# Thidiazuron and Explant Type Effects on High-frequency *In Vitro* Mass Propagation of Cherry Tomato

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**Abstract:** Cherry Tomato is one of the most important vegetable crops cultivated for export in Egypt. *In vitro* culture response was assessed in tomato (*Solanum lycopersicum L. var. cerasiforme*) cv. (Summer Cherry) for optimum callus induction and plantlet regeneration. Callus induction was achieved within eight to 12 days directly on the cut surfaces of hypocotyl, cotyledon and leaf disc explants cultured on Murashige and Skoog (MS) basal medium supplemented with various concentrations of Thidiazuron (TDZ)] and benzyl adenine (BA) alone, but not in hormone free-medium. The highest callusing index (3.9 and 3.7) was obtained on hypocotyl explants cultured on MS medium supplemented with TDZ(1.0 and 2.0 $mgl^{-1}$) followed by an index of 3.5 obtained from the same explant by using 0.5$mgl^{-1}$ BA. However, for the leaf disc explants, the highest callusing index (3.1) was obtained on MS medium supplemented with BAat 2.0 $mgl^{-1}$. After 8 weeks of culture, organogenesis was observed only on the explants cultured on medium containing different concentrations of TDZ and BA. The best shoot formation (93%) was obtained from leaf disc explant callus induced on MS medium containing TDZ. The highest number (13.4) of $shootsexplant^{-1}$ was found when cotyledon explant callus was sub cultured on MS medium supplemented with 2.0 $mgl^{-1}$ TDZ. Half strength of MS was found to be the best rooting medium, however, addition of IAA at 1.0 $mgl^{-1}$ and IBA at 2.0 $mgl^{-1}$ were found necessity to induce highest number of roots (22.5) and longer roots (11.0 cm), respectively. Acclimation of *in vitro* rooted plant is important for testing the post culture behavior of tissue culture regenerated plants. Cherry tomato derived from different explant sources under different concentration of TDZ and BA were not significantly different in their vegetative characters to those obtained from seed. However number of (raceme $plant^{-1}$, flower $raceme^{-1}$, fruit $raceme^{-1}$, fruit $plant^{-1}$) which produced by seed-derived plants was significantly less than those derived from *in vitro* propagated plants. This protocol would be valuable to create somaclonal variation and develop transgenic approaches for varietal improvement of cherry tomato.

***Keywords:*** *Solanum lycopersicum* L. var. *cerasiforme*, callus induction, organogenesis, TDZ, acclimatization

## INTRODUCTION

Cherry tomato (*Solanum lycopersicum L. var. cerasiforme*) previously called *Lycopersicon esculentum* Mill. (The Natural History Museum, 2011) is a cultivated variety of tomato. It is generally considered to be similar but not identical to the wild relatives of the domestic tomato. There are around 7500 tomato varieties grown for various purposes around the world (Wikipedia, 2011). Various tissue culture studies had been carried out on different varieties of tomato (Afroz *et al*., 2010; Chaudhry *et al*., 2010; Gubis *et al*., 2004; Jabeen *et al*., 2005), but still there are no results released on cherry tomato.

Cultivation of this valuable crop is expanding day by day because of its high economic values in the vegetables market. It is a rich source of minerals (iron), vitamins (A & C), organic acid, essential amino acids, and dietary fibers also can be used in preserved food stuffs like ketch-up, sauce, chutney, soup, paste etc. (Block *et al*., 1992; Gerster, 1997; Rao and Agarwal, 2000). Tomato cultivated area and productivity in Egypt is estimated by 180,000 hectares and 35.5 t/ha, respectively (FAO 2016). The current tomato productivity in Egypt is about half of USA productivity (66.57 t/ha). Cultivation of tomato suffers from serious losses because of infestation by insects and pests and the diseases they transmit. For that reason, there is an important for biotechnological interventions to rise the productivity of this crop. Culture of plant cell, tissues *in vitro* is an integral part plant biotechnology, which has been exploited for *in vitro* regeneration and genetic upgrading of this crop, as the first step towards genetic transformation of plants. Exclusive of a reliable, reproducible and well-organized system to regenerate genetically identical plants from a small mass of transformed cells, it is not possible to generate a complete genetically modified plant. There are several reports on adventitious *in vitro* cultivation in tomato from various explants (Moghaleb *et al*., 1999; Brichkova *et al*., 2002; Raziuddin *et al*., 2004; Mohamed *et al*., 2010; Liza *et al*., 2013). Nevertheless, improvement and standardization of *in vitro* regeneration and shoot multiplication protocols is still imperative due to its diverse morphogenic potential of different explants and genotypes (Tomsone *et al*., 2004). On the other hand, there are some limitations of *in vitro* technique because of the occurrence of spontaneous genetic or epigenetic changes leading to cytological irregularities, phenotypic mutations, sequence changes (Kaeppler *et al*., 2000), and DNA methylation in *in vitro* regenerated plants. These differences may affect the quality and quantity of plants as well as genetic transformation through various approaches. The presence of TDZ, either alone or in combination with other growth regulators, is important for shoot organogenesis in a wide variety of plant species (Jiang *et al*., 2005).

