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# Growth and Yield of Tomato and Cucumber Plants Grafted Onto *in vitro* and Seedling Rootstocks

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THIS study was carried out at the Plant Tissue Culture Laboratory and Greenhouse of the Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia Governorate, Egypt, during the period of 2017 – 2022. The main aims of the current study were to establish an efficient protocol for *in vitro* propagation of vegetable rootstocks and to compare the performance of rootstocks produced *in vitro* and those produced directly from seeds (*in vivo*), in terms of growth, yield and quality parameters. Two commercial rootstocks, namely Super Shintoza and Forza for Cucurbits and tomato grafting, respectively, were used. Also, the cucumber Hayel variety and tomato Assila F1 (T-186) were used as scions. The rootstock seeds of both genotypes were used as explants. The results showed that the micropropagation technique can be used efficiently to produce *in vitro* rootstocks, especially for tomato rootstocks, in order to decrease the high cost of the interspecific rootstocks for commercial production of grafted vegetables.

**Keywords:** Rootstock, Tissue culture, Cucumber, Tomato, Cucurbits, Grafting, Micropropagation.

## Introduction

Cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) are members of the *Cucurbitaceae and Solanceae* families, respectively. They are among the most economically important vegetable crops. Egypt is one of the major cucumber and tomato producing and exporting countries. Cucumber and tomato production in Egypt reached approximately 433440.85 tons and 6245787.13 tons, gained from a cultivated area of 19702 and 150109 hectares, respectively (FAOSTAT, 2023).

In the last decades, the vegetable grafting technique has emerged as a high potential technique to improve the efficiency of modern vegetable cultivation and improve plant tolerance and resistance to different abiotic and biotic stress types. At the present time, grafting is commercially practiced in some vegetable crops such as watermelon, melon, cucumber, tomato, eggplant and pepper in order to control soil-borne diseases and improve productivity and quality-related traits (Schwarz et al., 2010: Singh et al., 2020). Commercial cucumber and tomato hybrids are commonly grafted onto imported interspecific Cucurbits and Solanaceae rootstocks. However, interspecific rootstock seed production is expensive as well as its germination is problematic. The use of or search for some local genotypes to be used as suitable rootstocks for vegetable grafting as well as the breeding programs for appropriate interspecific rootstock production are still absent and have not gained much attention yet in Egypt.

Significant increase in traits of vegetative growth and yield and earliness was recorded when cucumber and tomato plants were grafted onto the right rootstock compared to non-grafted (Milenković et al., 2020 and Bayoumi et al., 2021), In the same regard, water use efficiency, N-uptake efficiency, N-utilization efficiency,

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and nitrogen use effeminacy were significantly increased, in grafted cucumber compared to non-grafted plants (Al-Harbi et al., 2017; Liang et al., 2021). Consequently, improved the net profit for growers. Asalm et al., 2020 reported higher gross and net profit for grafted cucumber plants (36.712 and 34.479 $/ha) than for non-grafted ones (27.110 and 24.877 $/ha).

The *in vitro* culture technique is a suitable method for obtaining a large number of genetically identical plants in a short period of time. However, *in vitro* plant proliferation has been found to depend on several factors, such as genotype, explant, composition of the basic medium, growth regulators, gelling agents, light intensity and quality, photoperiod, temperature, culture vessels and vessel covers (Kumar et al., 2022). In vitro tissue culture is used through various biotechnological techniques in cucumber and tomato such as genetic transformation, protoplast isolation and fusion, the production of virus-free plants and mass propagation of both crops (Bhatia et al., 2004, Aslibeigi, et al., 2022 and Salehian et al., 2023). The development of a rapid and efficient regeneration protocol for cucumber and tomato rootstocks may reduce the costs of rootstock seeds for cucumber and tomato grafting, which will have a strong benefit for farmers by reducing the costs of rootstock seeds and vegetable grafted seedlings.

Therefore, the objectives of this study were to:

1. establish an efficient protocol for *in vitro* propagation of the vegetable rootstocks.
2. study the effect of grafting on *in vitro* (produced from tissue culture) and *in vivo* (produced from the seeds) rootstocks on vegetative growth, yield and quality parameters of cucumber and tomato plants.

## Materials and Methods

The current investigation was carried out at the Plant Tissue Culture Laboratory and Greenhouse of the Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia Governorate, Egypt, during the years from 2017 to 2022.

*Plant materials*

Two commercial rootstocks; Super Shintoza (GSI Exports Seed Company) and Forza (Vilmorin Company), were used in this study. These two genotypes are among the most popular rootstocks for grafting Cucurbits and tomato, respectively. Also, the Hayel variety (Seminis company) and Tomato Assila F1, T-186 (Mirro Seeds Company) were used as scions in the current study. These genotypes are among the most popular varieties for cucumber and tomato production in Egypt.

*Preparation of Explants*

The rootstock seeds of both genotypes were soaked in running water for 30 minutes. Seeds were then surface sterilized with 70% ethanol for 1 minute, followed by 15% of sodium hypochlorite solution (2.5%) in addition to 2 drops of Tween-80 as a surfactant agent for 10 minutes. Seeds were shaken continuously for uniform sterilization and then rinsed four times with a plenty of sterile-distilled water.

*Culturing medium*

At the starting stage, a hormone-free Murashige and Skoog medium (MS; Murashige and Skoog, 1962) supplemented with 30 g/l sucrose was prepared. The pH of the medium was adjusted to 5.7±0.1 before the addition of 7 g/l agar. The medium was cooked for 10 minutes on a hot plate with a magnetic stirrer. Later, MS medium was poured into sterilized 350-ml glass jars, where each jar contained 40 ml of prepared MS medium and the jars were immediately capped with polypropylene plastic caps. Finally, the jars containing MS medium were autoclaved at 121°C at 1.5 lb/in$^2$ for 35 min and then stored at 25±1°C for contamination testing.

*Explant establishment*

Sterilized seeds of both rootstock genotypes were cultured on a hormone-free MS medium, as mentioned above at the starting stage. Cultured seeds were kept at 27±2 °C and 16/8 hours (day/night) light using white fluorescent lambs giving about 1500 Lux intensity. After 25 days, the shoot tips of germinated explants were transferred to the multiplication medium.

*Multiplication stage*

In order to study the effect of Benzyl Amino Purine (BAP) on vegetative parameters of Cucurbita and tomato rootstocks, Super Shintoza and Forza, respectively, MS medium supplemented with 30 g/l sucrose, 7.0 g/l agar and different concentrations of BAP (0, 1, 3 and 5 mg/l) was prepared. The multiplication cultural media were distributed to 350-ml glass culture jars, where each jar contained 40 ml of multiplication medium. The cultural jars were immediately capped with polypropylene caps and autoclaved at 121 °C at 1.5 lb/in$^2$ for 30 min. After one week of medium preparation, the established shoot tips

of both rootstock genotypes were subcultured on multiplication medium. BAP treatments were distributed in a completely randomized experiment with three replicates. Each replicate had 15 jars and each jar had 3 shoot tips. Then, cultured jars were incubated at 27±2 °C and 16/8 hours (day/night) light using white fluorescent lambs giving about 1500 Lux intensity. After 30 days of subculture, the following parameters were recorded per explant:

a) Number of shoots
b) Number of leaves
c) Shoot length
d) Shoot fresh and dry weight.

*Rooting stage:*

Another experiment was carried out *in vitro* to study the effect of Indole Butyric Acid (IBA) on the rooting of multiplied shoots of Cucurbita and tomato rootstocks. The MS rooting media was prepared and supplemented with 30 g/l sucrose, 7.0 g/l agar and different concentrations of IBA (0, 0.5, 2 and 4 mg/l). The rooting cultural media were distributed to 350-ml glass culture jars, where each jar contained 40 ml of rooting medium. The cultural jars were immediately capped with polypropulin caps and autoclaved at 121 °C at 1.5 lb/in$^2$ for 30 min. After one week, the established shoots of both rootstock genotypes were subcultured on rooting medium as mentioned above. IBA treatments were arranged in a completely randomized experiment with three replicates. Each replicate had 15 jars and each jar had 3 shoot tips. Then, the cultured jars were incubated at 27±2 °C and 16/8 hours (day/night) light using white fluorescent lambs giving about 1500 Lux intensity. After 30 days of subculture, the following parameters were recorded per explant:

a) Number of roots.
b) Length of roots.
c) Fresh and dry weight of roots.

