THE EFFECTS OF CYTOKININS AND THEIR INTERACTION WITH THIDIAZURON ON THE FORMATION OF AERIAL PLANTLETS IN *Asparagus officinalis L.*

ELSADIG HASSAN ELSADIG [1]

ABSTRACT

The effect of cytokinins on formation of aerial plantlets and production of spears in asparagus plants has been investigated. *Asparagus cultivars* UC 157,UC 66, 500W and Ida Lea were used as test plants. They were treated with benzyladenine and kinetin as spray, soil drench, and positional application. The effects of cytokinin on shoots of different ages and on initiation of rhizome buds were studied. Additionally, interaction of cytokinin and thidiazuron was also explored.

Spraying asparagus plants with varying concentrations of benzyladenine solutions, or positional application, resulted in formation of aerial plantlets; the number formed increased with an increase in concentration. Treatments as soil drench stimulated rhizome buds to grow and produce many spears. The results demonstrated that benzyladenine and thidiazuron have similar physiological activities in asparagus system. This suggests that thidiazuron possesses a high cytokinin activity. A physiological interaction between benzyladenine and thidiazuron was demonstrated. The mixture of benzyladenine and thidiazuron was significantly higher than benzyladenine alone and less than thidiazuron alone in a number of aerial plantlets.

ملخص

تم بحث تأثير السايتوكاينين على تكوين نبيتات هوائية وإنتاج حراب في نباتات الاسبرقص. الأصناف التي استعملت في هذه التجارب هي UC ١٥٧ و UC ٦٦ و W ٥٠٠ وإيديلي. لقد رشت النباتات بمحلول البنزايل أدنين والكاينتين، كما رويت تربة هذه الأصناف بالمحلولين، إضافة إلى استعمالهما موضعيًا. إلى جانب هذا فقد درس تأثير السايتوكاينين على سيقان نباتات الاسبرقص ذات أعمار مختلفة، وكذلك أثرها في تكوين براعم على الرايزومات. زيادة على ذلك فقد بحث التداخل بين السايتوكاينين والثيازيرون على هذه النباتات.

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[1] Professor of Horticulture and Deputy Vice-Chancellor (SUST)

رش نباتات الاسبرقص بتركيزات مختلفة من محلول البنزايل أدنين أو الاستعمال الموضعي لـه نتج عنه نبيتات هوائية، وأدى ذلك إلى زيادة في عددها مع زيادة التركيز. رى التربـــة حـــول النباتات بمحلول البنزيل أدنين أدى إلى تكوين براعم على الرايزوم وبالتالي إنتاج عدة حـــراب. أثبتت نتائج هذا البحث أن هناك معاملة في النشاط الفســـيولجي لكـــل مـــن البـــنزايل أدنيـــن والثديازيرون على نبات الاسبرقص وهذا يشير إلى أن للثيازيرون نشاطا سايتوكاينيني عاليا. عرض التداخل الفسيولجي بين السايتوكاينين والثديازيرون. إستعمال خليط من البنزايل أدنيـــن والثديازيرون زاد أعداد النبيتات الهوائية زيادة معنوية أكثر من البنزايل أدنين لوحـــده وأقـــل معنوياً من الثديازيرون لوحده.

INTRODUCTION

Cytokinins are a group of phytohormones that regulate cell division and differentiation [1]. Cytokinin activity may also be expressed in induction of organ formation and release of apical dominance [2]. The inhibition exerted by an intact apex on lateral buds was counteracted by kinetin, and bud release was obtained on intact plants of Alaska peas, Scabiosa, Coleus, *Helianthus annus* and *Helianthus tuberosus.*[3] BA increased the number of shoots per plant [4], and by using the cytokinin zeatinriboside, five to six times as many shoots per leaf explants were obtained [5].

The present study was undertaken to investigate the effect of cytokinins and thidiazuron on formation of aerial plantlets and spear production and their interaction in asparagus plant.