This study was aimed to formulate an efficient and reproducible regeneration system of cherry tomato by optimization of various factors viz., type of explants

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(hypocotyl, cotyledon and leaf disc), medium type (solide and half strength) and plant growth regulators (TDZ) and (BA) alone in different concentrations for *in vitro* multiplication and plant regeneration. Furthermore, the genetic stability of cherry tomato plants derived from in vitro developed plants was also assessed and compared with the same cherry tomato cultivar plants obtained from mature commercial seed. In the end of this experiment results presented if it is possible of using this protocole for secure micropropagation of valuable genotypes or breeding lines and hybrids in short time which might accelerate the breeding efforts of tomato plants.

## MATERIALS AND METHODS

### Experimental materials and surface sterilization

The current study was conducted at The Plant Tissue Culture Lab, Department of Horticulture, Suez Canal University, Ismailia, Egypt during the years 2017 and 2018, each experiment repeated twice the time. Mature seeds of cherry tomato (*Solanum lycopersicum L. var. cerasiforme*) (Summer Cherry) were obtained from the United States, USDA ARS Plant Genetic Resources Unit. Seeds were thoroughly washed under tap water in the laboratory for 30 min, followed by several washes with sterile ultrapure water. The seeds were surface sterilized by 5% Clorox (Sodium hypochlorite) for 10 min, followed by three times rinses with autoclaved distilled water (15 min.) under laminar air flow hood (Franklin and Dixon, 1994).

### Seed Germination: Culture Media and Conditions

The sterilized seeds were aseptically and cultured in 250 mL glass flask, containing 30 mL of MS (Murashige and Skoog 1962) medium without plant growth regulator (PGR), all media contained 3% sucrose, the medium pH was adjusted to 5.8 before adding 7 $gl^{-1}$plant agar prior to autoclaving at 110 kPa for 20 min at 121°C. Cultured explants were incubated in a controlled environment at 25±2°C and 50±10% relative humidity (RH). The cultured seeds were incubated in darkness for 48 h and thereafter maintained under 50 $\mu molm^{-2}$ $s^{-1}$ light provided by cool white fluorescent lamp for a photoperiod of 16 h. It was observed that germination was possible after 12 - 14 days of culture (Fig 1.).

**Fig (1):** In vitro germinated seedling of cherry tomato (Summer Cherry) after12 - 14 days of culture

### Callus and shoot induction media

MS medium (Duchefa Biochemie), was supplemented with different concentrations of TDZ (0, 1, 2, 3 and 4 $mgl^{-1}$) and BA (0, 1, 2, 3 and 4 $mgl^{-1}$), each plant growth regulator (PGR) was applied alone and not in combination. Explants derived from *in vitro* seed culture plantlet, were grown in 250 mL glass jars covered with clear plastic caps. Each jar contained 30 mL medium.

### Explant preparation and culture

Hypocotyls, cotyledon and leaf segments from 10 - 12 day-old in vitro raised seedlings were excised under aseptic conditions, the length of the hypocotyl (1 cm), cotyledonary leaves (0.5 ± 0.5 $cm^2$) and leaf (0.5 ± 0.5 $cm^2$) explants excised from four leaves in vitro seedlings grown from seeds. Hypocotyl explants were incubated horizontally, cotyledons and leaves incubated adaxial (upper surface facing down) (Bhatia *et al*., 2004) on callus and shoot induction media. Then, culture was kept at 24 ± 2°C conditions under 16 h photoperiod, light intensity of 50 $\mu mol\ m^{-2}\ s^{-1}$.