*Acclimatization stage*

At the end of the rooting stage, the produced plantlets of Cucurbita and tomato rootstocks were washed with tap water in order to remove the remains medium from the rootlets. The rooted plantlets were transplanted into plastic trays (50 cells) containing a mixture of peatmoss and vermiculite (1:1 v/v) Then, the trays were kept in a greenhouse at 25-28 °C under a mist water irrigation system for two weeks and covered with a polyethylene tunnel to maintain high relative humidity (about 80-90%). Seedlings were fertilized once with 1 g/l of NPK (19:19:19) and treated two times with 50 mg/l of antifungal (Previcure Energy; Syngenta Company).

*Production of in vivo seedlings of Cucurbita and tomato rootstocks:*

The seeds of Super Shintoza and Forza rootstocks were sown in Styrofoam trays filled with a mixture of peatmoss and vermiculite (1:1) for 15 and 25 days, respectively, to produce *in vivo* seedlings of cucumber and tomato seedlings for grafting.

*Production of the cucumber and tomato scions*

In the greenhouse, seeds of cucumber (Hayel variety) and tomato (Assila F1) were sown in Styrofoam trays filled with a mixture of peatmoss and vermiculite (1:1) for 30 days to produce cucumber and tomato seedlings ready for grafting and used as a scion.

*Grafting and Accalmization*

Cucumber and tomato grafting were performed onto *in vitro* and *in vivo* rootstock seedlings as mentioned above using the splice-grafting technique. Next, cucumber and tomato grafted seedlings were transferred and kept in a humidity chamber at 24-26 °C and 90-95% relative humidity until the scions were well connected to the rootstock (after 7 days).

*Field experiment*

The experimental soil was cleared, ploughed and harrowed. Then, organic manure and superphosphate (15.5% $P_2O_5$) were added at rates of 30 m$^3$/feddan and 150 kg/feddan, respectively. A drip irrigation system was used. After grafting success, the cucumber and tomato scion seedlings grafted on *in vitro* and *in vivo* Cucurbita and tomato rootstocks were transplanted into the field and arranged in a completely randomized block design with three replicates. The experimental unit area (plot) was 5 m in width and 3 m in length, each plot contained 5 ridges and included 20 plants. The grafted seedlings of cucumber and tomato were transplanted 60 cm between the plants in the same ridge. The other normal agricultural managements such as fertilization, control of insects and fungi as well as weed control were performed according to the recommendations of the Ministry of Agriculture for cucumber and tomato production under Ismailia Governorate conditions. The following vegetative and yield traits were recorded for grafted cucumber and tomato plants during the growth period and at the end of the growing season:

1. Plant height (cm),

2. No. of leaves per plant,
3. No. of fruits per plant,
4. Mean fruit weight (gm),
5. Yield/plant (kg).
6. Shoot fresh and dry weight (g).

*Statistical analysis*

Data were subjected to one-way analysis of variance (ANOVA) and differences among means of treatments were tested using the Duncan's multiple range test at the 5% level of significance.

## Results

*Effect of different BAP concentrations on Cucurbita rootstock "Super Shintoza" multiplication*

Data presented in Figures (1 and 2) indicated that there were significant differences among BAP concentrations on all studied traits except for the number of leaves per explant. The treatment of 3 mg/l of BAP gave the highest shoot number, fresh weight and dry weight (5.13, 1.53 g and 0.11 g, respectively), with significant differences compared to the other assigned concentrations. While control plantlets gave the highest shoot length and number of leaves (5.63 cm and 9.06, respectively). On the other hand, the lowest number of shoots (1.00), fresh weight (0.74 g) and dry weight (0.05 g) were produced by the control treatment (0 mg/l BAP), while the lowest shoot length (2.22 cm) and the number of leaves (7.95) were associated with the treatment of BAP at 1 mg/l.

*Effect of different BAP concentrations on tomato rootstock "Forza" multiplication*

Data in Figures (1 and 3) showed that there were significant differences among the BAP concentrations in all studied traits. It also showed that the treatments of 3 and 5 mg/l of BAP recorded the highest values of number of shoots (8.6 and 8.0, respectively) and fresh weight (0.94 and 0.90 g, respectively), without significant differences between them compared to the control treatment. Shoot length and the number of leaves recorded the highest values in control plantlets (10.13 cm and 16.80, respectively), with significant differences in comparison to all BAP treatments. The treatment of 5 mg/l of BAP recorded significantly the highest value of dry weight (0.16 g), compared to the control treatment, but without significant differences in comparison to the other BAP treatments. On the other hand, the lowest number of shoots (1.00), fresh weight (0.33 g) and dry weight (0.02 g), resulted from the control treatment, while the lowest shoot length (2.56 cm) and the number of leaves (9.56) were produced by the addition of 3 mg/l BAP to the multiplication medium.

*Effect of different IBA concentrations on rooting of multiplied shoots of Cucurbita rootstock "Super Shintoza"*

Data in Figures (4 and 5) showed that there were significant differences among the IBA concentrations in all studied traits. Data indicated that the number of roots significantly reached the highest value (4.86) when the concentration of 4.0 mg/l of IBA was used, compared to the control and other IBA treatments. Root length was the highest when the concentration of 0.5 mg/l IBA was applied to rooting medium without a significant difference when compared with the other treatments, except for the treatment of 4.0 mg/l IBA, which showed a significant decrease in root length compared to other IBA concentrations. The fresh and dry weight of roots showed that plantlets treated with 4.0 mg/l IBA concentration resulted in the highest values (1.30 g and 0.10 g, respectively) with significant differences relative to other IBA concentrations, which showed close values without significant differences among them. The data also showed that the lowest number of roots per plantlet (2.13) and root length (5.16 cm) were associated with IBA levels at 0.5 mg/l and 4.0 mg/l, respectively, while the control treatment produced the lowest fresh (0.10 g) and dry (0.008 g) weight of roots.

*Effect of different IBA concentrations on rooting of in vitro multiplied shoots of tomato rootstock "Forza".*

Data shown in Figures (4 and 6) showed that there were significant differences among the IBA concentrations in terms of number of roots and root fresh weight only. Data also showed that the treatment of IBA at 4.0 mg/l achieved the highest value of root number (6.86) compared to other IBA treatments, while the treatment of 2 mg/l of IBA resulted in the highest values of root length (8.83 cm), root fresh weight (0.23 g) and root dry weight (0.019 g), respectively). However, the differences between these two treatments were not necessarily significant for all studied traits. In contrast, the lowest number of roots (3.33) was produced by the treatment of 0.5 mg/l IBA, while the lowest root length, fresh and dry weight (5.67 cm, 0.06 g and 0.009 g, respectively) were associated with the control treatment (0 mg/l IBA).

