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REGULATION OF ADVENTITIOUS ROOTS FORMATION BY AUXIN AND CYTOKININ OF DEROOTED CUCUMBER SEEDLING IN RELATION TO AUXIN TRANSPORT

[29]

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ABSTRACT

The effects of application of synthetic cytokinin (CK) 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) and synthetic auxin Indole butyric acid (IBA) on adventitious root formation in cucumber hypocotyl were studied. Both plant growth regulators were supplied to derooted cucumber seedlings either as droplet of solution between the cotyledons (apical application) and/or through the hypocotyls stump (basal application).Epically applied CPPU or IBA increased auxin transport out of hypocotyls and stimulated adventitious root. In contrast basal applied CK or IBA decreased auxin transport and adventitious root formation. Several developmental parameters of derooted seedling (phenols, chlorophyll, sugars, and number of adventitious root growth) were studied in correlation to previous treatments and auxin transport. Furthermore anatomical structures of vascular bundles of treated hypocotyls were examined in relation to plant adventitious root formation and auxin transport.

Key Words: Chloropyridyl phenylurea (CPPU), Indole butyric acid (IBA), Cucumber, Hypocotyls, Adventitious root, Auxin transport-anatomy of vascular bundle, Biochemical constituents

INTRODUCTION

Different classes of plant-growth regulators, including auxin , cytokinins, gibberellins, brassinolide, as well as inhibitory substances such as abscisic acid, growth retardants and phenolics, influence root initiation (**Arteca 1996**). Several reports indicated that, auxin is involved in the initiation of adventitious roots and that division of root initials is dependent either upon exogenous or endogenous auxin. There are exceptions which auxin show no effect or inhibitory effect at higher concentrations on root formation, (**Hatrmann *et al* 1990**). Indole butyric acid IBA and Naphthalene acitc acid NAA are still the most commonly use auxins as a commercial basis for rooting (**Blazich 1989**). On the other hand, polarity observed with respect to adventitious root initiation has been correlated with auxin movement supporting the role of auxin in root initiation (**Hartmann *et al* 1990**). So, auxin transport inhibitors, 2,3.5,-triiodobenzoic acid

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(TIBA) inhibits rooting by inhibiting basipetal polar auxin transport and subsequent rooting. Another class of plant growth regulators is cytokinin, high cytokinin-to low auxin ratios promoted shoot growth and inhibited root development. These have been reported in the literature supporting that cytokinin inhibit root formation. Cuttings from species with high endogenous levels of cytokinins are more difficult to root than those with low cytokinin levels **(Okoro and Grace 1978).** Applied synthetic cytokinin to stem cutting inhibited adventitious root formation **(Moke & Moke 1994 and Hartmann *et al* 1990).** Also the interaction between auxin and cytokinin in regulating root formation still unclear. Therefore the present study was conducted to study the effect of both applied plant growth regulators on adventitious root formation and auxin transport. The reason of this research is to study the factors control adventitious root formation-inhibition. Derooted cucumber seedling is usually used as scion in grafting processes of cucumber. Adventitious root formation on scion in graft union caused graft failure **(Shehata *et al* 2000).** So this investigation offer important point for improving graft processes.

MATERIAL and METHODS

1. Plant material

Seeds of cucumber (*Cucumis sativus* cv. Beta alpha) were surfaced sterilized for 10 min in 10 % sodium hypochloride solution, then rinsed 3 h in running water and planted in seedling trays 84 holes. Under green-house of Agric. Botany Dept., Ain Shams Univ. Shoubra Elhkema. 500 uniformed cucumber seedling were derooted as plant material.

2. Treatment

Derooted cucumber seedling 8 days old after sowing were treated with auxin (indole butyric acid) IBA and synthetic cytokinin, CPPU (1-(2-chloro-4-pyridyl)-3 phenylurea. Derooted cucumber seedling were put in glass vial ∅ 2 mm × 5 mm contained aqueous solution of both previous plant growth regulators. The concentration of aqueous solution were 4 ppm and 2 ppm for IBA & Cppu respectively. Further treatment of plant growth regulators were applied as droplet at meristimatic apex between cotyledonary leaves.

Treatment could be summarized as follow:

1- Control (water only)
2- Auxin (IBA) at 4 ppm as droplet at shoot apex
3- Aqueous solution of auxin (IBA) at 4 ppm at the basal end of hypocotyls stump.
4- Cytokinin (CPPU) at 2 ppm as droplet at shoot apex
5- Cytokinin (CPPU) at 2 ppm at the basal end of hypocotyls stump.
6- Auxin (IBA) at 4 ppm at shoot apex and cytokinin (CPPU) at 2 ppm at the basal end of hypocotyls stump.
7- Cytokinin (CPPU) at 2 ppm at shoot apex and auxin (IBA) at 4 ppm at the basal end of hypocotyls stump.

