Australian Journal of Basic and Applied Sciences, 4(12): 6179-6184, 2010
ISSN 1991-8178

Regulating Cucumber Grafting by Interactions of Cytokinins in Xylem Exudates of Rootstock and Basipetol Polar Auxin Transport of Scion at Graft Union

S.A.M. Shehata and Amal M. El-Shraiy

Agriculture Botany Department Faculty of Agriculture Ain Shams University, Cairo, Egypt

Abstract: Trails were made to investigate the role of cytokinin in xylem exudates of different rootstocks of cucurbitaceous plants (squash – pumpkin - Bottle gourd –Figleaf gourd) on the progress of cucumber grafting. Also basipetal polar auxin transport out of hypocotyls of previous rootstocks and cucumber scion was also determined in relation to xylem exudates cytokinin. Pregrafted rootstocks showed great variation in cytokinin concentrations. Also the concentration of basipetal polar auxin transport out of hypocotyls of previous rootstocks was greatly varied a directly proportion between the concentration of two hormones was detected. Rootstock with higher cytokinin concentration in xylem exudates had higher auxin transport out of its own hypocotyls and vice versa was true. After grafting processes basipetal auxin transport through out grafting union could be used as indicator for grafting success. So the higher the auxin transport was the most successful grafting. Furthermore, cytokinin levels in xylem exudates 1 cm above the grafting union could be also used as second indicator for grafting success. The cytokinin in xylem exudates of rootstocks and basipetal polar auxin transport out of cucumber scion as hormonal signaling in rootstock-scion interactions was discussed.

Key words: cytokinin, Auxin transport, cucumber, grafting, rootstock. Scion, interactions, cucurbitaceae, xylem exudate

INTRODUCTION

Grafting technology is one of the widely applied techniques in vegetable crops. Cucumber is the most famous plant among grafted-vegetables. Grafting processes could be made by different methods and either by hand (hand grafting) or robot (mechanical grafting) Suzuki, *et al* (1995). Selected rootstocks to induce successful grafting in cucumber plant was investigated in different points of view such as anatomical development of grafting union Shehata, *et al* (2000), immunohistochemical localization of auxin in graft union La Shanfa, (2000).

There is a need for better understanding of endogenous factors which control rootstook-scion communication and processes which lead to the beneficial effects of grafting. Aloni, etal (2010) reported that phytohotmones are the most endogenous factor affect rootstock-scion communication in grafted plants. There are four aspects of hormonal signaling in rootstock-scion interactions: 1) formation of the rootstock-scion union; 2) rootstock-scion communication; 3) improvement of grafting interaction by hormonal manipulation; 4) hormonal influence on growth and development. However, little is known about hormonal interaction in graft union, Liu-Meiqin, *et al* (1996) revealed that cytokinins and IAA concentrations in the culture medium of autograft *Vicia faba* internode in vitro affected cell differentiation and the establishment of vascular connections between the rootstock and scion.

On the other hand, the role of cytokinins in plant growth and development was clearly demonstrated in literature including callus proliferation Susan, Capelle (1983); apical dominance Li, *et al* (1995); senescence and monocarpic senescence Nooden, *et al* (1990). Whereas basipetal polar auxin transport regulates vascular differentiation (Aloni, 1995 and Sachs, 1981), correlative dominance signal Bangertu, *et al* (2000), tropism Iino, (1995) and abscission Shehata, (1996).

The present study was conducted to explore the interaction effect of cytokinin in xylem exudates of rootstocks and basipetal polar auxin transport out of scion on development of cucumber graft union.

