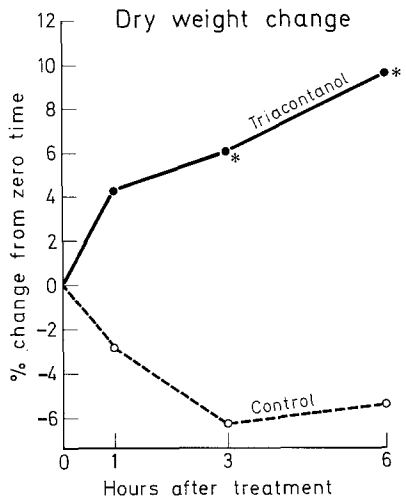

Fig. 4. Percent change in dry weight of whole 15-day-old "IR-8" rice seedlings treated with triacontanol (10 μg/l) in the dark. The dry weight at zero time was 37.3 mg per seedling. * indicates the F value for the difference between treatments at a harvest was significant at the 0.05 level

creased in triacontanol-treated plants relative to the dry weight after 8 h at both light intensities, as indicated by the LAR during the 9–16-h period.

Triacontanol Effect in Darkness

The marked response of rice seedlings to triacontanol under conditions of relative low light intensity prompted a study comparing the response of seedlings to triacontanol in complete darkness and in light. The response in the light was similar to that previously shown (Fig. 2). In the dark, the untreated plants decreased in dry weight as expected, but the triacontanol-treated plants were visually larger (Fig. 3), and had increased in weight compared to plants harvested at the initiation of the test at both the 6 h and 24 h harvest. A study conducted over a shorter time period, but only in the dark gave similar results. After both 3 h and 6 h, the triacontanol-treated plants con-

Table 4. Response to triacontanol of 17-day-old "IR-8" rice seedlings grown in the dark for 6 h with or without the normal content of CO_2 in air

Treatments started at end of light period

CO_2 level	Triacontanol (10 µg/l)	Dry wt. (mg/plant)		
		Expanded leaves	Roots	Whole plant
−	0	22.7	23.6	63.1
−	+	22.4	23.2	62.0
+	0	21.7	22.3	60.6
+	+	26.7	25.6	71.9
L.S.D.[a] at 0.05 level		1.5	1.0	3.3
L.S.D. at 0.01 level		2.1	1.4	4.6
Zero time		24.4	23.3	66.2
Coefficient of variation (%)		5.2	3.4	4.2

[a] Least significant difference

Table 5. Total protein (Kjeldahl nitrogen) content of 17-day-old "IR-8" rice seedlings grown in the dark for 6 h with or without triacontanol in the presence of CO_2

Triacontanol (10 µg/l)	Plant part				
	Fourth leaf	Leaves 1, 2 and 3	Sheaths	Roots	Entire plant
Protein (mg/g)					
0	356**	313**	210*	184**	239**
+	396	342	218	197	261
Zero time	341	308	202	182	242
Coefficient of variation (%)	4.1	3.7	2.6	2.4	1.4
Protein (mg/plant)					
0	1.25**	5.72**	3.42**	4.10**	14.49**
+	2.47	7.01	4.26	5.05	18.80
Zero time	1.26	6.38	3.73	4.63	16.00
Coefficient of variation (%)	25.3	4.5	6.5	4.4	5.8

* ** F value for difference between 0 and + triacontanol means significantly different at 0.05 and 0.01 level, respectively

tained more dry matter than they did at zero time and were significantly larger than the dark controls (Fig. 4). Although the total plant weight for triacontanol-treated seedlings was not significantly greater after 1 h, the difference in dry weight of the unexpanded leaves and leaf sheaths was significant. These studies indicate that triacontanol stimulates dark fixation of CO_2 by rice seedlings.

Effect of Presence and Absence of CO_2 on the Triacontanol Response

This hypothesis was tested by measuring the dry weight accumulation of triacontanol-treated and untreated seedlings grown in the presence or absence of the normal level of CO_2 in the air. The leaves, roots and entire plants from triacontanol treatments in the presence of CO_2 gained dry weight from zero time and weighed more than the plants grown in presence of CO_2 without triacontanol or grown in the absence of CO_2, with or without triacontanol (Table 4).

Effect of Triacontanol on Protein Content of Rice Seedlings in the Dark

Analyses of the total protein present in the different plant parts indicated that triacontanol-treated rice plants kept in the dark had both a higher concentration and more total Kjeldahl nitrogen than the controls in all parts of the plant, but this increase was most marked in the youngest leaves (Table 5). The increase in dry weight of these leaves accounted for a doubling of the total protein per leaf. With the exception of the fourth leaf, all plant parts in the dark control lost Kjeldahl-N as expected. The total increase in Kjeldahl-N per plant for the triacontanol-treated plants was 30% greater than in the control without triacontanol and 18% more than at zero time. This indicated that triacontanol-treated plants continued to synthesize protein in the dark.

Triacontanol has been shown, in six more experiments, to increase the dry weight of rice seedlings treated in the dark within 6 h compared to plants harvested at the start of the tests. Analysis of total nitrogen in three of these tests and analysis of free amino acids and nitrate in one tests indicated that triacontanol-treated plants synthesized more protein during this dark period.

Conclusions

Triacontanol at 2.3×10^{-8}M increased the transpiration of rice seedlings, but only because the leaf area increased. Triacontanol increased the dry weight and leaf area within 3 h. Growth analysis indicated that the direct effect of triacontanol occurred within 24 h: in 4 h in high light, and in 8 h in low light. Triacontanol increased the dry weight and protein content in the dark within 6 h.

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