3. Developmental Parameters

Six days after treatments derooted cucumber seedling were harvested to determine hypocotyls length, adventitious

root number, cotyledonary leaf area, meristematic apex activity (represented by the area of foliage leaf differentiation).Further more phenols, protein, chlorophyll, total carbohydrate and sugars were determined as follows:

Determination of Total Carbohydrates

One gram sample was randomly taken and added to 30 ml HCL 2N. The tubes were placed in a boiling water bath for 6 h. After cooling, the sample was transferred into a calibrated flask (100-ml). Total carbohydrates were estimated by the alkaline potassium ferricyanide method **(Shales and Schales, 1945)**.

Determination of total soluble sugars

One gram sample was ground in a mortar with ethanol 80% for 3 times. The extracts were combined and evaporated till dryness. The dried film was dissolved in 50 ml of 10 % aqueous isopropanol. Total soluble sugars determination was carried out according to the method by **(Shales and Schales, 1945).**

Determination of soluble protein

One gram sample was dried and mixed with 5 ml of extraction buffer (0.125 M tris borate, ph 8.9) then shaked for one hour and filtered . The supernatant was contained the soluble protein. A colorimetric determination of soluble protein was carried out by using the method of (**Bradford 1976**).

Determination of chlorophyll

0.1 gm Fresh weight (0.1 gm) was homogenized with 80% acetone and the extraction was obtained by filtration of the solvent in Buchner funnel. Total chlorophylls were determined spectophotometrically at 663 and 645 nm (Shimadzu UV-1601PC) using the method of **Arnon (1949)** and data were expressed as mg/g fresh weight.

Determination of Phenoles

one gram of fresh weight was taken and extracted with 80% cold methanol (v/v) for three times at 0oC. The combined extracts were collected and filtered (Wt. No. 1). Then , the volume of sample was raised up to 25 ml with cold methanol. Phenoles determination was carried out according to **Danial and George (1972).**

4. Auxin transport

Auxin transport out of hypocotyls was determined at 2, 4, 6, days after treatments. 10 replicates from each treatment with uniformed hypocotyls length were immediately put in 2 ml of receiver buffer (K2 HPO4 + KH2PO4 0.05mμ with PH 6.2) for 24 hr in dark at 25 °C.

The receiver buffer was analyzed to separate auxin by Sep-pak Cartridge 18 (C18) using aqueous solution of 40 % MeoH containing 0.1 N acetic acid **(Li and Bangerth 1992)**. Indirect ELISA was used to estimate auxin transport according to **(Weiler *et al* 1986)**

5. Anatomical studies

Samples of basal end of hypocotyls 5 mm in length were taken 6 days after treatment. They were immediately killed and fixed using F. A. A. solution for 24 hr. Paraffin method was followed up as

described by **Johanson (1940) and Gerlach (1989).** The prepared sections were examined using "Carl Zeiss" research light microscope. Photos were taken by especially fitted camera on top of the light microscope.

Statistical Analysis

The collected data were subjected to the proper statistical analysis of complete randomized design. L. S. D. at 5% level of probability was used to compare between means. **(Snedecor andCochran 1980).**

RESULTS

Developmental Parameters

Some developmental parameters of derooted cucumber seedling were studied 6 days after treatments of auxin (IBA) and cytokinin (CPPU), (Table 1). Apical application of auxin and untreated plants (control) stimulated adventitious root formation at the basal end of the hypocotyls. The adventitious root number could be morphologically detected and reached 5.6 and 3 roots by auxin application and control respectively. On the other, hand no adventitious roots formation could be morphologically detected by other treatments of both growth regulators. Emergence of the first foliage leaf from the meristematic tip followed the same pattern of adventitious root formation (Table 1), in addition to stimulating effects were shown by CPPU basal application and CPPU (basal) + IBA (apex). Apical application of IBA and basal application of CPPU enhanced cotyledonary leaves area, whereas apical application of CPPU + IBA (basal) reduced them. Six days after treatment no changeable was detected in hypocotyls length (Table 1).

