Physiological Responses and Grafting Success of In Vitro-Micrografted Cantaloupe (Cucumis melo L.*) onto Squash (Cucurbita pepo* L.*) Rootstocks.*

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Abstract

Soilborne fungal diseases pose a significant constraint to cantaloupe (*Cucumis melo* var. *ananas*) production. Grafting onto resistant rootstock, such as squash (*Cucurbita pepo* L.), offers an effective biological alternative to chemical control. This study integrates plant tissue culture with micrografting techniques to establish a protocol for producing grafted cantaloupe plants. Shoot tips of cantaloupe were surface-sterilized and multiplied on Murashige and Skoog (MS) medium supplemented with various concentrations of benzyladenine (BA) and kinetin (Kin). Micrografts were then performed using in vitro-derived cantaloupe shoots as scions and greenhouse-grown squash seedlings as rootstocks via hole insertion, splice/tube, and cleft grafting methods. Following a dark incubation period for wound healing, grafted plants were acclimatized under high humidity. The experiment was arranged in a Completely Randomized Design (CRD), with data analyzed by ANOVA and means compared using Duncan's Multiple Range Test ($p \leq 0.05$).

This integrated approach aims to combine the pathogen-free advantage of tissue culture with the stress tolerance conferred by squash rootstock, offering a sustainable strategy for improving cantaloupe productivity.

Keywords: *Cucumis melo* L., Micropropagation, micro grafting, Tissue culture, Shoot tips, Benzyladenine, Kinetin, Surface sterilization

Introduction

The origin of melon (*Cucumis melo* L.) is traced back to Africa, according to genetic studies, with wild melons possibly resulting from natural hybridization [1]. Melon cultivation began in China around 3000 BC, in India around 2000 BC, and in Egypt around 1500 BC [2]. Cantaloupe, a varietal group of *C. melo*, belongs to the genus *Cucumis* within the Cucurbitaceae family—a family of considerable global agricultural importance. Immature fruits are consumed fresh in salads or cooked in soups, while ripe fruits are eaten fresh, juiced, or processed into jam [3],

In general, due to the continuous use of agricultural land, it has led to the spread of many pests that affect vegetable crops, including cantaloupe, which is infected by n general, due to the continuous use of agricultural land, it has led to the spread of many pests that affect vegetable crops, including cantaloupe, which is infected by many types of fungi in the root area which leads to a lack of production or the collapse of the plant [4]. One of the common and good methods used for resistance to pests is the grafting technique. Agricultural techniques can be used to solve the problems that face us in growing cantaloupes or any other crops through a grafting technique using resistant rootstock [5],[6]. Grafting is an important agronomic technique widely used in cucurbit crops, including melon (Cucumis melo L.), watermelon, and cucumber, to control soilborne diseases and enhance

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tolerance to abiotic stresses such as low temperatur [7],[8]. This technique involves joining a scion (the desired above-ground variety) with a rootstock (the root-providing plant), typically from a resistant species or hybrid within the Cucurbitaceae family [9] One of the methods of grafting vegetables can be used to graft cantaloupe onto squash (Cucurbita pepo L.) rootstock to combat diseases that affect cantaloupe roots [10Squash (Cucurbita pepo L.) belongs to the Cucurbitaceae family and is one of the most economically important vegetable crops cultivated worldwide. Certain cultivars are known for their tolerance to soilborne diseases and salinity, making them promising candidates as rootstocks [11],[12].

Plant tissue culture technology represents a valuable approach for the production of virus-free and fungus-free plants [13]. Moreover, it is employed for the development of plants resistant to agricultural pests and for the large-scale multiplication of plants under aseptic conditions. Through the application of plant growth regulators (PGRs), plant regeneration can be achieved from callus, apical meristems (shoot tips), nodal segments, and other explant types [14].