### Parameters evaluation and data analysis

After 3 weeks of explant culture in callus and shoot regeneration media, data were observed at regular intervals. Scale rating from 0 to 5 was performed to set numerical values for callusing index. The scale was defined as following; "0- no tissue growth, 1- callus occurring from one explant end, 2- callus occurring from both explant ends, 3- callus occurring from both explant ends and double the original explant size, 4- callus occurring from both explant ends and triple the original explant size and 5- callus occurring from both explant ends and four times the original explant size"in addition data presented callus response %. After 6 weeks from explant culture. The following shoot regeneration parameters were recorded: shoot regeneration %, shoots number $explant^{-1}$ and shoot length cm.

### Root Induction

The best elongated normal shoots, which have no roots, were sub cultured and planted again on full

and half strength MS medium containing different concentrations of auxins IAA, IBA, NAA (0, 0.1, 0.5, 1.0 and 2.0 $mgl^{-1}$). (alone and not in combination) for root initiation. Data recorded included root initiation response (%), of roots length (cm.) and number of roots $shoot^{-1}$.

**Acclimization**

Eight weeks old rooted plantlets were acclimatized and out planted in pots contained peat moss and vermiculite (1: 1 v/v) after washing agar thoroughly with running tap water. The pots were covered with clear plastic bags having a few holes and were frequently watered to keep high humidity during 20 days. Hardened plantlets were out planted in a greenhouse set at a day temperature 25°C, a night temperature 18°C, relative humidity 85% and a day length of 14 h.

Mature seed derived plants were also grown under the same condition and the same time to compare the growth performance of *in vitro* plants with those of seed derived plants. The plantlets were irrigated immediately after planting and adequate soil moisture was maintained through daily watering and fertilized as the normal fertilizer program of commercial tomato plants. Data were recorded after 12 weeks from the initial culture in the greenhouse and the measurements included: Leaf number $plant^{-1}$, plant height cm., number of nodes to first flower, number of raceme $plant^{-1}$, number of flower $raceme^{-1}$, number of fruit $raceme^{-1}$ and total number of fruit $plant^{-1}$).

Pots were randomly arranged on the green house benches with four replicates (pots) for each plant types (plants derived from *in vitro* shoot regeneration of petiole, cotyledon and leaf disc under different TDZ and BA concentrations) and from mature cherry tomato seeds.

**Statistical analysis**

All *in vitro* experiments were repeated twice with at least five replications. Data were combined and subjected to ANOVA using Costat computer program (CoStat Statistical Software; CoHort Soft-ware version 6.4, Berkeley, California) and the differences between means were separated using Duncan's multiple-range test at 5% significance level.

## RESULTS AND DISCUSSION

The in vitro morphogenetic responses of the cultured explants are affected by different components of the culture media and therefore, it is essential to evaluate their effects on plant callus induction and shoot regeneration (Gubis *et al.,* 2004). In the current study callus was initiated within 8 - 12 days directly on the cut surfaces of hypocotyl, cotyledon and leaf disc explants cultured on MS basal medium supplemented with different concentration of (TDZ and BA) alone, but not in hormone free- medium (Table 1). Callus response was markedly affected by types of explant and growth regulators used. Different concentrations of TDZ and BA had a distinct effect on callus induction from explants. Pal *et al*. (2007) reported that in vitro callus induction depends on the endogenous concentration of plant growth regulator as well as exogenously supplied growth regulator. Moreover, Nikam and Shitole (1998) reported that the growth regulator requirements for callus induction vary depending on the source of explant.

**Table (1):** Effect of different concentrations of benzyladenine (BA) and thidiazeron (TDZ) on callus induction of cherry tomato (*Lycopersicon esculentum* Mill., c.v. Summer Cherry)

