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Mycorrhiza

The effect of cytokinin and thidiazuron on tomato inoculated with endomycorrhiza

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Abstract. Seeds of tomato (*Lycopersicon lycopersicum* var. Peto 86) were planted in soil inoculated with two vesicular-arbuscular mycorrhizal isolates (*Glomus intraradices* Schenck & Smith). After 7 weeks, plants were transplanted to the field and sprayed twice with cytokinin and thidiazuron, at 50% flowering and 3 weeks later. These treatments significantly increased the percentage of infected roots (30–43%) and chlorophyll content (8–13%). Mycorrhizal plants had less carbohydrates in the roots than in the leaves. Total protein and phenylalanine contents showed pronounced increases, while proline content decreased. Treatment of the mycorrhizal plants with thidiazuron or cytokinin significantly increased plant dry weight (51–54%), and the tomato yield significantly increasecd after inoculation with both isolates (18–35% and 14–32%).

Key words: Tomato plants – Vesicular-arbuscular mycorrhizal infection – Cytokinin – Thidiazuron

Introduction

Mycorrhizal fungi are cosmopolitan beneficial fungi associated with the roots of most crops. They have been shown to stimulate consistently plant absorption of P, Zn and Cu, but they can also enhance uptake of K, Ca, Fe, Mn, Mg and S (Gerdemann 1968; Kleinschmidt et al. 1972; Mosse 1973). As a result of improved mineral nutrition, such plants normally grow more rapidly and appear healthier than non-mycorrhizal plants, especially on low fertility soil (Menge 1982).

The beneficial effects of vesicular-arbuscular mycorrhizae (VAM) are not only found in undisturbed natural habitats, but also in intensive agricultural and horticultural areas (Schönbeck and Dehne 1981; Menge 1983). In practice, the development of VAM is influenced by soil type, genotype of the host plant, naturally occurring mycorrhizal fungi and fertilization, as well as by the application of pesticides. Therefore, early infection of host roots by mycorrhizal fungi will be less sensitive to detrimental chemicals and the beneficial effects on plant growth and stress tolerance will be most pronounced.

The purpose of the present work was to check the effect of the application of a phytohormone (the cytokinin kinetin) and a urea derivative (thidiazuron) on the early infection by VAM and the subsequent beneficial symbiotic response of infected tomato plants.

Materials and methods

Seed germination and transplanting were carried out in the experimental area of the Faculty of Agriculture, Alexandria University, Saba Bacha, during the 1991 growing season.

Two VA mycorrhizal isolates of *Glomus intraradices* Schenck & Smith were used in this study. The first, given the symbol "D", was introduced from the Institute of Plant Pathology, University of Hannover, Federal Republic of Germany, and the second was isolated from the Experimental Station of Alexandria University and given the symbol "A".

Seeds of Peto 86 tomato variety *Lycopersicon lycopersicum* L. were germinated in 25-cm dishes and then transplanted after 2 weeks to plastic pots, 10 cm in diameter, with two seedlings per pot. Plants were transplanted to the field 7 weeks after sowing with one plant per hill in rows 70 cm apart and with a hill spacing of 50 cm. Each treatment included at least 22 plants. Fertilization and pesticides were applied according to usual practice.

Three inoculation treatments [uninoculated (U), inoculated with "A" and "D"] and three spraying treatments [not sprayed (control), sprayed with cytokinin, sprayed with thidiazuron] were laid out in a factorial experiment as follows:

1. Control not treated with either inoculum or chemicals
2. Plants not inoculated with VAM but sprayed with cytokinin
3. Plants not inoculated with VAM but sprayed with thidiazuron
4. Plants inoculated with VAM "A"
5. Plants inoculated with VAM "A" and sprayed with cytokinin
6. Plants inoculated with VAM "A" and sprayed with thidiazuron
7. Plants inoculated with VAM "D"
8. Plants inoculated with VAM "D" and sprayed with cytokinin
9. Plants inoculated with VAM "D" and sprayed with thidiazuron

Inoculation with VAM was carried out before seeding in dishes. To ensure suitable infection, plant were transplanted after 2 weeks to plastic pots containing 1:1:1 by volume mixture of sand, pitmus and inoculum; the latter consisted of a mixture of sand and maize roots (Aboul Nasr 1987).

Cytokinin and thidiazuron were sprayed twice, at 50% flowering and 3 weeks later (i.e. the plants were 11 and 14 weeks old) using a concentration of 10 ppm on the leaves.