Material and Methods

This study was carried out at the Horticulture and Landscape Design Laboratory of Plant Cells and Tissue Culture/ College of Agriculture/University of Kirkuk. The Shoot tips were used as an explant of the melon plant, On March 14, 2026, seeds of pineapple melon (*Cucumis melo* var. *ananas*) were sown in peat moss. The seeds were obtained from the Turkish company (Bursa Tohum). The sowing was carried out in a specialized seedling tray. Subsequently, the seedlings were transferred to a nursery, where agricultural practices were monitored. Following germination and upon reaching the true leaf stage, the shoot tips of the seedlings were excised for planting in MS (Murashige and Skoog) medium After sterilizing the tools, we placed a 1000 ml beaker on the magnetic stirring device and added 400 ml of boiled distilled water. The medium was then weighed according to the research requirements (4.43 mg). After dissolving it in a small amount of distilled water, it was added to the beaker. The required growth regulators (cytokines and kinetin) were then added according to the research specification. next 30 g of sugar was weighed, dissolved in 100 ml of distilled water, and added to the beaker additionally, 1 g of active charcoal was weighed and gradually added to the beaker finally 6gm of agar was added and adding as small amounts. the volume was then adjusted to 100 ml with distilled water, and the mixture was left to achieve homogeneity. After thoroughly mixing the components, The PH was set at 5.7- 5.8 by adding sodium hydroxide (NaOH) to increase the PH or hydrochloric acid (HCL) to decrease it. The medium was then distributed into 240 ml jars, with 25 ml per jar. They were then placed in a beaker and washed under tap water for 15–20 minutes. Subsequently, surface sterilization was performed using commercial bleach NaOCl sodium hypochlorite. Before this, the laminar airflow cabinet was cleaned with 99% ethanol. Necessary tools and materials for sterilization were placed inside the laminar airflow cabinet and exposed to UV radiation for 15–20 minutes for surface sterilization of the equipment. Thereafter, plant explants were surface-sterilized using sodium hypochlorite at concentrations of (2%, 4%, and 6%) for durations of (2, 4,

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and 6) minutes[16] respectively. The explants were then rinsed three consecutive times with sterile distilled water, each rinse lasting 5 minutes. They were subsequently cultured on MS medium prepared in advance After four weeks, the results of surface sterilization were recorded. For the multiplication stage [17], [18]. shoot tips were excised from the plants and sterilized using 2% sodium hypochlorite for 2 minutes. They were then cultured on MS medium supplemented with different concentrations of benzyladenine (BA: 0, 0.5, 1.0, 1.5, 2.0 mg/L1-) and kinetin (Kin: 0, 2.0, 4.0, 6.0, 8.0 mg/L1-). The cultures were placed in a growth room at a temperature of 25 ± 2°C, with a photoperiod of 16 hours light / 8 hours dark provided by fluorescent lamps at a light intensity of 3000 lux. 15 days after culturing the melon shoot tips, pumpkin (*Cucurbita pepo* L., 'Kousa' type) seeds were sown in peat moss and placed in the nursery until the emergence of three true leaves, which occurred after 15 days, making them ready for grafting. Using the micro grafting technique, shoots resulting from melon multiplication were grafted onto Squash (*Cucurbita pepo* L.). using the hole insertion grafting, Splice/Tube Grafting and Cleft Grafting method. Initially, the shoot tip of the pumpkin seedling was excised using a razor blade. Then, a hole was made in the rootstock using a sharp metal or wooden tool, The melon shoot was inserted into the rootstock and secured with a clip. After grafting, the seedlings were placed in a dark chamber for 3–5 days to promote callus formation, wound healing, and vascular reconnection. Relative humidity was maintained at 85–90%, and temperature at 20–25°C. After five days [19][20], the seedlings were transferred to a growth room for acclimatization. Acclimatization was performed by covering the grafted plants with transparent plastic covers. The cover was removed for 10 minutes daily, with the duration gradually increased until complete removal of the cover. Following acclimatization, response percentage, number of leaves, and plant height were recorded. Once acclimatization was completed, the seedlings were ready for transplantation into the field. The experiment was conducted using a Completely Randomized Design (CRD). Data were subjected to Analysis of Variance (ANOVA), and the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$. Each treatment consisted of ten replicates, with each replicate containing a single explant.

Table (1) The effect of using commercial bleach for surface sterilization with different concentrations and time periods.