MATERIALS AND METHODS

PLANT MATERIAL

The test plants used were 3 to 12 months old UC 157, UC 66, 500 W and Ida Lea asparagus plants grown in 3.5 liter plastic containers filled with UC soil mix and raised in a greenhouse under ambient light conditions. Temperature ranged from 19.4 degrees to 26.7 degrees Celsius.

CHEMICALS

Benzyladenine (6[benzyl-amino]–purine) and kinetin (6[furfurylamino]–purine) were the cytokinins used in this study. Stock solutions of benzyladenine (4.4mM) and kinetin (4.64mM) were prepared by dissolving the chemicals in IN HCL (10:1, distilled H_2O: HCL), and distilled water, (30 ml IN HCL and 500 ml distilled water/1000mg) and then making to one liter volume. Thidiazuron (N-phenyl–N–1,2,3 – thidiazol-5 ylurea) is an urea derivative with empirical formula $C_9H_8M_4O_5$ and molecular weight 220. A stock solution of 9 mm (2000 ppm) was prepared by dissolving 4 g of chemical (50% active ingredient)

in 5 ml dimethyl sulfoxide (DMSO) plus 10 ml buffer X (principal functioning agents: alkyarypolyethoxy ethanol, free and combined fatty and phosphatic acids and isopropanol), and then making the volume to one liter with double distilled water in a I-L volumetric flask. Lower concentrations were made by dilutions from these stock solutions.

SPRAY APPLICATIONS

The cultivars UC 57, UC 66, 500 W and Ida Lea were sprayed to run off with solutions of concentrations 0, 0.88, 1.7, 2.6 and 3.52mm benzyladenine, and 0, 0.9, 1.86, 2.7and 3.72mM kinetin. The numbers of lateral buds that grew and formed aerial plantlets were counted with a hand tally counter.

SOIL DRENCH APPLICATION

These treatments were performed to examine the effect of cytokinins on promotion of rhizome bud growth. The soil surrounding each plant was drenched with 100 ml of each of the treatment chemical concentrations. Concentrations and cultivars used were the same as in spray treatments. The number of spears produced and aerial plantlets formed were recorded.

EFFECTS OF CYTOKININS ON SHOOTS OF DIFFERENT AGES

These experiments were undertaken to test response of shoots of asparagus plants of different ages to cytokinin. Shoots 6, 8 and 10 weeks old were identified by tagging 10 spears on each of 6- month old UC 157 asparagus plants every two weeks. Pieces of cotton saturated with 1.32mM benzyladenine solution were wrapped at the middle of each shoot. The cotton piece was covered with a thin polyethylene strip to minimize evaporation and to increase absorption of the chemical. The numbers of lateral buds that grew and formed aerial plantlets were counted.

EFFECTS OF CYTOKININS ON RHIZOME BUD INITIATION

The objective of this experiment was to evaluate influence of cytokinins on promotion of existing buds and on initiation of new buds. Four- month-old UC 66 asparagus plants grown in 3.5 liter plastic pots were used as test plants. The plants were removed from pots and soil washed off to expose roots and crown. Buds and shoots on each crown were counted and plants repotted and watered to let the soil settle. The next day, five plant–sets were treated with concentrations of 0, 0.44, 0.88, 1.32 or 1.76mM benzyladenine solution. Each plant received 100ml of the chemical solution as soil drench. After 5 weeks plants were removed from pots and soil washed off, and a recount of rhizome buds and shoots formed was done. The number of new buds formed was determined by subtracting the number of buds plus shoots before treatment from their number after treatment

INTERACTION OF THIDIAZURON AND BENZYLADENINE

This investigation was undertaken to study interaction between thidiazuron and benzyladenine in formation of aerial plantlets and production of spears in asparagus plants. UC 66 asparagus plants, 4.5 months old were used as test plants. Thidiazuron (0.9lmM) and benzyladenine (0.88mM) were used.