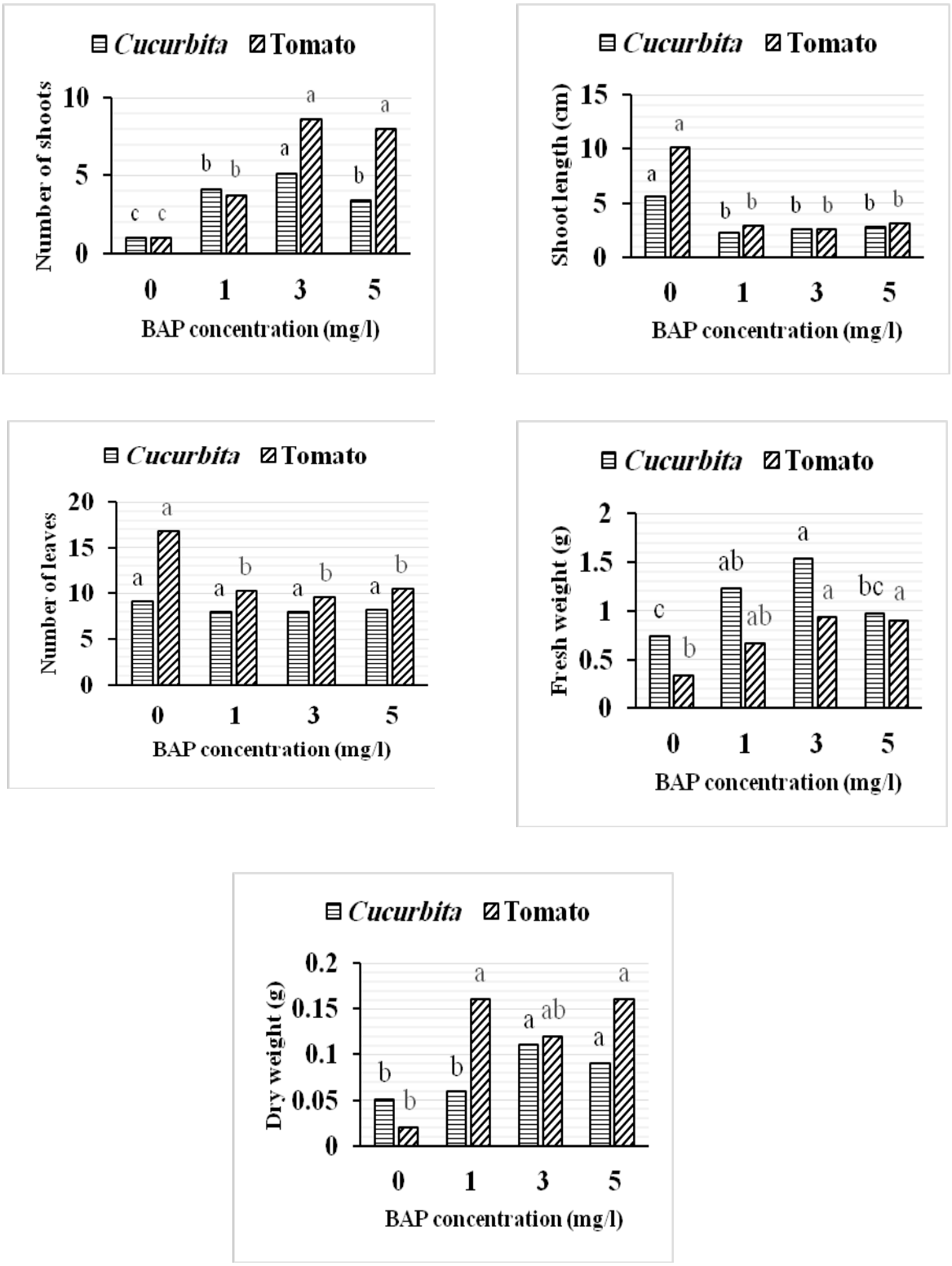


**Fig. 1. Effect of different concentrations of BAP on the number of shoots, shoot length, number of leaves and fresh and dry weights of Cucurbita rootstock "Super Shintoza" and tomato rootstock "Forza" micro-propagated *in vitro* during multiplication stage.**