Corresponding Author: Amal M. El-Shraiy Nafeh Agriculture Botany Department Faculty of Agriculture Ain Shams University
E-mail: amal_nafeh@yahoo.com. Fax: 002 02 44444460

MATERIALS AND METHODS

Grafting Procedures:

Seeds of four rootstocks, i. e. *Ccurbia ficifolia* (fig leaf gourd); *Lagenaria sicerorita* (bottle gourd); *Cucurbita maxima* (pumpkin) and *Ccurbita pepo* (squash) as well as seeds of *Cucumis sativus* (cucumber) passandra hybrid were surface sterilized in sodium perchlorate (0.12 %)for 5 min washed in running tap water and swon in seedling trays 84 holes. Sowing dates was monitor according to the differences in germination rate to obtain uniform seedlings of pervious seeds. The seedlings were watered with complete nutrient solution (Jung, 1972). Seedling of rootstocks and scion were ready for grafting at the appearance of the second foliage leaf. At this stage the thickness of both rootstock and scion were approximately similar. Slint grafting were carried out according the method described by Seong, *et al* (2003).

Cytokinins in Xylem Exudates (Sap):

Cytokinin in xylem exudates was determined in pre and post grafted plants. Pre grafted plants (10 days after germination) were cut 2 cm below two cotyledon leaves, whereas post grafted plants were decapitated 1 cm above graft union. A piece of silicon rubber tubing (5 cm long) was fixed over the top of the cut surface and exudates was collected by Pasteur pipettes three time per timepoint, i. e. three time in four hours; three time in eight hours and so on. Whereas post grafted cytokinin exudates was collected 2, 4, 6 days after grafting. The exudates was immediately frozen in liquid nitrogen and stored at -20 until analysis. For analysis of zeatin and zeatin riboside (Z/ZR), xylem exudates purified by adgusting the pH to (8.5) and passing it first over a polyvinyl pyrolidone colum then, after readjusting PH to (3.0) over a waters. C-18 Sep-pak cartridge (waters Milford, Mass USA). The cartridge was then washed with 0.1 M acetic acid and Z/ZR eluted with 4 ml of 25 % (v/v) methanol in 0.1 N acetic acid (Bangerth, 1994). Indirect ELISA was used to estimate Z/ZR concentrations according to the method described by Weiler, *et al* (1986).

Auxin Transport:

The scion of decapitated plants with 3-4 cm hypocotyls length were cut and immediately immersed in 2 ml phosphate buffer (K_2HPO_4 +KH_2PO_4 0.05 mM with PH 6.2) and kept in dark for 24 hr at 25 C°. The same procedure was carried out to determine auxin transport throughout graft union, grafted plants were cut 1 cm below graft union at 2, 4, 6 days time intervals. For determination auxin transport, phosphate buffer PH (6.2) read gusted to (3.0) and run over sep-pak cartridge, then auxin (IAA) was eluted with 4 ml of 40 % (v/v) methanol (Shehata, 2001). Indirect ELISA was used to estimate the concentration of auxin transport as described by (Weiler, *et al* 1986).

Grafting Progress:

After grafting processes, the successful grafting was recorders as resuming of scion growth and estimated in percent. Furthermore, adventitious root formation on scion was also recorded and represented in percent (Shehata, *et al* 2000)

Statistical Analysis:

The statistical analysis of data were carried out using the SPSS base 16.0 (SPSS Inc., Chicago, IL) packages.

RESULTS AND DISCUSION

Two major hormonal signaling interactions were evaluated during grafting progress. The first signal was cytokinins in xylem exudates of rootstock, meanwhile, the second one was the basipetal polar auxin transport throughout scion. Pregrafted different rootstocks showed great variation of cytokinins (Z/ZR_ concentrations in xylem exudates (Fig. 1).