Table 1. Effect of auxin (IBA) and cytokinin (CPPU) 6 days after treatments on some growth characters of derooted cucumber seedlings

Treatments	Hypocotyls length (cm)	Adventitious roots number	Cotyledonary leaves area (cm^2)	First foliage leaf area (cm^2)
Control	4.6	3.0	10.4	3.1
Auxin at apex	4.1	5.6	12.8	2.1
Auxin at basal	4.3	0.0	10.2	0.0
CK at apex	4.0	0.0	11.2	1.26
CK at basal	4.2	0.0	12.2	0.9
Auxin at apex + Ck at basal	4.3	0.0	11.4	0.0
CK at apex + auxin at basal	4.7	0.0	7.20	0.0
LSD (0.05	0.61	1.52	0.65	0.07

Biochemical Constituents

IBA application at basal end of hypocotyls significantly increased phenol compounds in derooted seedling recording 472 ug/g. whereas CPPU applied below decreased phenol compounds to half amount comparing with IBA application (Table 2). Less amount of phenol in derooted seedling was detected by application of CPPU at tip plus IBA at basal. In contrast, the same treatment significantly increased soluble proteins and chlorophyll concentration. Also, an increase in chlorophyll were detected by basal application of IBA and CPPU. Reduction in total carbohydrates and sugars were found by all treatments.

Table 2. Effect of auxin (IBA) and cytokinin (CPPU) 6 days after treatments on some biochemical constituents of cotyledonary leaves of derooted cucumber seedlings

Treatments	Phenols (ug/g)	Soluble proteins (mg/g)	Total chlorophyll (mg/g)	Total carbohydrates (mg/g)	Total soluble sugars (mg/g)	Reduced sugars (mg/g)
Control	153.07	2.75	1.48	67.90	28.51	22.50
Auxin at apex	207.8	2.55	1.74	32.00	26.88	9.11
Auxin at basal	472.15	2.32	2.44	35.45	21.98	10.81
CK at apex	173.6	2.58	1.63	22.12	10.29	9.10
CK at basal	257.72	2.94	2.22	36.75	22.66	6.62
Auxin at apex + Ck at basal	177.09	2.25	2.09	21.30	8.70	8.11
CK at apex + auxin at basal	113.61	4.74	2.31	29.73	18.20	10.19
LSD (0.05)	28.98	0.28	0.07	1.11	0.65	0.41

Auxin Transport

Auxin transport out of hypocotyls was increased by apical applied cytokinin (CPPU) or by apical treated auxin (IBA). Apical applied cytokinin stimulated auxin transport more than apical applied auxin. In contrast, auxin transport was significantly reduced by auxin and cytokinin applied at basal stump of derooted seedling. This was found to be more pronounced by basal application of auxin than that of cytokinin.Combination treatments, i.e.cytokinin at shoot apex with auxin at basal end or auxin at shoot apex with cytokinin at basal end reduced also auxin transport out of hypocotyls (Fig.1).