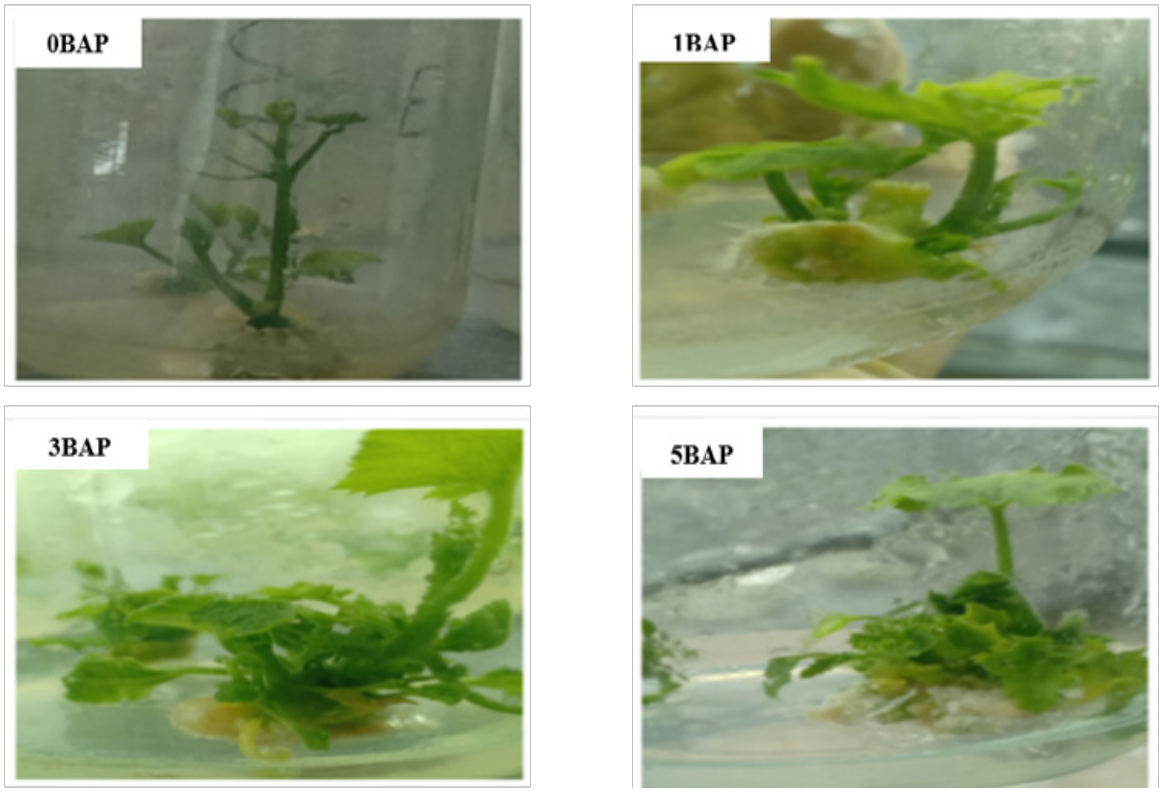


**Fig. 2. Multiplication stage of *Cucurbita.***

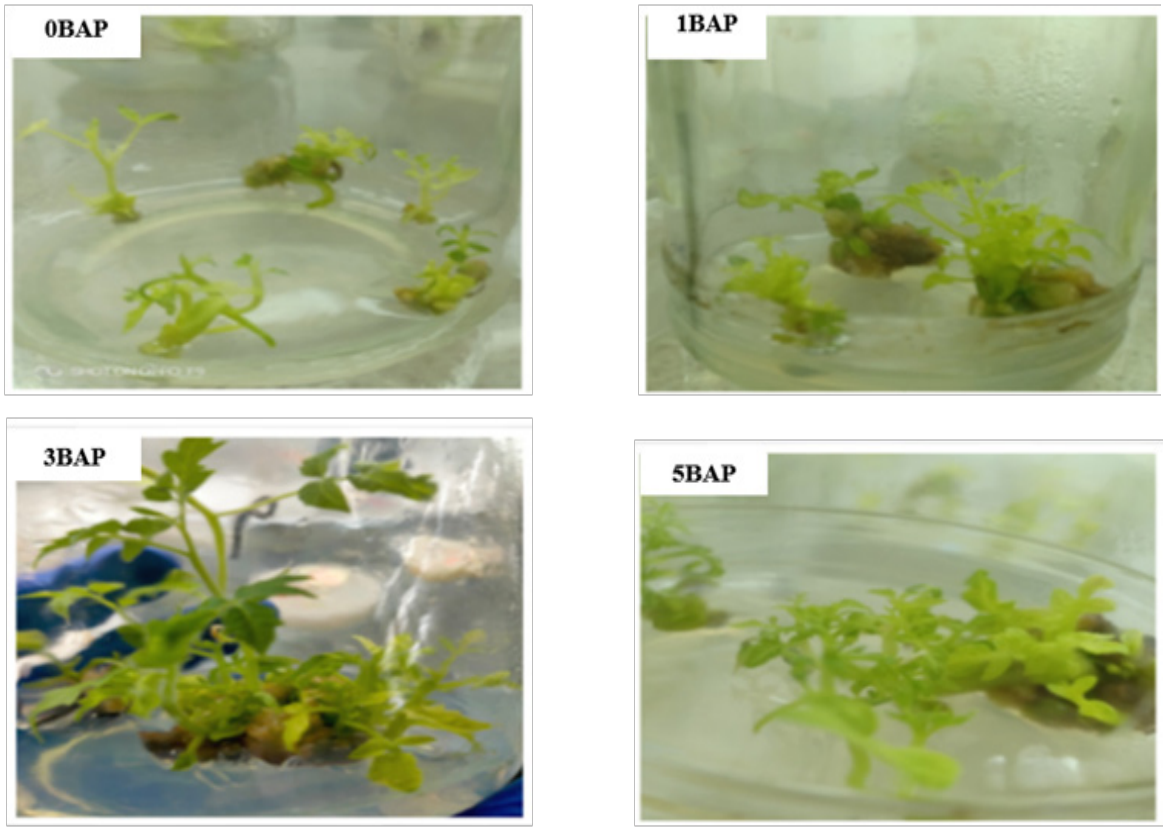


**Fig. 3. Multiplication stage of tomato**

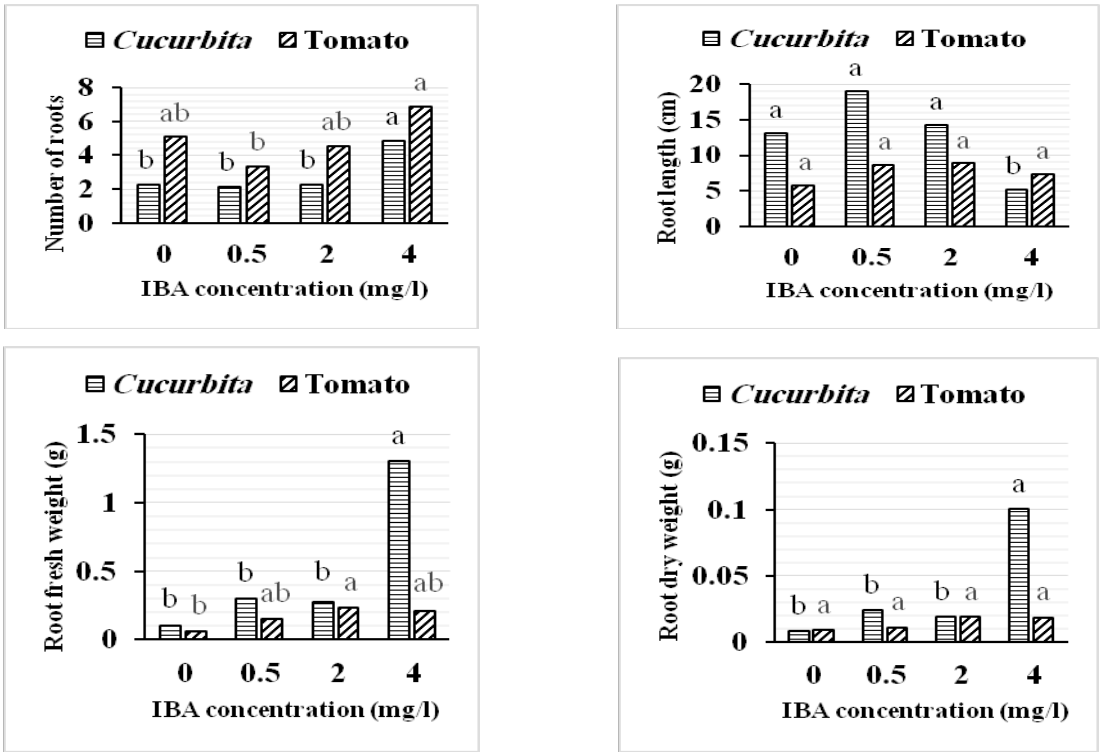


**Fig. 4. Effect of different IBA concentrations on the number of roots, root length and fresh and dry weights of Cucurbita rootstock "Super Shintoza" and tomato rootstock "Forza", during *in vitro* rooting stage.**