According to rootstocks the concentrations could be arranged in descending order as follow pumpkin < fig leaf gourd < squach < bottle gourd. However cytokinins in xylem exudates was synthesized in root (Chen, *et al* 1985) and translocate via xylem elements (Van Staden and Davey, 1979). So the amount and concentration of exudates cytokinin depended on root growth vigor. After decapitation the rootstock, the concentration of xylem exudates cytokinin was increased with prolonged the time after decapitation. In this connections (Bangerths, 1994) reported that, when bean plants were decapitated about 16 hour before the xylem exudates was collected, an almost 25 fold increase in cytokinin concentration was observed. This

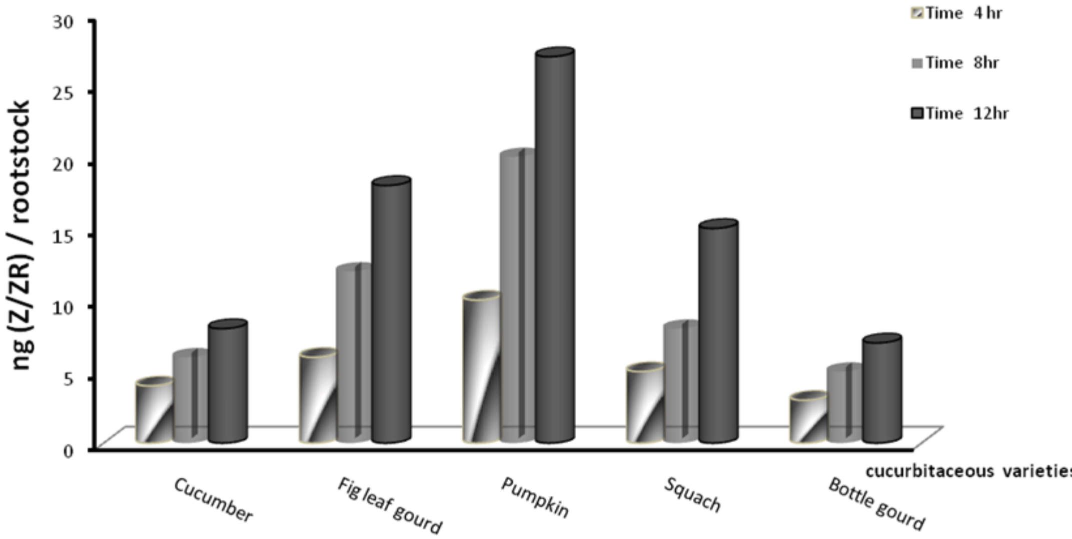

Fig. 1: Cytokinins concentrations (Z/ZR) in xylem exudate of different root stocks after decapitation (10 days after germination) collecting at 4, 8 and12 hours.

increase could be stopped when naphthalene acetic acid (NAA) was applied to cut surface of decapitated plant. This result leads to suggest that cytokinin in xylem exudates of intact plant are under the control of polar auxin transport system. Data in (Fig. 2) revealed that pumpkin had the highest level of auxintransport followed by fig leaf gourd, squash, bottle gourd and cucumber. It could be deduced from (Figs. 1 & 2) that a positive correlation between the level of basipetal polar auxin transport out of different scion and the levels of cytokinin in xylem exudates of its own rootstock was observed. This results indicate that there was a directly proportion between the level of auxin transport and the level of cytokinin (CK). So the higher the auxin transport was the higher the cytokinin in xylem exudates. Such correlations indicate that high cytokinin concentration encounter high auxin transport to depress increasing xylem exudates cytokinin. Bangerth, (1994) showed that an feed back mechanism exist between auxin and cytokinin which a decrease in the polar flow of auxin would resulted in a less-depressive effect on CK concentration in xylem sap.