To determine the chlorophyll content, leaf samples were taken 2 weeks after the first and the second sprays according to Schopfer (1970). Two weeks after the second spray, leaf and root samples were also collected and dried at 90° C for 90 min for determination of carbohydrates and two amino acids, proline and phenylalanine (Umbreit et al. 1972). Protein content was measured according to Bradford (1976). Fresh root pieces from all treatments were cleared and stained following the method of Philips and Hayman (1970) for rapid assessment of mycorrhizal fungal infection, and the percentage of root infection determined. Tomato fruits were collected from all plants in each treatment every 2-3 days and weighed. At the end of the experiment, the average weight of fruits per plant and their total dry weights were measured.

The experiment was carried out in a split plot design. The main plots were allocated to VAM isolates and the sub-plots were spraying treatments. Statistical analysis was according to Mudra (1958).

Results and discussion

The effect of cytokinin and thidiazuron on the induction and development of VAM in tomato roots

Figure 1 shows that the proportion of infected roots in control plants was 2.7% due to natural soil VAM. Treatment with either thidiazuron or cytokinin stimulated VAM root infection in control plants by 55.6% and 133%, respectively.

A pronounced increase in infection was also found in plants inoculated with isolate A and treated with thidiazuron or cytokinin (30% and 43% more than the control, respectively). Similar results were obtained in plants inoculated with isolate D (36% and 38% more than the control, respectively).

These results agree partially with the findings of Dehne (1985), who showed that treatment with thidiazuron efficiently increased symbiosis.

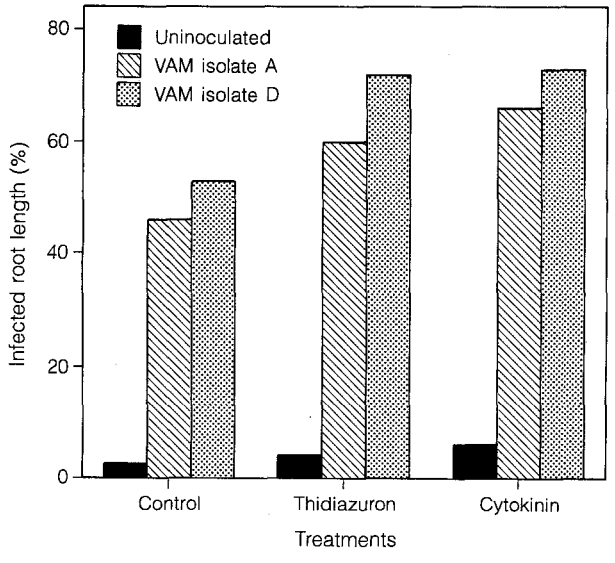

Fig. 1. Effect of thidiazuron and cytokinin on the development of vesicular-arbuscular mycorrhiza (VAM). Plant age = 16 weeks

Table 1. Effect of thidiazuron and cytokinin on chlorophyll content. U, Uninoculated; A, vesicular-arbuscular mycorrhizae (VAM) isolate "A"; D, VAM isolate "D". The data are the means of four samples

Treatment	Chlorophyll content (mg/g fresh wt.)					
	After 1st treatment[a]			After 2nd treatment[b]		
	U	A	D	U	A	D
Control	22.4	23.0	26.0	7.0	8.0	6.0
Thidiazuron	27.1	26.0	28.0	10.0	9.0	9.0
Cytokinin	26.0	26.0	24.0	8.0	11.0	6.0

[a] Plants were 13 weeks old
[b] Plants were 16 weeks old

The effect of cytokinin and thidiazuron on some components of treated plants

Chlorophyll content. According to the data presented in Table 1, the chlorophyll content in plants inoculated with isolate A and D showed an increase of 3% and 16%, respectively, compared with uninoculated ones. The first treatment with thidiazuron and cytokinin increased the chlorophyll content of uninoculated plants by 21% and 16% over the control, respectively.

The first treatment with thidiazuron and cytokinin in both cases increased the chlorophyll content 13% in plants inoculated with isolate A. Plants inoculated with isolate D and treated with thidiazuron showed an 8% increase in chlorophyll content, whereas application of cytokinin led to an 8% reduction in chlorophyll compared to control plants.

Two weeks after the second spray it was found that inoculation with isolate A had led to a remarkable increase in chlorophyll content (14% more than non-mycorrhizal plants), whereas the chlorophyll content in plants inoculated with isolate D was 14% less than the control.