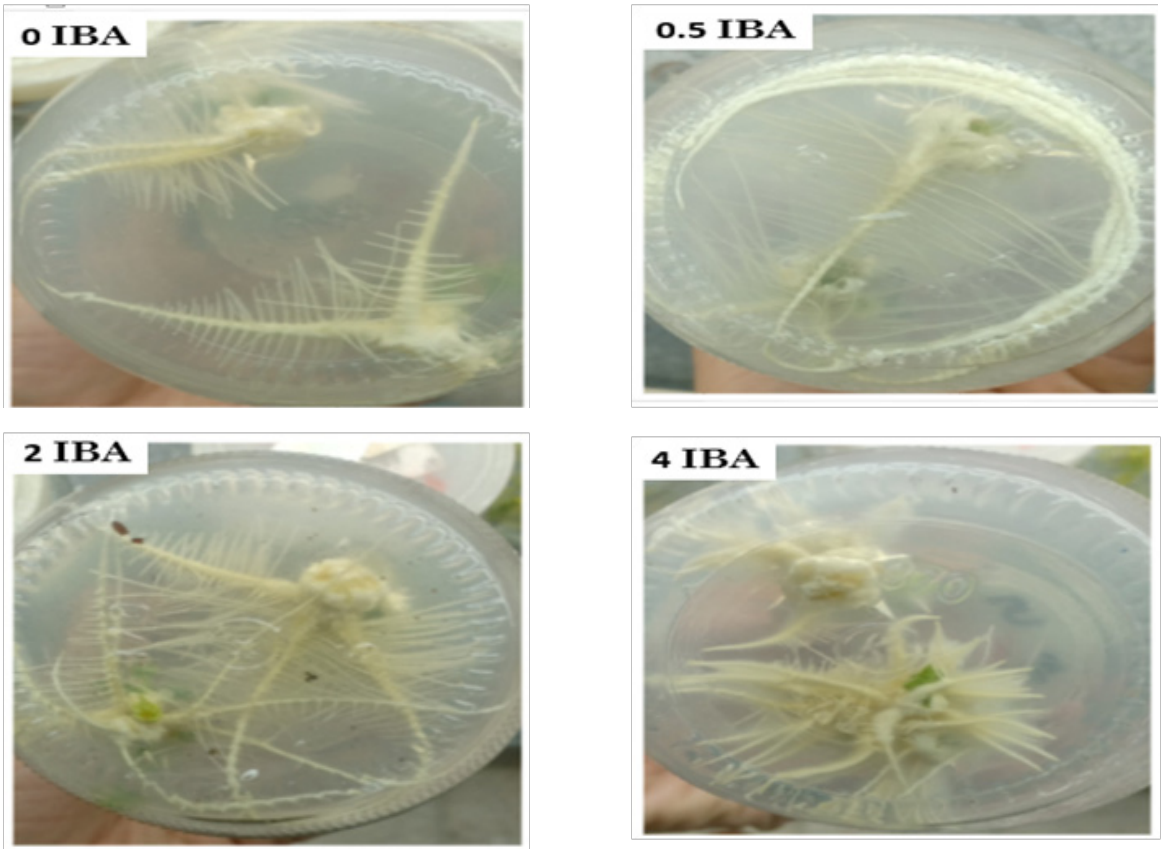


**Fig. 5. Rooting stage of Cucurbita**

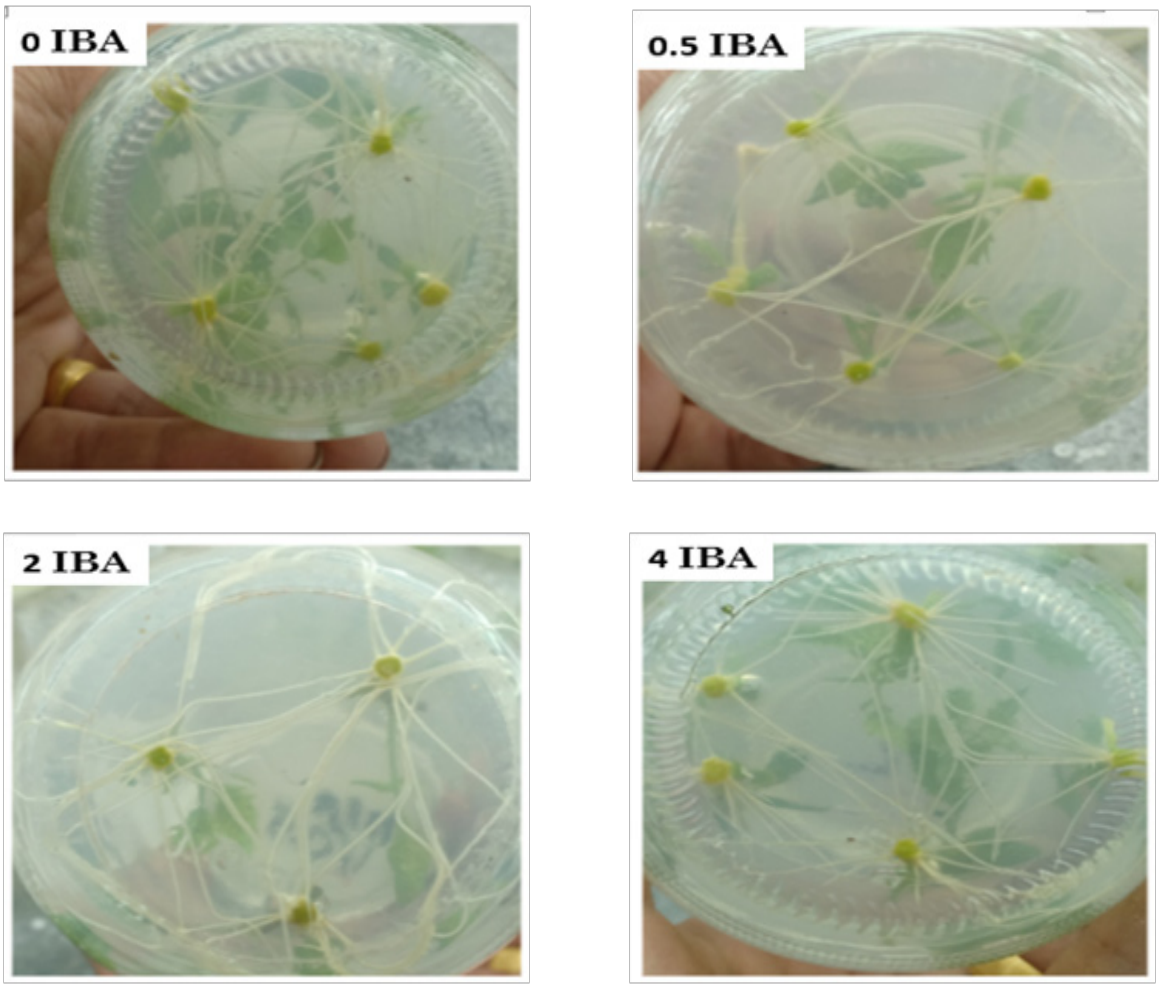


**Fig. 6. Rooting stage of tomato.**

*Cucumber grafting experiment*

Data presented in Table 1 showed the effect of cucumber grafting onto *in vitro* and *in vivo* "Super Shintoza" rootstock seedlings. The presented results indicated that there were significant differences between grafted cucumber plants onto *in vitro* and *in vivo* "Super Shintoza" rootstock in terms of plant height, number of fruits per plant and total yield per plant. While there were no significant differences between grafted cucumber plants on *in vitro* and *in vivo* rootstock in terms of the number of leaves per plant, plant fresh and dry weights, and mean fruit weight. Generally, it could be stated that the cucumber cv. 'Hayel' grafted plants onto *in vivo* rootstock recorded higher means for all studied traits in comparison to those grafted onto *in vitro* rootstock. However, these differences between these two treatments were not necessarily significant except only for plant height (159.11 cm), number of fruit per plant (26.66) and total yield per plant (2959.98 g) compared to the cucumber-grafted plants onto *in vitro* seedlings (151.11 cm, 19.76 and 2134.44 g, respectively).

*Tomato grafting experiment*

The data shown in Table 2 displayed the effect of tomato grafting onto *in vitro* and *in vivo* "Forza" rootstock seedlings. The obtained results clearly indicated that there were no significant differences between grafted tomato plants onto *in vitro* and *in vivo* "Forza" rootstock in all studied traits. Grafted tomato plants onto *in vivo* seedlings of "Forza" rootstock had higher plant height, number of leaves per plant, plant fresh and dry weights, the number of fruits per plant, mean fruit weight and total yield per plant than those grafted onto *in vitro* seedlings of "Forza" rootstock. However, these differences were not significant for all studied traits.

**TABLE 1. Vegetative traits, fruit yield and fresh and dry weights of grafted cucumber cv. 'Hayel' onto *in vivo* and *in vitro* "Super Shintoza" rootstock seedlings.**

| Treatment | Plant height (cm) | No. of leaves/ plant | Plant fresh weight (g) | Plant dry weight (g) | No. of fruits/ plant | Mean fruit weight (g/fruit) | Total yield (g/ plant) |
|---|---|---|---|---|---|---|---|
| ***in vivo* rootstock** | 159.11a | 34.22a | 582.22a | 112.66a | 26.66a | 112.16a | 2959.98a |
| ***in vitro* rootstock** | 151.11b | 31.44a | 536.66a | 83.66a | 19.76b | 108.13a | 2134.44b |

Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

**TABLE. 2. Vegetative traits, fruit yield and fresh and dry weights of grafted tomato onto *in vivo* and *in vitro* "Forza" rootstock seedlings.**

| Treatment | Plant height (cm) | No. of leaves/ plant | Plant fresh weight | Plant dry weight | No. of fruits/ plant | Mean fruit weight (g/fruit) | Total yield (g/ plant) |
|---|---|---|---|---|---|---|---|
| ***in vivo* rootstock** | 87.33a | 78،00a | 705.88a | 212.00a | 62.15a | 76.69a | 4121.66a |
| ***in vitro* rootstock** | 79.88a | 69.73a | 680.55a | 182.66a | 56.13a | 71.15a | 3950.66a |

Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

## Discussion

Commercial cucumber and tomato hybrids are commonly grafted onto imported interspecific Cucurbits and Solanaceae rootstocks. However, interspecific rootstock seed production is expensive as well as its germination is problematic. The use of or search for some local genotypes to be used as suitable rootstocks for vegetable grafting as well as the breeding programs for appropriate interspecific rootstock production are still absent and have not gained much attention yet in Egypt. The micropropagation technique might be used to overcome these limitations. Therefore, it is necessary to develop a suitable protocol for mass propagation of Cucurbits and Solanaceae rootstocks.

*Effect of BAP concentrations on Cucurbita rootstock multiplication*

The obtained results clearly indicated that BAP is an effective growth regulator in the growth and shoot proliferation of Super Shintoza rootstock. These results are in harmony with the results of Nasarudin and Mansor (2021) who reported that BAP was essential for the growth of shoot or shoot proliferation in *Momordica charantia*. Also, Lee et al. (2003) reported that adventitious shoot bud regeneration was effectively achieved on MS medium supplemented with BAP alone for *Cucurbita maxima*.