At grafting time CK (Z/ZR) in xylem exudates of rootstock increased considerably and reached maximum peak after 12 hour (Fig. 2). In the same concern, Li *et al* (1995) revealed that decapitated pea plant showed increase in cytokinin Z/ZR and IAdo (Isopentyl adeninosine) in xylem sap after 6 hours from decapitation. This increase reached its maximum value 12 hr after treatment later on, after 48 hr the CK concentration dropped to its orginal value. However during grafting processes there is available amount of CK derived from rootstock but did not derive from scion. So, the available ck stimulate cell division in both rootstock and scion at graft union and lead to formation of callus tissue. The callus tissue was observed during the anatomical studies on cucumber grafting (Shehata, *et al*. 2000). The link between ck and callus building was documented in tissue culture (Susan, capelle *et al*, 1983). The amound of callus proliferation depended on ck concentration. So the higher the ck concentration was the higher callus proliferation. The callus cell interacted with auxin transport which transported throughout scion at graft union. Transported auxin was found to be nesseccary for differentiation the callus tissues to vascular tissue. The interaction effect of ck and auxin on callus differentiation to conductive tissue was documented in autografted *Vicia faba* internode in vitro (Lia, *et al* 1996). Auxin (IAA) and cytokinin (kinetin) concentrations in culture medium affect cell and establishment vascular connection between rootstock and scion in cultured. Addition 1.4 mg IAA and 0.3 mg kinetin could changed 30 % from callus tissues to vascular tissues. Therefore the amount of auxin transported throughout scion determined the amount of vascular tissues at graft union Sachs, 1981. Moreover, high cytokinin concentration with low auxin concentration resulted in highest phloem element than xylem element (Aloni, 1995). However the role of polar auxin transport on vascular differentiation was documented by (Aloni and Zimmermann 1983). At graft union, the only way of auxin to be transported in basipetal manner is the occurrence of phloem parenchyma or cambium derivatives. These tissues are responsible for polar basipetal auxin transport (Morris and Thomas, 1978).

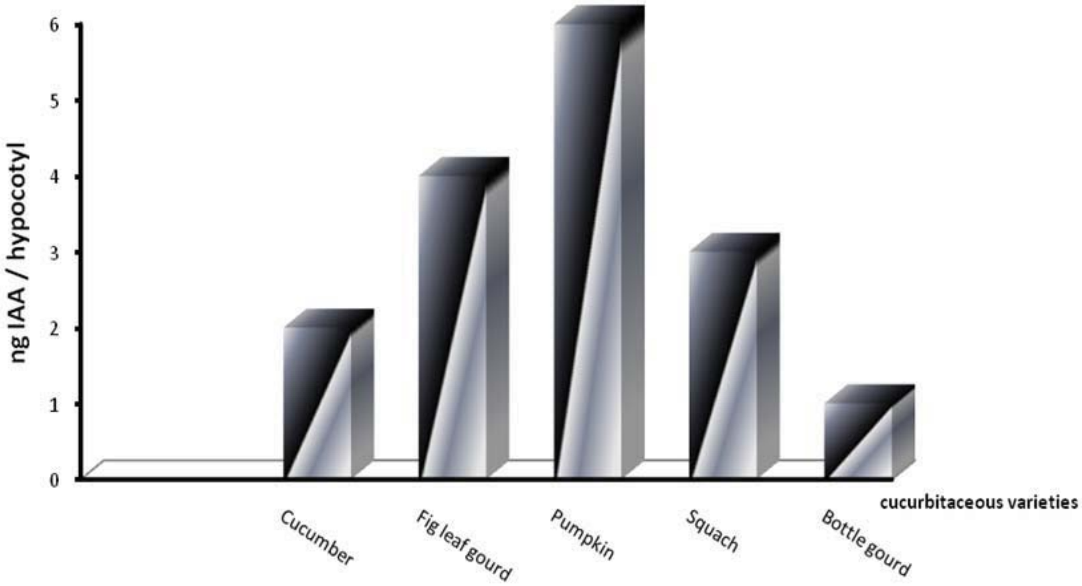

Fig. 2: Auxin transport out of hypocotyl of different cucurbitaceous varieties at grafting time (10 days after germination).