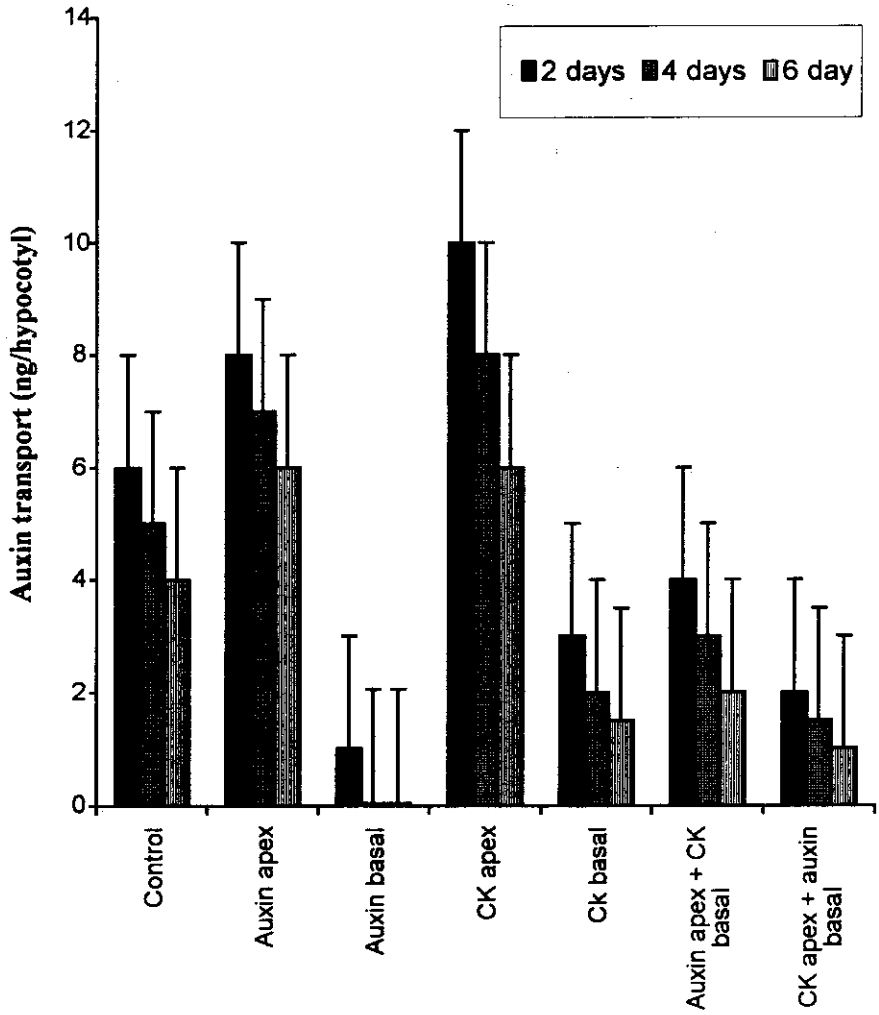


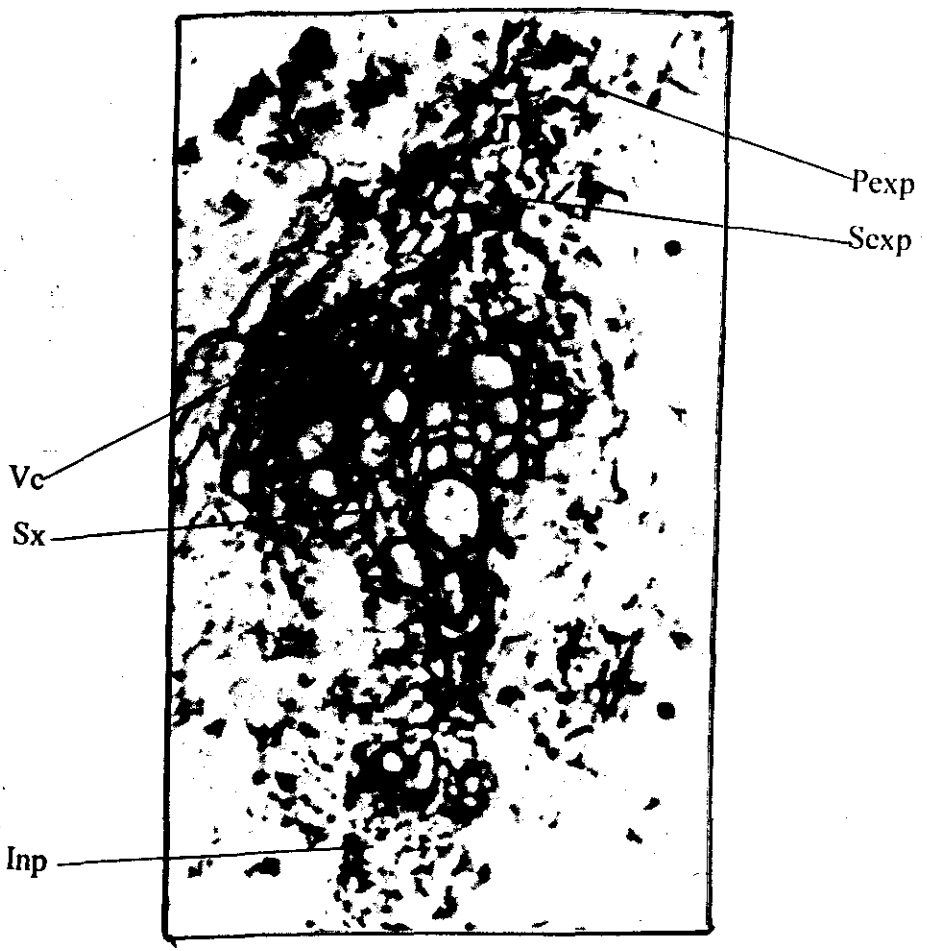
Fig. 1. Effect of different applications of Auxin and Cytokinin (CK), at shoot tip or basal of hypocotyls on Auxin transport out of hypocotyls of derooted cucumber seedlings at 2,4,6 days from treatment.