The results of the current study also reported that the treatment of 3 mg/l of BAP had the highest shoots number, fresh weight and dry weight (5.13, 1.53 g and 0.11 g, respectively) compared to the control and other BAP concentrations. A similar result was reported by Sarowar et al. (2003) who found that the longest shoots and the highest percentage of shoot formation (2.25±0.44 cm and 84%, respectively) were recorded for the treatment of 3 mg/l of BA in Super Shintoza rootstock. Also, Mahzabin et al. (2008) reported that the longest shoots and the highest percentage of *Cucurbita maxima* shoot formation (6.1±0.85 cm and 90.45%, respectively) were associated with the treatment of 3.0 mg/l BA after 30 days of culture.

Nevertheless, another study on cucumber found that MS medium augmented with 1.5 mg/l BAP produced the maximum number of shoots as compared to other tested concentrations of BAP and the number of shoots proliferated decreased by increasing concentrations above 1.5 mg/l BAP (Joyia et al., 2019). In addition, Mali and Chavan (2016) reported that treatment of 2 mg/l of BAP was the most efficient in *Cucumis trigonus* multiplication, with an average of 4.93±0.77 shoot numbers and 4.90±0.25 cm shoot length. The difference between the results of the current study and the previous studies might be due to the response of different plant species and/or different cultivars and experimental conditions used.

Generally, several previous studies reported that the BAP in a range of 1.0 - 3.0 mg/l gave good results in terms of multiplication traits in different Cucurbits, including *Cucurbita Sp*. (Mahzabin et al., 2008; Sangeetha and Venkatachalam, 2011; Faria et al., 2013). The positive effect of BAP on the proliferation process could be explained by its known effect on cell division and differentiation. This is because BAP activates the enzymes responsible for cell division and promotes the synthesis of new proteins and nucleic acids, which are essential for cell growth and differentiation (Jameson and Song**,** 2016). BAP also plays an important role in chloroplast development, which is essential for the photosynthesis process. BAP increases the number of chloroplasts per cell and enhances the development of chloroplasts, resulting in increased photosynthetic activity and plant growth (Arigita and Gotor, 2018).

*Effect of BAP concentrations on tomato rootstock multiplication*

The results of the current study generally indicated that the treatment of 3 and 5 mg/l of BAP recorded the highest values of shoot number (8.6 and 8, respectively) and fresh weight (0.94 g and 0.90 g, respectively), while the 3 mg/l treatment resulted in the lowest shoot length (2.56) and number of leaves (9.56) compared to control treatments and other tested BAP concentrations in tomato rootstock "Forza". A similar result was reported by Baye et al. (2019) who stated that the lowest shoot length (3.31 cm) and leaf numbers (2.48) were obtained on MS + 3 mg/l BAP. However, they found that among BAP concentrations (0.0, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) the highest number of shoots/explants was achieved on MS medium supplemented with 2 and 2.5 mg/l BAP, depending on varieties.

In contrast to our results, Yesmin et al. (2022) found that the maximum number of shoots per explant (3.18 and 2.46) was obtained with 1.0 and 1.5 mg/l BAP in BINA tomato-3 and BINA tomato-4 genotypes. They also observed that the number of shoots increased with the increase of BAP up to 1.5 mg/l and decreased slightly with higher concentrations (2.0 - 3.0 mg/l). Also, Mohamed et al. (2010) found that the MS medium supplemented with 2.0 mg/l BAP had the best number of shoots and shoot length in two tested tomato cultivars (pearl and beril). Similarly, Sarker (2013) reported that the highest number of shoots in tomato was noted in MS medium containing 2.0 mg/l BAP.

Interestingly, the addition of BAP to multiplication medium resulted in a decrease in shoot length from 10.13 cm (control treatment) to 2.56 cm (BAP concentrations) and a decrease in the number of leaves from 16.80 (control treatment) to 9.56 (BAP concentrations). This may be due to the role of BAP in enhancing shoot formation and releasing lateral buds, but it has lower impacts on shoot length through controlling various distinctive processes such as cell growth and differentiation (George and Sherrington,1984).

*Effect of IBA concentrations on Cucurbita rootstock rooting*

Our results clearly indicate that IBA is an effective growth regulator in the root regeneration of Super Shintoza rootstock. This result is in harmony with several previous reports, which showed similar results in other cucurbitaceous species (Thomas and Sreejesh, 2004; Hoque et al., 2007; Baskaran et al., 2009; Meena et al., 2010; Thiruvengadam et al., 2010; Sangeetha and Venkatachalam, 2014).

The results of the current study also reported that the treatment of 4 mg/l of IBA had the highest root number, fresh weight and dry weight (4.86, 1.30 g and 0.10 g, respectively), and the lowest root length (5.16 cm) compared to the control and other tested IBA concentrations. In harmony with this result, Sarowar et al. (2003) reported that 1.0 mg/l of IBA was effective for root induction in an interspecific hybrid of Cucurbita.

In contrast to our results, Sarowar et al. (2003) reported that the best result for root formation was obtained when 1 mg/l of IBA was applied, and either higher (3.0 mg/l) or lower (0.5 mg/l) concentrations of IBA resulted in lower

percentages of root initiation. Also, Selvaraj et al. (2007) reported that 0.7 mg/l of IBA was the best treatment for root induction of cucumber. In contrast, Dhumal et al. (2020) found that MS media without hormones induced multiple shoots with a good number of roots. The obtained results may be due to the fact that IBA works by binding to specific receptors on the surface of plant cells, which triggers a signaling cascade that leads to changes in gene expression and the activation of various growth-related processes such as root formation. One of the key pathways activated by IBA is the auxin response pathway, which involves the activation of genes that regulate cell division and cell elongation. Another unique characteristic of IBA is that it can be metabolized by plants into other auxins, such as indole-3-acetic acid (IAA), which is the most common and well-studied auxin. This means that IBA can have a longer-lasting effect on plant growth and development than other auxins, as it can be converted into other active forms as needed by the plant (Jameson and Song, 2016; Arigita and Gotar, 2018).

*Effect of IBA concentrations on tomato rootstock rooting*

The results of the current study generally showed that the treatment of 4.0 mg/l of IBA recorded the highest value of root number (6.86) compared to other tested IBA treatments. While the treatment of 2 mg/l of IBA recorded the highest significant values of root length, root fresh and dry weights (8.83 cm, 0.23 g and 0.019 g, respectively) in tomato rootstock "Forza". Similar results were reported by Yesmin et al. (2022) who found that IBA was more effective than IAA and that maximum root induction and development in tomato plants were found in half- strength MS medium supplemented with 0.2 mg/l IBA. In contrast to our results, Yesmin et al. (2022) and Jawad et al. (2020) reported that the combination of 0.5 mg/l IAA + 0.5 mg/l IBA was found to be more efficient than other auxin combinations (IAA + IBA) and separate treatments (IAA alone or IBA alone) in the induction and development of tomato roots.

*Grafting experiment*

The main problems facing cucumber and tomato production may include soil salinization and degradation, groundwater pollution, drought and heat stresses (Liang et al., 2021) as well as soil-borne pathogens such as bacteria, fungi and nematodes (Punja et al., 2019). The vegetable grafting technique makes a distinguished relationship between the scion and rootstock, which should be tolerant or resistant to stressful conditions, biotic and abiotic and its root system has the ability to improve the uptake of water and nutrients, making the grafted plant stronger than non-grafted plants (Wang et al., 2017). Fortunately, grafting has been applied successfully as a tool to improve the fruit yield and quality parameters of cucumber and tomato under normal and stressful conditions, such as salinity and drought (Venema et al., 2008; Usanmaz and Abak, 2019; Elsheery et al., 2020; Fu et al., 2022; Shalaby et al., 2022). The work done on grafting of tomatoes and cucurbits has shown a significant positive effect on yield, quality, resistance to soil-borne diseases, water stresses, salinity and tolerance to toxic chemicals in the soil due to grafting (Yassin and Hussen, 2015). Generally, grafting has resulted in yield increases of up to 80% in the *Solanaceae* family and 60-90 percent in the *Cucurbitaceae* family (Dash et al., 2021).