The results in (Fig. 3) revealed a decrease in auxin transport throughout graft union 2 days after grafting and increase gradually with increasing time (4, 6 days after grafting). This indicated that an increase in conductive tissue (phloem parenchyma) needed for auxin transport. La Shanfa (2000) revealed that, at the later stage of graft union development, IAA content decreases gradually in step with the reconnection of the cut vascular bundles of scion and rootstock and continuation of the transport system. The amount of auxin transport below the grafting union reflect the amount of new developed phloem elements (sive tube and phloem parenchyma). So the higher the auxin transport was higher the phloem tissue. In (Fig. 3) revealed that pumpkin had the highest auxin transport flowed by fig leaf gourd; squash and bottle guard. These results matched well with the concentration of cytokinin in xylem exudates during 2, 4 and 6 days after grafting (Fig. 4). The amount and concentration of xylem exudates cytokinin reflect the amount of new developed xylem element. Therefore auxin transport and below graft union (Fig. 3) and cytokinin in xylem sap above graft union (Fig. 4) could be used as indicator to the connection between rootstock and scion which finally reflect the state of grafting failure or successful (Fig. 5)

On the other hand, adventitious root formation at the banal end of cucumber scion prohibited graft connection and reduced the percentage of graft successful (Fig. 5). On the physiological basis, here again cytokinin auxin interaction regulate this phenomenon. The results showed that rootstock with high concentration of xylem exudates cytokinin had less adventitious root formation and vice versa was true (Fig. 4 & 5). In the same concern, Zaghlool, Sanaa and Shehata (2002) reported that basal applied cytokinin to derooted cucumber hypocoty decreased both auxin transport and adventitious root formation. Petridou and Bangerth (1997) revealed that cytokinin application caused a decrease in rooting of pea cuttings. So the endogenous cytokinins had regulatory role of adventitious root formation, when rooting occurred cytokinin content was low (Bollmark, *et al* 1988). The present results revealed that pumpkin rootstock had the highest cytokinin concentration in xylem exudates either pregrafted (Fig. 1) or post grafted (Fig. 4) with lowest adventitious root formation (Fig. 5) as compared to bottle guard.

Finally manipulation hormonal signaling of both cytokinin and in xylem exudates and auxin transport at graft union could be a new approach to obtain successful grafting.

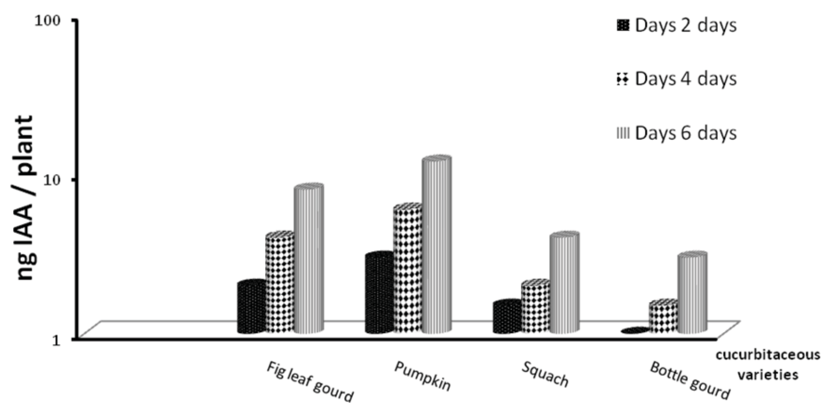

Fig. 3: Auxin transport though out graft union at 2, 4 and 6days after germination.