The plants were treated as follows:-

1. Foliage sprayed with distilled water (control).
2. Foliage sprayed with thidiazuron solution alone.
3. Foliage sprayed with thidiazuron solution and after the solution dried, the plants were re-sprayed with benzyladenine solution.
4. Foliage sprayed with benzyladenine solution alone.
5. Foliage sprayed with benzyladenine solution and after the solution dried the plants were re-sprayed with thidiazuron solution.
6. Foliage sprayed with a mixture of thidiazuron and benzyladenine solutions (1:1, v:v).
7. Soil drenched with water alone.
8. Soil drenched with thidiazuron solution alone.
9. Soil drenched with thidiazuron solution and after the solution has been drained the pots were re-drenched with benzyladenine solution.
10. Soil drenched with benzyladenine solution alone.
11. Soil drenched with benzyladenine and after drainage the pots were re- drenched with thidiazuron solution.
12. Soil drenched with a mixture of the two chemical solutions (1:1,v:v).

In spraying treatments plants were sprayed to run off, while in soil drenching treatments, each plant was drenched with 100ml of solution. The number of aerial plantlets formed and spears produced were recorded 5 weeks after treatments.

APICAAL DOMINANCE EXPERIMENTS

These experiments examined effects of presence or absence of main shoots apices of asparagus, or its replacement with auxin, on formation of aerial plantlets on shoots of asparagus plants treated with thidiazuron. Five-month-old UC 66 asparagus plants were used as test plants. The plants were raised in the same conditions as mentioned earlier. Thidiazuron (0.03%) in lanolin was prepared by dissolving it in few milliliters of dimenthyl sulfoxide and then mixing with melted lanolin. Indolebutytric acid (0.01% and 0.1 %) were prepared by dissolving in ethanol and mixing with melted lanolin. The plants were treated as follows:-

1. Control (no treatment).

2. Apex removed from the main shoot.
3. Apex removed and replaced with lanolin alone.
4. Apex intact, thidiazuron in lanolin placed on lateral buds.
5. Apex removed, replaced with 0.01% IBA, and thidiazuron placed on lateral buds.
6. Apex removed, replaced with 0.1% IBA and thidiazuron placed on lateral buds.
7. Apex intact and the plants sprayed to run off with thidiazuron.
8. Apex removed, replaced with 0.01% IBA and plants sprayed to run off with thidiazuron.
9. Apex removed and replaced with 0.1% IBA and plants sprayed to run off with thidiazuron.

The number of aerial plantlets formed on each shoot was recorded 5 weeks after treatment.

RESULTS AND DISCUSSION

FOLIAGE SPRAY TREATMENTS

Lateral buds on asparagus shoots were promoted to grow and form aerial plantlets when sprayed to run off with a solution containing different concentrations of benzyladenine (Table 1). As in the case of thidiazuron [6] an aerial plantlet first appeared as a single stem with a swollen tip containing several buds each sub-tended by a reddish bract. This stem elongated and formed an aerial plantlet, which was easily detachable from the mother plant. The plants were responsive to a range of concentrations: The number of aerial plantlets increased slightly with increasing concentrations of benzyladenine up to 2.6 mM (Table1). In this respect benzyladenine and thidiazuron had similar effects on asparagus system. A noted difference, however, between effects of benzyladenine and thidiazuron was that foliage spray with the former led to release of rhizome buds and production of many spears simultaneously, (Table 2), while in the case of foliage spray by thidiazuron there was no effect on rhizome buds, (Table 3). This suggests that benzyladenine or some secondary chemical or metabolic product moves from aerial shoots to the rhizome where it exerts its effect in promoting rhizome buds to grow. Another difference between the two substances was that thidiazuron was more effective at lower concentrations than benzyladenine in formation of aerial plantlets.

Table (I): Effect of benzyladenine applied as foliage spray on aerial plantlet formation in UC 157asparagus plants, 4 months old.