In commercial production of grafted cucumber and tomato, growers currently use interspecific rootstocks in cucumber (*Cucurbita maxima* x *Cucurbita moschata*) and tomato (*Solanum lycopersicum* × *Solanum habrochaites*), which are very vigorous as a consequence of heterosis, leading to a higher yield and tolerance to different stressful conditions. Nevertheless, breeding such interspecific rootstocks faces several challenges such as, crossing barriers, low fruit set and low fertile seeds (Karaagac and Balkaya, 2013; Lu et al., 2020). These factors cause high seed costs and prevent farmers from using the advantages of grafted plants. Therefore, micropropagation of rootstocks may reduce the costs of rootstock for cucumber and tomato grafting, which may have a strong benefit for small-scale farmers of cucumber and tomato.

In this study, two commercial rootstock genotypes, one for cucumber and the other for tomato, were used to establish an efficient protocol for *in vitro* propagation of both rootstocks and to compare the efficiency of grafting onto *in vitro* and *in vivo* rootstock seedlings of "Super Shintoza" and "Forza" in terms of vegetative growth and yield parameters. The results of the current study indicate that the cucumber cv. 'Hayel' grafted onto "Super Shintoza" rootstock seedlings produced *in vivo* recorded higher means for all studied traits in comparison to those grafted onto "Super Shintoza" rootstock seedlings produced *in vitro*. While there were no significant differences between grafted

tomato plants onto "Forza" rootstock seedlings even produced *in vitro* or *in vivo* in all studied traits. Therefore, the micropropagation technique could be recommended as an efficient method for the production of "Forza" rootstock for tomato grafting. While it is not recommended for the production of "Super Shintoza" rootstock for cucumber grafting.

## Conclusion

To the best of our knowledge, no or minor studies have been performed to compare the response of grafted cucumber and tomato plants onto *in vitro* and *in vivo* rootstocks. The current study provides new evidence that the micropropagation technique can be used efficiently to produce *in vitro* vegetable rootstocks, especially tomato rootstocks, in order to decrease the high cost of the interspecific rootstocks for commercial production of grafted vegetables.

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*Conflicts of interest*

The authors declare that they have no conflict of interest.

## References

Al-Harbi, A. R., Al-Omran, A. M., and Alharbi, K. (2018) Grafting improves cucumber water stress tolerance in Saudi Arabia. *Saudi J. Biol. Sci.,* **25**(2), 298-304.

Arigita, L. and Gotor, V. (2018) Benzyladenine metabolism in *Arabidopsis thaliana*. *Phytochem.,* **1**(147), 54-61.

Aslam, W., Noor, R. S., Hussain, F., Ameen, M., Ullah, S., and Chen, H. (2020). Evaluating morphological growth, yield, and postharvest fruit quality of cucumber (*Cucumis sativus* L.) grafted on cucurbitaceous rootstocks. *Agri,* **10**(4), 101.

Aslibeigi, A., Haddad, R., Garoosi, G., Hossaini, S. M., and Nazari, M. R. (2022) *In vitro* ovary culture of cucumber (*Cucumis sativus* L.) for haploid plant production. *Iran. j. genet. plant breed.,* **11**(1), 87-98.

Bayoumi, Y., Abd-Alkarim, E., El-Ramady, H., El-Aidy, F., Hamed, E. S., Taha, N., and Rakha, M. (2021) Grafting improves fruit yield of cucumber plants grown under combined heat and soil salinity stresses. *Hortic.,* **7**(3), 61.

Bhatia, P., Ashwath, N., Senaratna, T., and Midmore, D. (2004) Tissue culture studies of tomato (*Lycopersicon esculentum*). *Plant Cell Tissue Organ Cult.,* **78**, 1-21.

Baskaran, P., Velayutham, P. and Jayabalan, N. (2009) *In vitro* regeneration of *Melothria maderaspatana* via indirect organogenesis. *In Vitro Cell. Dev. Bio. Plant,* **45**, 407-413.

Baye, E., Matewos, T. and Belew, D. (2019) Effect of 6-benzyl amino purine on *in vitro* multiplication of tomato (*Lycopersicon esculentum* Mill.) varieties using shoot explant. *J. Plant Sci. Agric. Res.,* **4**(1), 32.

Dash, R., Jena, C., Pramanik, K. and Mohapatra, P.P. (2021) Vegetable grafting: A noble way to enhance production and quality. *The Pharma Innov. J.,* **10** (8): 1580-1584.

Dhumal, S.S., Naik, B.V. and Nimbalkar, M.S. (2020) Advances in tissue culture of Cucurbits: A review. *Int. J. Curr. Microbiol. App. Sci.,* **9**(8), 2887-2910.

Elsheery, N.I., Helaly, M.N., Omar, S.A., John, S.V., Zabochnicka-Swiątek, M., Kalaji, H.M. and Rastogi, A. (2020) Physiological and molecular mechanisms of salinity tolerance in grafted cucumber. *S. Afr. J. Bot.,* **130**, 90-102.

FAOSTAT (2023). Food and Agriculture Organization of the United Nations, Rome, Italy. Retrieved January, 2023 on the world Wide Web: http://www.fao.org/faostat/en/#data/QC

Faria, L.A., Ara, T., Karim R., Islam, R. and Hossain, M. (2013) Rapid *in vitro* clonal propagation of two hybrid muskmelon cultivars and their field evaluation in agro climatic condition of Bangladesh. *J. Genet. Environ. Resour. Conserv.,* **1**(3), 247-253.

Fu, S., Chen, J., Wu X., Ga, H. and Lu, G. (2022) Comprehensive evaluation of low temperature and salt tolerance in grafted and rootstock seedlings combined with yield and quality of grafted tomato. *Horticulturae,* **8**(7), 595.

George, E.F. and Sherrington, P.D. (1984) *Plant Propagation by Tissue Culture*: Handbook and directory of commercial laboratories. Exgetics Ltd., England.

Hoque, A., Hossain, M., Alam, S., Arima, S. and Islam, R. (2007) Adventitious shoot regeneration from immature embryo explant obtained from female-a female *Momordica dioica*. *Plant Tissue Cult. Biotechnol.,* 17(1), 29-36.

Jameson, P.E. and Song, J. (2016) Cytokinins and auxins in plant growth and development. *Annu. Rev. Plant Biol.,* 67, 379-405.

Jawad, Z.A., Turker, M. and Ozdemir, F.A. (2020) Effect of different plant growth regulator on *in vitro* propagation of endangered plant; yellow tomato (*Lycopersicon esculentum* Mill.). *Int. J. Agric. For. Life Sci.,* 4(1), 92-98.

Joyia, F.A., Mustafa, G., Zahra R., Munawar, S., Anjum, M.N. and Khan, M.S. (2019) Exploiting shoot tips as an efficient explant for *in vitro* regeneration of cucumber (*Cucumis sativus* L.). *Pure Appl. Biol.,* 8(2), 1824-1829.

Karaagac, O. and Balkaya, A. (2013) Interspecific hybridization and hybrid seed yield of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) lines for rootstock breeding. *Sci. Hortic.,* 149, 9-12.

Kumar, M., Sirohi, U., Malik, S., Kumar, S., Ahirwar, G. K., Chaudhary, V., and Prakash, S. (2022) Methods and factors influencing in vitro propagation efficiency of ornamental tuberose (Polianthes species): A systematic review of recent developments and future prospects. *Hortic.,* 8(11), 998.