The data shown in Table 1 clearly indicate that the second treatment with thidiazuron enhanced chlorophyll formation in all non-mycorrhizal plants as well as those inoculated with isolates A and D (43%, 13% and 50% more than control, respectively). However, the effect of cytokinin was limited to the non-mycorrhizal plants and those inoculated with isolate A (14% and 38% more, respectively). These increases might be expected to lead to increased light absorption and more efficient plant nutrition and growth (Hayman 1974).

Total carbohydrates. The results in Tables 2 and 4 show that the carbohydrate content of both leaves and roots of mycorrhizal plants was low compared to non-mycorr-

Table 2. Effect of thidiazuron and cytokinin on the carbohydrate and protein contents of tomato roots 2 weeks after the 2nd treatment, i.e. of plants 16 weeks old. Otherwise as Table 1

Treatment	Carbohydrate (mg/g dry wt.)						Protein (mg/g dry wt.)					
	U	%[a]	A	%	D	%	U	%	A	%	D	%
Control	19.2		11.1		16.1		27.9		40.7		42.8	
Thidiazuron	25.1	+31.0	2.7	−76.0	12.1	−25.0	34.7	+24.0	45.8	+13.0	47.2	+10.0
Cytokinin	15.5	−19.0	5.0	−55.0	9.5	−41.0	38.0	+36.0	44.7	+10.0	50.9	+19.0

[a] Percentage difference to the control

Table 3. Effect of thidiazuron and cytokinin on the proline and phenylalanine contents of tomato roots 2 weeks after the 2nd treatment, i.e. of plants 16 weeks old. Otherwise as Table 1

Treatment	Proline (mg/g dry wt.)						Phenylalanine (mg/g dry wt.)					
	U	%[a]	A	%	D	%	U	%	A	%	D	%
Control	1.17		1.22		0.94		17.51		19.77		22.03	
Thidiazuron	1.51	+29.0	1.15	−6.0	1.04	+11.0	18.64	+ 6.0	20.90	+6.0	22.60	+3.0
Cytokinin	1.00	−15.0	1.20	−2.0	1.15	+22.0	19.21	+10.0	21.47	+9.0	23.16	+5.0

[a] Percentage difference to the control

Table 4. Effect of thidiazuron and cytokinin on the carbohydrate and protein contents of tomato leaves 2 weeks after the 2nd treatment, i.e. of plants 16 weeks old. Otherwise as Table 1

Treatment	Carbohydrate (mg/g dry wt.)						Protein (mg/g dry wt.)					
	U	%[a]	A	%	D	%	U	%	A	%	D	%
Control	34.3		21.4		11.7		39.4		101.2		120.2	
Thidiazuron	45.7	+33.0	20.7	−3.0	13.5	+15.0	56.5	+43.0	139.3	+38.0	168.2	+40.0
Cytokinin	16.3	−52.5	21.3	−0.5	23.4	+12.0	72.3	+84.0	156.4	+55.0	171.0	+42.0

[a] Percentage difference to the control

Table 5. Effect of thidiazuron and cytokinin on the proline and phenylalanine contents of tomato leaves 2 weeks after the 2nd treatment, i.e. of plants 16 weeks old. Otherwise as Table 1

Treatment	Proline (mg/g dry wt.)						Phenylalanine (mg/g dry wt.)					
	U	%[a]	A	%	D	%	U	%	A	%	D	%
Control	2.8		3.0		3.2		38.4		46.3		47.7	
Thidiazuron	2.9	+ 3.6	2.7	−10.0	3.9	+22.0	40.4	+ 5.0	42.9	− 7.0	57.6	+21.0
Cytokinin	2.4	−14.0	2.4	−20.0	3.6	+13.0	33.6	−13.0	40.1	−13.0	56.2	+18.0

[a] Percentage difference to the control

hizal plants. This may be the result of higher respiration rates characteristic of mycorrhizal plant roots. The results are in agreement with those obtained by Nehemiah (1977). Treatment with thidiazuron and cytokinin enhanced the development of mycorrhiza-infected roots and consequently reduced carbohydrate content.

Total protein, proline and phenylalanine. The results in Tables 2–5 show marked increases in protein, proline and phenylalanine in mycorrhizal roots. Moreover, treatment of tomato plants with either thidiazuron or cytokinin clearly increased these components. Similar results were recorded by Baltruschat (1975), who also showed that the development of mycorrhizal roots in most cases led to the accumulation of free amino acids in the root system.