Number of aerial plantlets/shoot

Plant	Concentration				
	0	0.88mM	1.76Mm	2.64mM	3.52mM
1	0	1.07	9.8	12.8	11.0
2	0	8.5	10.4	11.5	11.7
3	0	13.6	13.6	14.0	12.0
4	0	10.0	10.0	20.8	08.8
Mean[z]	0c	10.7 b	10.9 b	14.8 a	10.9 b

[z] Mean separation of concentrations by Duncan' s Multiple Range Test, 5% level

Table(2): Effect of benzyladenine applied as foliage spray on spear production in UC 157 asparagus plants, 4 months old.

Number of spears/shoot

Plant	Concentration				
	0	0.88mM	1.76Mm	2.64mM	3.52mM
1	4	9	8	9	9
2	4	11	10	9	8
3	4	10	8	10	8
4	5	10	10	10	9
MEAN[z]	4.25c	10.00a	9.00ab	9.50ab	8.25b

[z] Mean separation of concentrations by Duncan' s Multiple Range Test, 5% level

Table (3): Effect of thidiazuron applied as foliage spray on initiation of rhizome buds

Number of rhizome buds before and after treatment

Plant Number	Treatment									
	0(Control)		0.45 mM		0.91 mM		1.36 mM		1.82mM	
	after	before	after	Before	after	before	after	before	after	Before
1	9	8	10	9	11	11	12	7	17	11
2	5	4	19	11	21	11	9	9	19	10
3	6	6	12	8	15	11	15	9	16	12
4	17	17	14	10	10	8	16	10	12	9

Z Not significant at 5 %level

[Z] Mean separation of concentrations by Duncan' s Multiple Range Test, 5% level

SOIL DRENCH TREATMENTS

Many rhizome buds were stimulated to grow simultaneously and produce many spears when plants were treated with benzyladenine as soil drench, (Table 4). The higher the concentration, the higher the number of spears produced up to 2.64 mM concentration. This finding is similar to what has been reported for thidiazuron.[6]. Under normal conditions there is a periodic production of spears by asparagus plants; interval between appearance of two spears on same plant varies with extent of maturity to which the first spear is allowed to reach.[7]. This periodic spear production appears to be due to some sort of regional inhibition. This might also be the case with benzyladenine, where this periodic spear production was interrupted by benzyladenine application resulting in promotion of growth of many buds. In spite of this explanation, the mode of action of cytokinins is not well understood[8] and it is not possible to explain their exact physiological effects[9].

In contrast to effect of thidiazuron applied as soil drench on formation of aerial plantlets on asparagus shoots, no aerial plantlets were formed on asparagus shoots as a result of benzyladenine soil drench application. This suggests that benzyladenine or some secondary substance did not move from rhizome and root system to aerial shoots to cause aerial plantlet formation. Unlike thidiazuron and benzyladenine, kinetin did not stimulate lateral buds on asparagus shoots to grow and produce aerial plantlets, or rhizome buds to grow and form spears, whether applied as spray (Table 5) or soil drench (Table 6). This difference in physiological action of hormones in the same class is not unusual. It is known that in several test systems where kinetin is highly effective, zeatin, a naturally occurring cytokinin, is completely ineffective.

Table (4): Effect of benzyladenine applied as soil drench on spear production in UC 157 asparagus plants, 4 months old.

Number of spears / plant

Plant	Concentration				
	0	0.88mM	1.76Mm	2.64mM	3.52mM
1	2	3	5	6	2
2	2	4	3	6	3
3	2	3	4	6	2
4	2	3	3	6	4
MEAN [z]	2c	3.25b	3.75b	6a	2.75bc

[z] Mean separation of concentrations by Duncan's Multiple Range Test, 5% level

Table (5): Effect of kinetin applied as foliage spray on aerial plantlets formation and spear production in asparagus planets.

Number of aerial plantlets per shoot and spears per plant.