Lee, Y.K., Chung, W.I. and Ezura, H. (2003) Efficient plant regeneration via organogenesis in winter squash (*Cucurbita maxima* Duch.). *Plant Sci.,* 164(3), 413-418.

Liang, J., Chen, X., Guo, P., Ren, H., Xie, Z., Zhang, Z. and Zhen, A. (2021) Grafting improves nitrogen-use efficiency by regulating the nitrogen uptake and metabolism under low-nitrate conditions in cucumber. *Sci. Hortic.,* 289, 110454.

Lu, X., Liu, W., Wang, T., Zhang, J., Li, X. and Zhang, W. (2020) Systemic long-distance signaling and communication between rootstock and scion in grafted vegetables. *Front. Plant Sci.,* 11, 460.

Mahzabin, F., Parvez S. and Alam M.F. (2008). Micropropogation *of Cucurbita maxima* Duch. through shoot tip culture. *J. Bio-Sci.,* 16, 59-65.

Mali, A.M. and Chavan, N.S. (2016) *In vitro* rapid regeneration through direct organogenesis and *ex-vitro* establishment of *Cucumis trigonus* Roxb. An underutilized pharmaceutically important cucurbit. *Ind. Crops Prod.,* 83, 48-54.

Meena, M.C., Meena, R. and Patni, V. (2010) High frequency plant regeneration from shoot tip explants of *Citrullus colocynthis* (Linn.) Schrad. An important medicinal herb. *Afr. J. Biotechnol.,* 9(31), 5037-5041.

Milenkovic, L., Mastilovic, J., Kevresan, Z., Bajic, A., Gledic, A., Stanojevic, L., and Ilic, Z. S. (2020) Effect of shading and grafting on yield and quality of tomato. *J. Sci. Food Agric,* 100(2), 623-633.

Mohamed, A.A.N., Ismail, M.R. and Rahman, M.H. (2010) *In vitro* response from cotyledon and hypocotyls explants in tomato by inducing 6-benzylaminopurine. *Afr. J. Biotechnol.,* 9(30), 4802-4807.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* 15, 473-497.

Nasarudin, S.S. and Mansor, F.N. (2021) A review on the effects of auxin and cytokinin on the growth of *Momordica charantia* using *in vitro* propagation technique. *ASEAN J. Life Sci.,* 1(2), 29-33.

Punja, Z.K., Tirajoh, A., Collyer, D. and Ni, L. (2019) Efficacy of *Bacillus subtilis* strain QST 713 (Rhapsody) against four major diseases of greenhouse cucumbers. *Crop Prot.,* 124, 104845.

Salehian, H., Shahnazi, S., and Nazari, M. (2023) Production of doubled haploid plants in cucumber (*Cucumis sativus* L.) via parthenogenesis. *In Vitro Cell. Dev. Biol. Plant,* 59(4), 467-474.

Sangeetha, P. and Venkatachalam, P. (2011) Induction of multiple shoots from shoot tip explants of cucumber (*Cucumis sativus* L.). *Plant Cell Biotech. Molec. Biol,* 12, 1-4.

Sangeetha, P., and Venkatachalam, P. (2014) Induction of direct shoot organogenesis and *in vitro* flowering from shoot tip explants of cucumber (*Cucumis sativus* L. cv.'Green long'). In Vitro Cell. Dev. Bio. Plant, 50, 242-248.

Sarker, U. (2013) Effect of growth regulators on plant regeneration and shoot induction in tomato (*Solanum lycopersicum* L.). MSc thesis, BRAC University, Bangladesh.

Sarowar, S., Oh, H.Y., Hyung, N.I., Min, B.W., Harn, C.H., Yang, S.K. and Shin, J.S. (2003) *In vitro* micropropagation of a Cucurbita interspecific hybrid cultivar–a root stock plant. *Plant Cell, Tissue Organ Cult.,* 75, 179-182.

Schwarz, D., Rouphael, Y., Colla, G., and Venema, J. H. (2010) Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Sci. Hortic.,* **127**(2), 162-171.

Selvaraj, N., Vasudevan, A., Manickavasagam, M., Kasthurirengan, S. and Ganapathi, A. (2007) High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis. *Sci. Hortic.,* **112**(1), 2-8.

Shalaby, T.A., Taha, N.A., Rakha, M.T., El-Beltagi, H.S., Shehata, W.F., Ramadan, K.M. and Bayoumi, Y.A. (2022) Can grafting manage *Fusarium* wilt disease of cucumber and increase productivity under heat stress? *Plants*, **11**(9), 1147.

Singh, H., Sethi, S., Kaushik, P., and Fulford, A. (2020) Grafting vegetables for mitigating environmental stresses under climate change: a review. *J. Water Clim. Chang.,* **11**(4), 1784-1797.

Thiruvengadam, M., Rekha, K.T., Yang, C.H., Jayabalan, N. and Chung, I.M. (2010) High-frequency shoot regeneration from leaf explants through organogenesis in bitter melon (*Momordica charantia* L.). *Plant Biotechnol. Rep.,* 4, 321-328.

Thomas, T.D. and Sreejesh, K.R. (2004) Callus induction and plant regeneration from cotyledonary explants of ash gourd (*Benincasa hispida* L.). *Sci. Hortic.,* **100**(1-4), 359-367.

Usanmaz, S. and Abak, K. (2019) Plant growth and yield of cucumber plants grafted on different commercial and local rootstocks grown under salinity stress. *Saudi J. Biol. Sci.,* **26**(6), 1134-1139.

Venema, J.H., Dijk, B.E., Bax, J.M., van Hasselt, P.R. and Elzenga, J.T.M. (2008) Grafting tomato (*Solanum lycopersicum*) onto the rootstock of a high-altitude accession of *Solanum habrochaites* improves suboptimal-temperature tolerance. *Environ. Exp. Bot.,* **63**(1-3), 359-367.

Wang, Q., Men, L., Gao, L. and Tian, Y. (2017) Effect of grafting and gypsum application on cucumber (*Cucumis sativus* L.) growth under saline water irrigation. *Agric. Water Manag.,* **188**, 79-90.

Yassin, H. and Hussen, S. (2015) Review on role of grafting on yield and quality of selected fruit vegetables. *Global J. Sci. Front. Res.,* **15**,1-15.

Yesmin, S., Mollika, S.R., Islam, M.N. and Nasrin, S. (2022) *In vitro* regeneration of two BINA tomato (*Lycopersicon esculentum* Mill.) varieties of Bangladesh. *Plant Tissue Cult. Biotechnol.,* **32**(1), 43-51.

## نمو ومحصول نباتات الطماطم والخيار المطعومة على أصول تطعيم بذرية ومكثرة معملياً

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أجريت هذه الدراسة فى معمل زراعة الأنسجة وصوبة قسم البساتين كلية الزراعة، جامعة قناة السويس، محافظة الإسماعيلية، مصر خلال الفترة من ٢٠١٧ وحتى ٢٠٢٢ لوضع بروتوكول كفء لإنتاج أصول تطعيم لنباتات الخضر معملياً ولمقارنة تأثير التطعيم علي أصول ناتجة من زراعة الأنسجة و أصول ناتجة من زراعة البذرة مباشرة على نمو نباتات الخيار والطماطم المطعومة والمحصول. وقد تضمنت هذه التجربة استخدام أصل لتطعيم القرعيات «سوبر شنتوزا» واصل لتطعيم الطماطم «فورزا» وقد تم استخدام صنف خيار «هايل» وهجين الطماطم أصيل «تي-١٨٦» كطعم للتطعيم علي الأصول المستخدمة. ولقد أوضحت النتائج المتحصل عليها أنه يمكن استخدام تكنيك الإكثار الدقيق بكفاءة لإنتاج أصول تطعيم معملياً وخاصة أصول الطماطم؛ وذلك بغرض تقليل التكاليف العالية لأصول التطعيم للإنتاج التجاري للخضروات المطعومة.