Anatomical Structres

Cross sections through the basal end of hypocotyls of derooted cucumber seedling were made to investigate the anatomical structure, particularly vascular bundles which influenced by application of growth regulators. The vascular bundles of hypocotyls are bicolleteral as a common feature of family cucurbitaceae (**Esau 1960**). The bicolleteral vascular bundles of untreated plant showed vascular cambium activity which differentiated to secondary phloem outwardy and xylem inwardy (Plate 1- A).Plants treated with auxin at meristematic apex, their vascular bundles showed highly vascular cambium activities with smaller xylem vessels diameter comparing with control .Furthermore, adventitious root primordial could be detected at lateral side of vascular bundles (Plate 1- B). Few secondary phloem with highly number of secondary xylem vessels could be observed in vascular bundles of treated plants with auxin at basal stump of hypocotyls (Plate 1- C). This bundle showed less cambial activity comparing with control or auxin treated at shoot apex. Examined bicolleteral bundles of treated plants with cytokinin at shoot apex revealed that vascular cambium activity differentiated more secondary phloem than secondary xylem. This effect was more pronounced when cytokinin was applied below at hypocotyls stump (Plate 1-D, E). In this case the vascular cambium give rise to differentiate high amount of secondary phloem. The vessels number of secondary xylem were reduced to half as compared to plant treated with cytokinin at shoot apex.Suplied auxin from above(shoot apex) or below (at hypocotyls stump) with plants treated with cytokinin revealed that the vascular cambium of treated plants with cytokinin at basal and auxin at shoot apex induced few secondary xylem vessels with high secondary phloem (Plate 1-F). No reduction in secondary xylem vessels could be observed in bicolleteral bundle of plants treated with cytokinin at shoot apex and auxin supply at basal end (Plate 1-G).

DISCUSSION

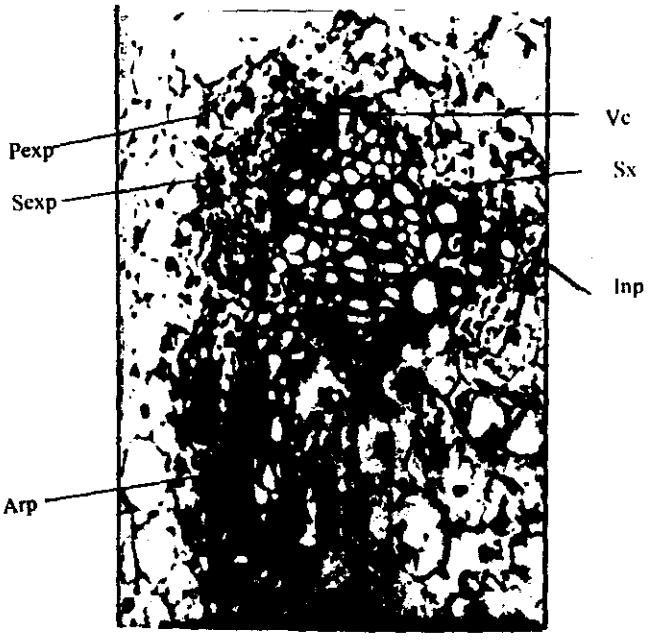
Several evidence in literature indicated that, auxin transport involved in adventitious root formation. Polarity observed with respect to adventitious root initiation has been correlated with auxin movement supporting the role of auxin in root initiation (**Blakesley *et al* 1993**). The role of basipetal polar auxin transport in adventitious rooting could be confirmed by using auxin transport inhibitors such as 2,3,5-triiodbenzoic acid (TIBA). Application of IBA at the basal end of plant cutting inhibits rooting by inhibiting basipetal transport of IAA and subsequent rooting. Obtained data indicated that auxin transport out of hypocotyls correlated with adventitious root formation. So non disturbance auxin transport out of hypocotyls either treated with auxin IBA on shoot apex or untreated revealed that high number of adventitious root arised on these hypocotyls. (Table 1 and Fig. 1). Whereas decrease auxin transport out of hypocotyls could be prevented adventitious root formation. This effect could be observed in basal applied auxin and cytokinin. Inhibition of basipetal polar auxin transport by basal applied auxin at hypocotyls stump resulted from autoinhibition (**Bangerth 1989 and Shehata 2000**). Furthermore basal applied cytokinin at hypocotyls stump reduced also



A- control

Plate (1) : Effect of auxin (IBA) and Cytokinin (CPPU) applied either epically or basipetaly to derooted cucumber seedling on vascular bundles development at basal end of hypocotyls, 6 days after treatments, (200 X).