Plants	Concentration									
	0		0.93mM		1.86mM		2.78mM		3.72mM	
	Aerial Plantlets	Spears	Aerial Plantlets	Spears	Aerial Plantlets	Spears	Aerial Plantlets	Spears	Aerial Plantlets	Spears
1	0	0	0	5	0	4	0	1	0	1
2	0	4	0	2	0	3	0	3	0	4
3	0	2	0	4	0	3	0	3	0	4
4	0	6	0	5	0	6	0	2	0	4
NS[Y]	0	3	0	4	0	4	0	2.3	0	3.25

[z] Mean separation of concentrations by Duncan's Multiple Range Test, 5% level.

[Y] NS = non - significant

Table (6): Effect of kinetin applied as soil drench on aerial plantlet formation and spear production in asparagus plants. Number of aerial plantlets per shoot and spears per plant.

Plant	Concentration									
	0		0.93mM		1.86mM		2.78mM		3.72mM	
	Aerial plantlet		Aerial plantlet		Aerial plantlet		Aerial plantlet		Aerial plantlet	
		Spear		Spear		Spear		Spear		Spear
1	0	3	0	2	0	4	0	5	0	2
2	0	2	0	5	0	0	0	3	0	3
3	0	0	0	4	0	4	0	4	0	3
4	0	4	0	4	0	4	0	6	0	5
Mean [z] NS[y]	0	3	0	3.8	0	3	0	4.5	0	3.3

[z] Mean separation of concentration by Duncan's Multiple Range Tests.5% level

[y] NS = non - significant

EFFECT OF CYTOKININ ON RHIZOME BUD INITIATION

The treatments of shoots of different ages with benzyladenine solution led to different responses. Younger shoots were more responsive than older ones (Table 7). This shows that the age response of asparagus plants to benzyladenine is similar to their response to thidiazuron [6].

Table (7): Effect of 1.3 mm benzyladenine applied in cotton pieces at middle of asparagus shoots of different ages. Number of aerial plantlets formed.

Shoot	6weeks old	8 weeks old	10 weeks old
1	12	10	5
2	12	10	6
3	13	11	6
4	14	10	6
Mean [z]	12.75a	10.25b	5.75c

[z] Mean separation of shoot age by Duncan Multiple Range Test, 5%level.

EFFECT OF CYTOKININ ON RHIZOME BUD INITIATION

The treatments of asparagus plants with different concentrations of benzyladenine resulted in formation of new buds, (Table 8). This result suggests that the action of benzyladenine in asparagus system was similar to action of kinetin, which has been reported to cause bud initiation in several plant systems (such as tobacco tissue culture[10]; in *Isatis tinctoria* roots, [11]; in Convolvulus roots, [12]; in Begonia leaves, [13]. The effect of benzyladenine on bud initiation in asparagus rhizome is similar to the effect of thidiazuron on bud initiation in the same system as has been reported earlier[6].

INTERACTION OF THIDIAZURON AND BENZYLADENINE

Table (9) shows results of interaction of thidiazuron and benzyladenine in formation of aerial plantlets. Plants treated with thidiazuron alone gave the highest number of aerial plantlets, whereas benzyladenine alone produced the lowest number. Thidiazuron applied prior to or in a mixture with benzyladenine produced an intermediate response in aerial plantlet formation. Table (10) shows that similar interaction occurred when chemical solutions were applied as soil drench. The results suggest that a physiological interaction between thidiazuron and benzyladenine occurs, but the exact cause of this interaction cannot conclusively be determined from these results.

Table (8): Effect of benzyladenine applied as soil drench on initiation of new buds in UC 66 asparagus plants, 4months old.

Number of new buds

Plant Nymber	Concentration				
	0	0.46mM	0.93mM	1.4mM	1.8mM
1	1	8	5	6	0
2	2	9	6	5	2
3	2	9	7	0	0
4	2	6	4	3	2
Mean[z]	1.75c	8.0a	5.5b	3.5bc	1.0c

[z] Mean separation of concentrations by Duncan's Multiple Range Test, 5% level.