Total dry weight. The data in Fig. 2 indicate that the dry weight of mycorrhizal plants was significantly greater than that of non-mycorrhizal ones (54% and 101% for those inoculated with isolates A and D, respectively). Treatment of the mycorrhizal plants with thidiazuron or cytokinin increased the dry weight by 51–54% more than the control. Thus these compounds probably stim-

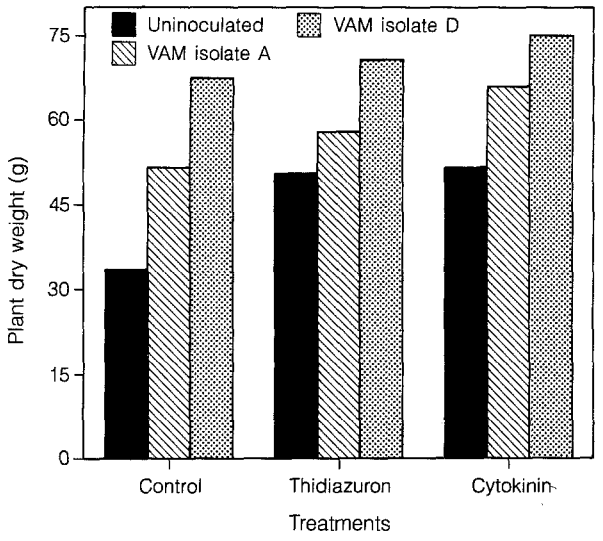

Fig. 2. Effect of thidiazuron and cytokinin on plant dry weight. The data are the means of 22 plants

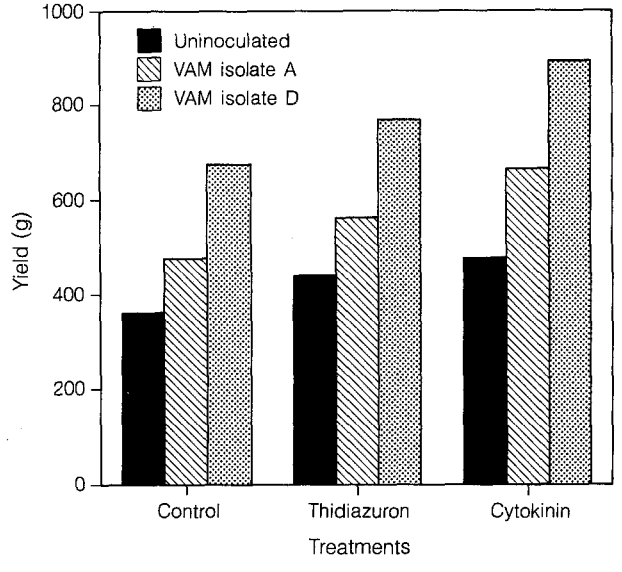

Fig. 3. Effect of thidiazuron and cytokinin on tomato yield. The data are the means of 22 plants

ulated the formation of mycorrhizae and this led to the beneficial effects of the symbiotic host-fungus association.

Tomato yield. The results shown in Fig. 3 indicate that the average tomato yield in untreated, non-mycorrhizal plants was approximately 364 g/plant. Treatment of non-mycorrhizal plants with thidiazuron and cytokinin increased the yield by 21% and 31% above that of the untreated plants.

An increase in yield of 18% and 35% was recorded when mycorrhizal plants (isolate A) were treated with thidiazuron and cytokinin, respectively. Tomato mycorrhizal plants inoculated with isolate D produced 86% and 42% more fruits than the non-mycorrhizal plants and those inoculateld with isolate A, respectively. Treatment of isolate D mycorrhizal plants with thidiazuron improved the yield to approximately 14% more than the untreated control. The increase in yield was much more pronounced with cytokinin treatment (32% more than the control).

The results obtained clearly suggest a positive correlation between application of thidiazuron and cytokinin to tomato plants and yield. These findings are in agreement with Dehne (1985) and Aboul Nasr (1987), who reported that application of urea derivatives at low concentrations leads to a most pronounced increase in mycorrhizal infection. Thidiazuron, which can be used at higher application rates as a defoliant, enhanced the development of the symbiosis without any visible influence on host plant growth. The stimulation of infection was independent of the fungal isolate and proved to be a general characteristic of these chemicals.

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