Arp = Adventitious root primordial
Pexp = Primary external phloem
Sexp = Secondary external phloem
Inp = Internal phloem
Vc = Vascular cambium
Sx = Secondary xylem



B- Auxin at apex

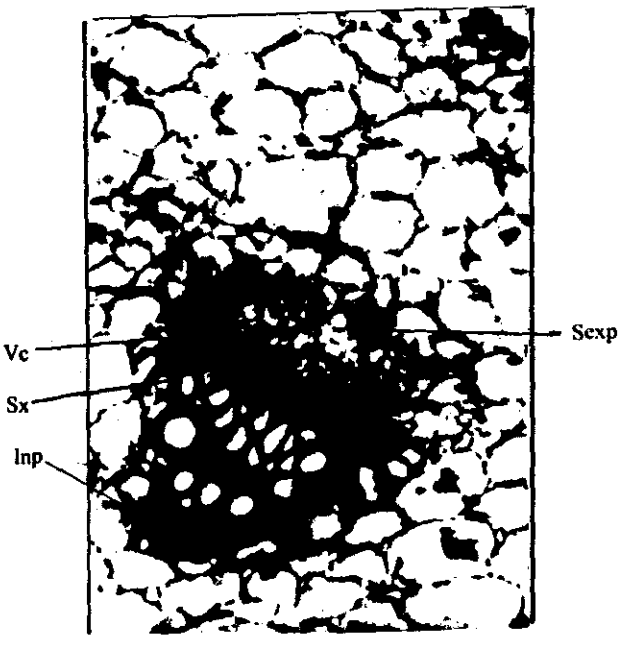
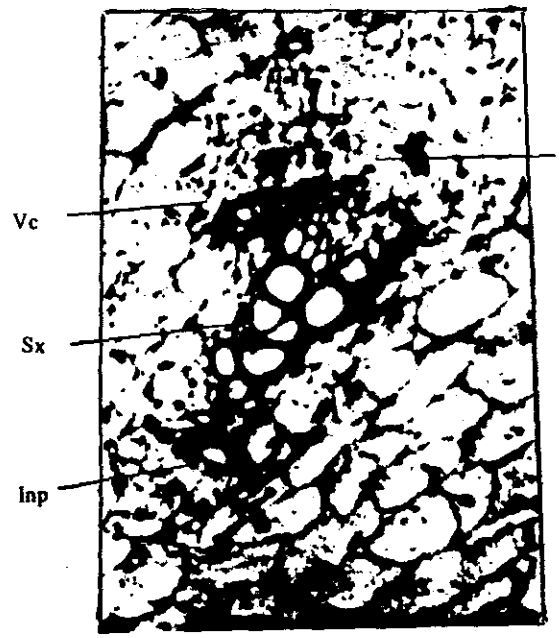
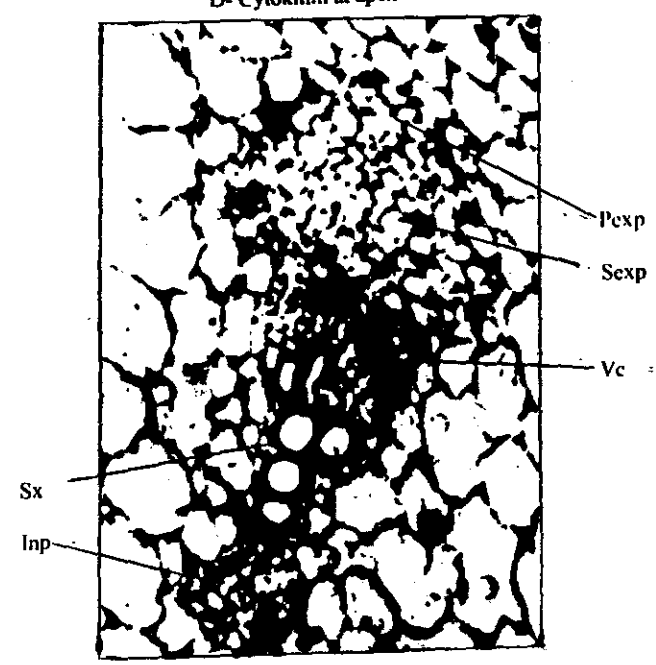