Table(9): Interaction of thidiazuron (thid.) and benzyladenine applied as foliage spray on aerial plantlet formation in asparagus plants

Number of aerial plantlets

Plant	Treatments					
	H_2O	thidiazuron	Thidiazuron Followed by BA	BA	BA followed By thid.	Mixture of thid.&BA.
1	0	30.6	20.5	7.8	16.25	105
2	0	20.5	19.5	7.5	10.0	20.0
3	0	20.8	15.3	8.4	16.2	18.8
4	0	30.5	16.7	8.1	9.2	15.5
Mean [z]	0 d	25.6 a	18.25 b	7.95c	12.9 be	16.2 b

[z] Mean separation of Duncan's Multiple Range Test, 5% level.

Table (10): Interaction of thidiazuron and benzyladenine applied as soil drench in the formation of aerial plantlets in asparagus plants

Number of aerial plantlets

Plant	Treatments					
	H_2O	thidiazuron	Thidiazuron Followed by BA	BA	BA Followed By Thid.	Mixture of Thid.&BA
1	0	9.6	5.0	0	7.25	6.0
2	0	7.2	5.0	1.25	5.0	6.6
3	0	8.5	4.25	0.8	7.5	5.6
4	0	7.2	7.0	1.1	8.5	10.5
Mean [z]	0	8.1a	5.3 b	0.78 c	7.0 ab	7.1 ab

[z] Mean separation of treatments by Duncan's Multiple Range Test, 5% level.

APICAL DOMINANCE EXPERIMENTS

Removal of apex from main shoot or removal of apex and treating the wound with lanolin did not promote lateral buds to grow and form aerial plantlets (Table 11). Table (11) also shows that placing thidiazuron on lateral buds of shoots with intact apices, or shoots in which apices were replaced with auxin, promoted lateral buds to grow and form aerial plantlets. Likewise, spraying thidiazuron onto shoots of intact apices or shoots in which apices were replaced with auxin led to formation of aerial plantlets. So, removal of apices, without thidiazuron treatment, had no effect on aerial plantlets formation, while thidiazuron treatments led to

formation of aerial plantlets. The inhibition of lateral buds might not be under sole control of main apex, but needlelike branches (cladophylls) might also have contributed to inhibition. If so when apex was removed, the lateral buds might still be under inhibition by the cladophylls. When thidiazuron was applied it was able to counteract inhibition exerted on lateral buds by apices and cladophylls or by exogenously applied auxin and cladophylls, and promote formation of aerial plantlets on asparagus shoots.

Table (11): Effect of removal of shoots apices and treatment with indolebutyric acid and Thidiazuron on formation of aerial plantlets in UC 66 asparagus plants, 5.5 months old

Number of aerial plantlets per shoot

Shoots Number	Treatments								
	A*	B*	C*	D*	E*	F*	G*	H*	I*
1	0	0	0	27	8	20	45	35	25
2	0	0	0	16	14	13	24	20	29
3	0	0	0	22	6	12	47	27	23
4	0	0	0	15	9	25	27	30	20
5	0	0	0	20	10	15	25	25	28
6	0	0	0	17	7	16	44	41	26
MEAN[z]	0f	0f	0f	19.5cd	9.0e	16.8d	35.6a	29.6ab	25 bc

[z] Mean separation of treatments by Duncan's Multiple Range Tests. 1% level.

A* = Control, no treatment.

B* = Apex removed from main apex

C* = Apex removed replaced with lanolin

D* = Apex intact, thidiazuron on lateral buds

E* = Apex removed replaced with 0.01% IBA, thidiazuron put on lateral buds.

F* = Apex removed replaced with 0. 1% IBA, thidiazuron put on lateral buds.

G* = Apex intact, thidiazuron spray on plants.

H*= Apex removed replaced with 0.01% IBA, thidiazuron spray on plants.

I* = Apex removed replaced with 0. 1% IBA, thidiazuron spray on plants.

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