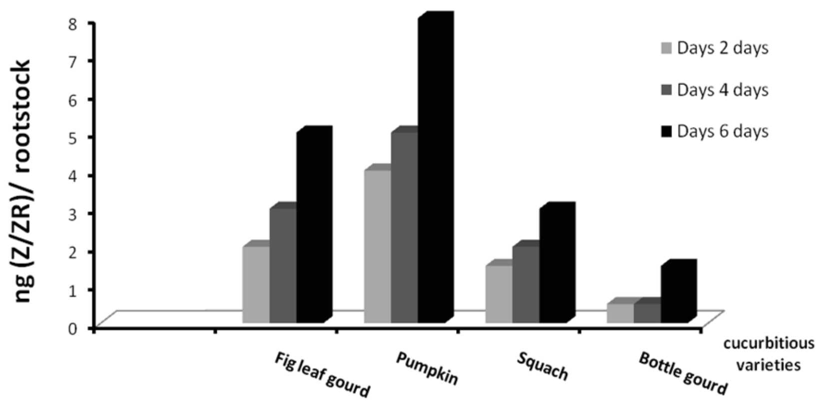

Fig. 4: Concentration of xylem exudate cytokinins (Z/ZR) 1cm above grafting union at 2, 4 and 6 days after grafting.

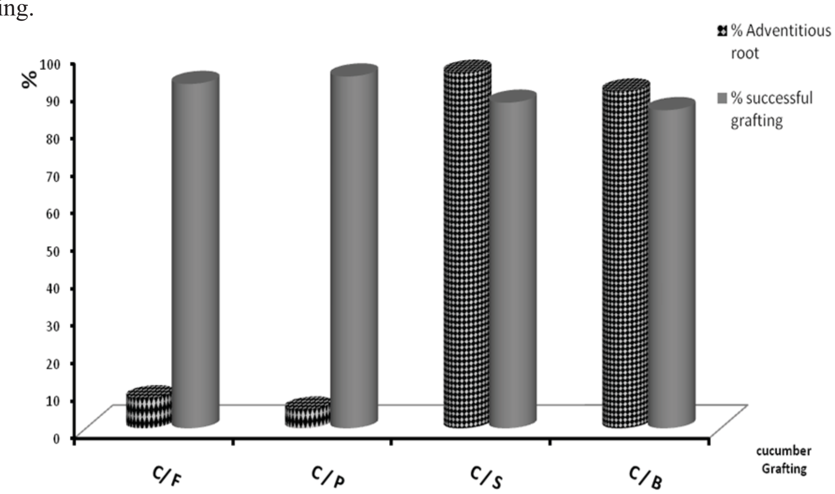

Fig. 5: Percentage of adventitious root formation in graft union and grafting successful percentage of different root stocks.

C/F = cucumber/ fig leaf gourd; C/P = cucumber/ pumpkin; C/S = cucumber/ squash; C/B = cucumber/ bottle gound.

REFERENCES

Aloni, R., R. Cohen, L. Karni, H. Aktas and M. Edeistein, 2010. Hormonal signaling in rootstock-scion interactions. Sci. Horti., 127: 119-126.

Aloni, R., 1995. The induction of vascular tissues by auxin and cytokinin In: plant hormones, physiology, biochemistry and molecular biology, Davies, P. J. ed., pp: 531-546. Kulwer Academic Publishers.

Aloni, R. and M.H. Zimmermann, 1983. The control of vessel size and density along the plant axis. Differentiation, 24: 203-208.

Bangerth, F., C.J. Li and J. Graler, 2000. Mutual interaction of auxin and cytokinins in regulating correlation dominance. Plant Growth Regul., 32: 205-217.

Bangerth, F., 1994. Response of cytokinin concentration in xylem exudates of bean (*Phaseolus vulgaris.* L.) plants to decapitation and auxin treatment and relationship to apical dominance. Planta, 194: 439-442.

Bollmark, M., B. Kubat and L. Eliasson, 1988. Variation in endogenous cytokinin content during adventitious root formation in pea cuttings. J. plant Physiol., 132: 262-265.

Chen, C. M., J.R. Ertl, S.M. Leisner and C.C. Chang, 1985. Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiol., 78: 510-513.

Iino, M., 1995. Gravitropism and Phototropism of maize coleoptiles: Evaluation of the cholodny-went theory through effects of auxin application and decapitation. Plant Cell Physiol., 36(2): 361-367.