Plate (1) Cont. C- Auxin at basal

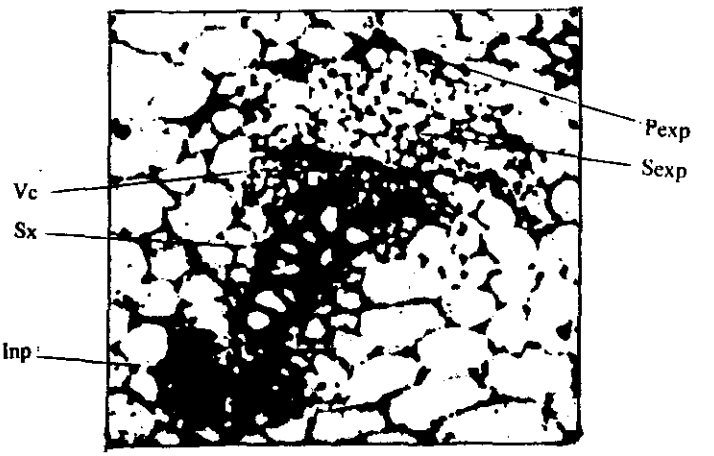


D- Cytokinin at apex



E- Cytokinin at basal

Plate (1) Cont.



F- Auxin apex + Cytokinin basal

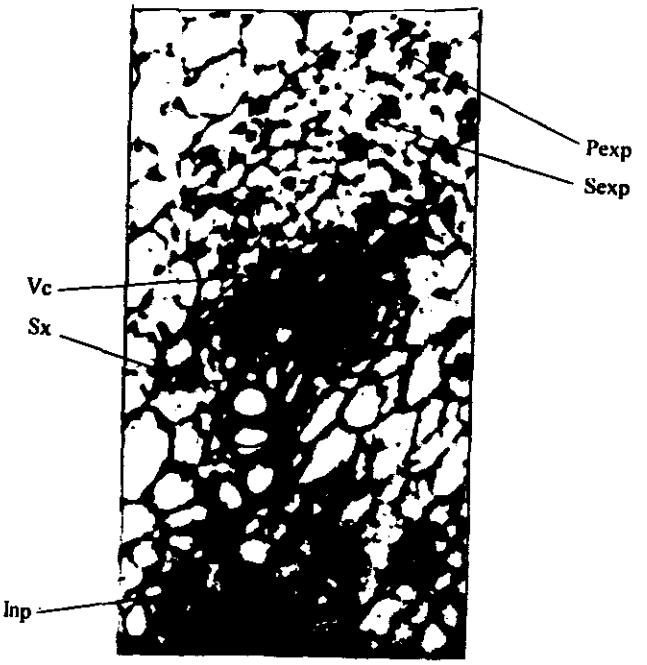


Plate (1) Cont. G- Cytokinin apex + auxin basal

auxin transport. These results were found in agreement with **(Bangerth 1989).** Feed back mechanism existed between cytokinin and auxin; high auxin transport from shoot apex decreased cytokinin concentration in xylem exudate. In the other words, increased cytokinin at basal end decreased auxin transport. **(Bangrth, 1994).** In contrast epicaly applied cytokinin at shoot stimulated auxin transport (Fig. 1). **Li and Bangerth (1992),** found that application of synthetic cytokinin (CPPU) to the pea apex had been significantly stimulated the polar auxin transport out of that apex. Untreated plant (control) showed adventitious lateral root formation near hypocotyls stump because auxin tends to accumulate immediately above any wound site in shoots as result of polar auxin transport. Another function of IAA transport is correlative signal in dominance phenomena **(Bangerth, 1989).** Meristematic tip and cotyledonary leaves of hypocotyls provided example for correlative signal of auxin transport, high auxin transport from apex enhanced cotyledonary leaves senescence by decreasing chlorophyll and protein conents (Table 2). In this concern, positive correlation was found between auxin transport out of wheat ears and chlorophyll concentration in flag leaf, higher auxin transport enhanced chlorophyll degradation in flag leaf **(Shehata, *et al* 2001).** The site of application of growth regulators *i.e.* proximal or distal of shoot apex act differentially in relation to abscission in this respect. The action of transport out of apex could be mediated by at least three possibilities. 1- altering senescence by decreasing CK level in xylem. 2- by direct assimilate transport to apex and compete for leaf petiole. 3- decreased IAA export out of leaf petiole by autoinhibition **(Bangerth, 1989).** Obtained results showed that, phenol contents increased with decreasing auxin transport. Phenols is well known as auxin transport and adventitious root inhibitors. **(Arteca, 1996).** On the other hand, the involvement of auxin transport in vascular differentiation was reported **(Aloni, 1987).** Auxin application stimulated cambial activities and xylem differentiation **(Zakrzewski, 1983).** Whereas cytokinin decreased xylem differentiation and promoted phloem differentiation, these effects could be obviously detected in (Plate 1 D, E). **Blakesley *et al* (1991),** described the process of the formation of adventitious roots. These appears to be consists of four stages, dedifferentiation coupled with the formation of meristematic locus, cell division to form a radially symmetrical culture of cells, further division coupled with organization into a bilaterally symmetrical meristem and finally growth of cells in the basal part of meristem which causes its protrusion through the epidermis. **Houck and LaMotte (1977)** reported that zeatin or zeatin riboside (cytokinin) in aqueous solution, applied to the bases of excised internodes of coleus receiving auxin (IAA) at their apical ends, restored phloem regeneration to the level of that found in whole plants. Generally apical application of both plant growth regulators stimulated vascular cambium activities of vascular bundles than those of basal applied.