T / C	2	4	6	concentration
2	1.00 a	0.90 a	0.90 a	0.90 a
4	0.90 a	0.80 a	0.70 b	0.80 a
6	0.90 a	0.80 a	0.70 b	0.70 b
time	0.90 a	0.8 b	0.80 b	

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* Values with similar characters for each factor are not significantly different according to the Duncan's Multiple Range Test below the 5% probability level**.**

Table (2) Effect of BA on melon shoot tips for Initiation and multiplication stages after 4 of cultivation

BA mg.L^{-1}	After 4 weeks (Initiation)			
	Response(%)	Shoots No.	Shoots Length cm	Leaves No.
0.0	0.80 b	0.9 b	0.71 b	1.8 b
0.5	100 a	2.8 a	1.35 a	9.6 a
1.0	100 a	2.4 a	1.31 a	8.1 a
1.5	100 a	2.4 a	1.56 a	8.3 a
2.0	100 a	2.8 a	1.42 a	7.9 a

* Values with similar characters for each factor are not significantly different according to the Duncan's Multiple Range Test below the 5% probability level**.**

Table (3) Effect of KNon melon shoot tips for Initiation and multiplication stages after 4 of cultivation

KN mg.L^{-1}	After 4 weeks(Initiation)			
	Response(%)	Shoots No.	Shoots Length cm	Leaves No.
0.0	0.80 b	0.9 b	0.70 c	1.8 b
2.0	100 a	2.1 a	1.67 a	8.5 a
4.0	100 a	2.2 a	1.21 b	8.2 a
6.0	0.80 a	0.9 b	0.59 c	2.2 b
8.0	0.70 a	0.8 b	0.47 c	1.6 b

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* Values with similar characters for each factor are not significantly different according to the Duncan's Multiple Range Test below the 5% probability level.

Table (4) A comparison of grafting technique types used for grafting melon (*Cucumis melo*) onto squash (*Cucurbita pepo*) rootstock After 7 days.

NO.plant	Splice grafting	Hole insertion grafting	Cleft grafting
1	0	1	1
2	1	1	0
3	0	1	1
4	0	1	1
5	1	1	0
6	0	1	1
7	0	1	1
8	1	1	0
9	0	0	0
10	1	0	1
response rate	40%	80%	60%

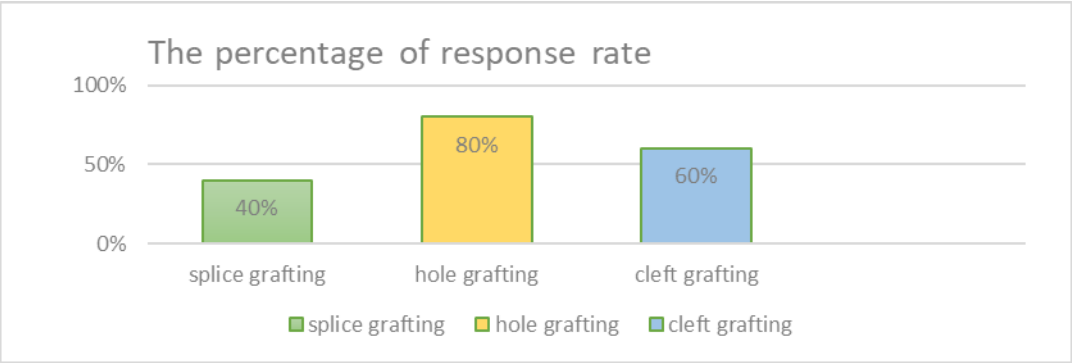

Table (5) Measurement of response rate, plant height, and number of leaves 11 days after grafting using the hole-insertion method

No. plant	response	Hight of plant	leaf number
1	1	5	2
2	1	6	3
3	1	6.5	3
4	1	5	2
5	1	5.5	2
6	1	4	2
7	1	6.5	2
8	1	5	2
9	0	0	0
10	0	0	0
mean	%80	5.43	3

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Results and Discussion:

The data presented in Table (1) demonstrate the effect of sodium hypochlorite (NaOCl) concentration and exposure duration on the survival of melon (*Cucumis melo*) shoot tips. The 2-minute exposure duration was superior to all other durations, achieving a 100% survival rate. Regarding NaOCl concentrations, both 2% and 4% were equally effective with no significant difference between them; however, both significantly outperformed the 6% concentration. The interaction effect revealed that the combination of 2% NaOCl with a 2-minute exposure duration yielded the highest survival rate among all treatments

The data in Table (3) reveal that kinetin at concentrations of 0.2 and 0.4 mg L^{-1} resulted in 100% survival, significantly exceeding the control and higher concentrations. The highest shoot numbers (2.1 and 2.2 shoots) were obtained at 2.0 and 4.0 mg L^{-1} kinetin, respectively. Maximum shoot length (1.67 cm) was achieved at 2.0 mg L^{-1} kinetin. Regarding leaf production, concentrations of 2.0, 4.0, and 6.0 mg L^{-1} kinetin were significantly superior, producing 8.5, 8.2, and 8.2 leaves, respectively

The results in Table (4) demonstrate that the hole-insertion grafting method recorded the highest success rate (80%), followed by cleft grafting (60%), and finally splice grafting (40%). Table (5) further confirms that the hole-insertion method achieved an 80% success rate, with a mean plant height of 5.43 cm and a mean leaf number of 3 leaves per plant.

Discussion:

Surface Sterilization Optimization

The superiority of 2% NaOCl for 2 minutes reflects an optimal balance between disinfection and explant viability. NaOCl releases hypochlorous acid (HOCl), which disrupts microbial cell walls and denatures proteins [21]. At 2%, the disinfectant eliminates surface contaminants without phytotoxicity, whereas 6% overwhelms antioxidant defenses (SOD, CAT), causing cellular damage [22]. Longer exposures (4–6 min) damage meristematic cells. Similar results were reported in watermelon [23] and cucumber [24].

Physiological Role of BA in Shoot Proliferation

The 100% response rate and enhanced proliferation confirm cytokinins' role in promoting meristematic activity. BA upregulates cyclin genes (*CYCD3*), driving the G1/S cell cycle transition [25], and promotes axillary bud outgrowth by suppressing apical dominance (Matsumoto-Kitano et al., 2008). The concentration-dependent response reflects the classic biphasic effect—optimal BA (0.5–2.0 mg L^{-1}) enhances organogenesis, while supra-optimal concentrations induce oxidative stress [26]. Similar findings were reported in cassava [27].

Physiological Role of Kinetin in Shoot Proliferation

Kinetin promotes cell division and delays senescence, but its lower stability and receptor affinity explain lower proliferation rates compared to BA [28]. Low concentrations (0.2–0.4 mg L^{-1}) maintain viability, while higher concentrations (4.0–6.0 mg L^{-1}) trigger stress responses. Kinetin at 2.0 mg L^{-1} produced the longest shoots (1.67 cm), indicating optimal balance between cell division and elongation. The lower shoot

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numbers with kinetin (2.2 vs 2.8 shoots) align with studies where BA outperformed kinetin [29].

Physiological Basis for Grafting Method Success

The superior performance of hole-insertion grafting (80%) compared to cleft (60%) and splice (40%) is explained by: (1) larger vascular cambium contact, facilitating faster vascular reconnection [30] reduced mechanical damage, minimizing wound ethylene and oxidative stress [31] and better vascular bundle alignment, critical for water and nutrient translocation. These findings align with studies on watermelon [32], tomato [33], No graft incompatibility was observed between *Cucurbita* rootstocks and *Cucumis* scions [34].

Conclusion:

In conclusion, surface sterilization of melon shoot tips is optimally achieved using 2% sodium hypochlorite for 2 minutes, yielding a 100% survival rate. Benzyladenine (BA) at concentrations of 0.5–2.0 mg L^{-1} is effective for shoot proliferation, producing up to 2.8 shoots per explant, while kinetin at 2.0–4.0 mg L^{-1} supports optimal survival and shoot development. Among the grafting methods evaluated, hole-insertion grafting is the most effective technique, achieving an 80% success rate, superior plant height, and greater leaf production compared to cleft and splice grafting. These findings provide a robust protocol for in vitro propagation and micrografting of cantaloupe, with potential applications for disease management and crop improvement

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