Jung, J., 1972. Die Wasserkultur hoherer pflanzer in handbuch der pflanzenernahrang und dungung. Vol. ½ (H. Linser. Ed.) P1136 , Springer verlag wien.

Li, C.J., E. Guevara, J. Herrera and F. Bangerth, 1995. Effect of apex excision and replacement by 1-naphthylactic acid on cytokinin concentration and apical dominance in pea plants. Physi. Plant., 94: 465-469.

Liu-Meiqin, Y. Wang, S. Yang, Liu-Ma, Y.Q. Wang and S.J. Yang, 1996. The effect of plant hormones on the formation of the auto graft of *Vicia faba* internode in vitro. Acta Horti. Sinica, 23(3): 264-268.

La, Shanaf, 2000. Immunohistochemical localization of IAA in graft union of explants internode grafting. Chinese Sci. Bull., 45(19): 1767-1771.

Morris, D.A. and A.G. Thomas, 1978. A microautoradigraphic atudy of auxin transport in stems of intact pea seedlings (*Pisum sativum* L.). J. Exp. Bot., 29: 147-157.

Nooden, L.D., S. Sontokh and D.S. Lentham, 1990. Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. Plant Physiol., 93: 33-39.

Sachs, I., 1985. Cellular patterns determined by polar transport In: plant growth substances, Bopp, M. ed., pp: 231-235, Springer Verlag, Berlin.

Sachs, T., 1981. The control of the patterned differentiation of vascular tissues. Adv. Bot. Res., 9: 152-255.

Seong, K., M. JiHye, L. SangGyu, K. YongGu, K.K. Yong and S. HyoDuck, 2003. Growth, lateral shoot development, and fruit yield of white of white-spined cucumber (*Cucumis sativus* cv. Baekseong-3) as affected by grafting methods. J. Korean Soci. Hort. Sci. Hort. Sci., 44(4): 478-482.

Shehata, S.A.M., 1996. The role of calcium in regulating abscission induced by thidiazuron and its relations to auxin transport out of cotton bolls. Egypt J. Appl. Sci., 11(1): 183-194.

Shehata, S.A.M., U.M. Salama and S.M. Eid, 2000. Anatomical studies on cucumber grafting. Ann. Agric. Sci. Moshtohor, 38(4): 2413-2423.

Shehata. S.A.M., 2001. Regulation of gravitropic response in water hyacinth (*Eichornia carassipes* (mart.) solm) peduncle by calcium and auxin. Arab. Univ. J. Agric. Sci.,Ain Shams univ., Cairo, 9(2): 523-535.

Susan, C. Capelle, W.M. Oarid, C.K. Sandra and C.M. Machted, 1983. Effect of thidiazuron on cytokinin autonomay and the metabolism of N- isopentyl- F-c14, adenosine in callus tissues of *Phaseolus lunatus* L. Plant Physiol., 73: 796-802.

Suzuki, M, K. Kobayashi, K. Inooka and Miurak, 1995. Development of grafting robot for cucurbitaceous vegetables. J. Japanese Soci. Agric. Machinery, 57(3): 103-110.

Van Staden, J. and J.E. Davey, 1979. The synth esis, transport and metabolism of endogenous cytokinin. Plant cell Environ., 2: 93-106.

Weiler, E.W., J. Eberle, R. Mertns, R. Atzorn, M. Feyerabendl, P.S. Jourdan, A. Arnsheidt and U. Wieczorek, 1986. Antisera and monoclonal antibody-based immunoassay of plant hormones In: immunology in plant sciences (Wang T. L. ed) pp: 27-58. Cambridge univ. press.

Zaghlool, Sanaa, A.M. and S.A.M. Shehata, 2002. Regulation of adventitious roots formation by auxin and cytokinin of derooted cucumber seedling in relation to auxin transport. Ann. Agric. Sci., Ain Shams Univ., Cairo, 47(2): 445-459.