It could be concluded that regulation of auxin transport and subsequent adventitious root formation by auxin/ck application and their interaction could be provided a tool for improvement cucumber grafiting, by preventing adventitious root formation at cucumber scion which

caused graft failure (**Shehata 2000**). Control adventitious root formation on cucumber scion is important from commercial point of view.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ،جامعة عين شمس ، القاهرة، م (٤٧)، ع (٢)، ٤٤٥-٤٥٩، ٢٠٠٢

تنظيم تكوين الجذور العرضية بواسطة الاوكسين و السيتوكينين على بادرات الخيار منزوعة الجذور وعلاقة ذلك بانتقال الأوكسين

[٢٩]

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١- قسم النبات الزراعي – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر

- تم دراسة تاثير اضافة السيتوكينين الصناعي CPPU بتركيز ٢ جزء فى المليون و الاوكسين الصناعى IBA بتركيز ٤ جزء فى المليون على تكوين الجذور العرضية على السويقة الجنينية السفلى فى بادرات الخيار منزوعة الجذور وقد تم إضافة كلا من منظمات النمو السابقة اما على القمة النامية بين الأوراق أو عند قاعدة السويقة.
- تم دراسة تاثير هذة المعاملات على عدد من قياسات النمو مثل عدد الجذور – طول السويقة – مساحة الأوراق الفلقية

ومساحة الورقة الأولى بالإضافة الى بعض المركبات الحيوية مثل الفينولات والبروتينات الذائبة والكلوروفيل والكربوهيدرات الكلية والسكريات و تم دراسة العلاقة بين هذه المركبات و انتقال الاوكسين.

- تم عمل دراسة تشريحية لدراسة التغيرات الحادثة فى الحزم الوعائية وارتباط ذلك بالمعاملات وانتقال الاوكسين.

وتتلخص أهم النتائج فيما يلى

- إضافة السيتوكينين و الاوكسين عند القمة زاد من انتقال الاوكسين فى السويقة وشجع تكوين الجذور. وعلى العكس إضافة السيتوكينين والاوكسين عند القاعدة قلل من انتقال الاوكسين وتكوين الجذور العرضية.
- إضافة الأوكسين عند القمة أو السيتوكينين عند القاعدة أدى الى زيادة مساحة الأوراق الفلقية فى حين أن إضافة الاثنين معا بحيث يكون السيتوكينين عند القمة والأوكسين عند القاعدة أعطى تأثير عكسى.
- المعاملة بالاوكسين عند القاعدة أدت إلى زيادة واضحة فى مستوى الفينولات فى حين نفس المعاملة بالسيتوكينين أدت إلى نقص الفينولات إلى نصف الكمية الموجودة فى حالة المعاملة بالاوكسين.
- المعاملة بالسيتوكينين عند القمة بالإضافة إلى الاوكسين عند القاعدة أدت زيادة تركيز البروتين الذائب والكلورفيل.
- المعاملة بالاوكسين عند القمة أدت إلى زيادة في نشاط الكامبيوم الوعائى وصغر فى قطر أوعية الخشب مقارنة بالكنترول بجانب ظهور بداءات الجذور العرضية.
- المعاملة بالأوكسين عند القاعدة أظهرت قلة فى اللحاء الثانوى وزيادة فى عدد أوعية الخشب الثانوى فى الحزم الوعائية. وقد لوحظ أن نشاط الكامبيوم أقل فى هذه الحزم.
- المعاملة بالسيتوكينين من القمة أدت إلى تكشف الكامبيوم الوعائى إلى مزيد من اللحاء الثانوى مقارنة بالخشب الثانوى.

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