| Type of explants | Callus response % | | | | Callus index | | | |
|---|---|---|---|---|---|---|---|---|
| PGR ($mgl^{-1}$) | Hypocotyls | Cotyledon | Leaf disc | Mean | Hypocotyls | Cotyledon | Leaf disc | Mean |
| **TDZ** | | | | | | | | |
| **0.5** | 55d | 58.3d | 55d | **56.1** | 2.8b | 1.8c | 1.6c | **2.1b** |
| **1.0** | 100a | 75.0c | 95a | **90.0** | 3.7a | 2.2bc | 2.3ab | **2.7a** |
| **2.0** | 100a | 86.6b | 92ab | **92.9** | 3.9a | 2.1bc | 2.0bc | **2.7a** |
| **3.0** | 100a | 92.3a | 100a | **97.4** | 3.0a | 2.0bc | 2.4ab | **2.5a** |
| **4.0** | 100a | 60.3de | 100a | **86.8** | 2.2bc | 1.6c | 1.6c | **1.8b** |
| **Mean** | 91a | 74.5b | 88.4ab | | 3.12a | 1.94b | 1.98b | |
| **BAP** | | | | | | | | |
| **0.5** | 67.9c | 51.0d | 69.9c | **62.9** | 3.5a | 0.0d | 0.0d | **1.2c** |
| **1.0** | 80.6b | 91.6a | 82.6b | **84.9** | 2.5b | 1.9c | 1.8c | **2.1b** |
| **2.0** | 100a | 66.6cd | 80a | **82.2** | 2.0bc | 2.6b | 3.1a | **2.6a** |
| **3.0** | 78.5c | 75.0c | 83.5b | **79.0** | 2.7b | 2.5b | 2.9ab | **2.7a** |
| **4.0** | 72.5c | 48.0e | 74.5c | **65.0** | 2.7b | 1.8c | 1.7c | **2.4ab** |
| **Mean** | 79.9a | 66.44b | 78a | | 2.7a | 1.8b | 1.9b | |

Data represent the mean of four replicates with six explants for each treatment. Means followed by same letter do not differ statistically at p=0.05 according to the Duncan's multiple range test

Our results indicated that the highest callusing index (3.9 and 3.7) was obtained on hypocotyl explant cultured on MS medium supplemented with TDZ at 1.0 and 2.0 $mgl^{-1}$ (Table 1), followed by (3.5) obtained from the same explant by using 0.5 $mgl^{-1}$ BA. However MS medium supplemented with BA at either 2.0 or 3.0 $mgl^{-1}$ presented the highest callusing index for both cotyledon and leaf disc explants. In general, it was found that hypocotyls produced the highest values of both callus response and callus index than cotyledons and leaf disc. Moreover, it was noticed that even though more callus were produced by hypocotyls, their shoot regeneration ability was lower than that produced by cotyledons and leaf disc explants (Table 2). These results are coherent with the findings of former investigations (Ali *et al*., 2012). A significant increment of callus proliferation was observed over the culture period. The origination of callus and shoots was noticed in the middle part of explants and on cut edges. In terms of morphology, the callus was fragile and characterized by a pale yellow or green color.

The primary mode of regeneration is shoot organogenesis, which can retrieve directly or indirectly from organ explants (Bhatia *et al.,* 2004; Chaudhry *et al.,* 2010). Most of the reports about shoot organogenesis in tomato are related to the induction of regeneration from hypocotyls or cotyledon explants (Asakura *et al.,* 1995; Icjimura and Oda, 1995; Moghaleb *et al.,* 1999). In this study, it was found that using hypocotyls, cotyledons and leaf discs as explants to study the effect of various concentrations of TDZ and BA for high cherry tomato regeneration frequencies. MS media containing BA and TDZ were tested individually for shoot induction. Generally, No responses were found in control medium without TDZ and BA. It shows that the presence of BA and TDZ is essential for shoot organogenesis. It was observed that all explants responded significantly to presence of TDZ and BA. However, the best responses to regeneration (100%) was recorded on leaf disc and cotyledon at TDZ (2.0, 3.0 and 4.0 $mg^{-1}$) and BA (2.0 $mg^{-1}$). Hypocotyle showed the lowest regeneration % at different TDZ and BA concentration. (Figs. 2 (a,b), 3(a,b) and 4(a,b)) are shown different regeneration responses among the three explants and two PGR (TDZ & BA). These results are in agreement with Chaudhry *et al.* (2010), who have reported the best explants for regeneration are respectively leaf disc, cotyledon and hypocotyl. In addition, other reports indicated that cotyledon regenerated better than hypocotyls explants (Shutze and Wieczorrek, 1987; Hamza and Chupeau, 1993). However, Mohamed *et al.* (2011) have reported that there was no significant difference between cotyledon and hypocotyl explants in the same treatment on regeneration.

The highest number of shoots $explant^{-1}$ (13.4) was observed in medium containing 2.0 mg $L^{-1}$ TDZ with a maximum in vitro shoot length of about 8 cm. BA-supplemented medium produced 11.9 shoots $explant^{-1}$ at 3.0 mg $L^{-1}$, and the highest shoot length of 7.2 cm was observed at 2.0 mg $L^{-1}$ (Table 2). Also, the best explant for shoot number $explant^{-1}$ was the cotyledon, and highest shoot length was observed in leaf disc explant for both PGRs, (TDZ and BA) at different concentrations. These results are consistent with Chaudhry *et al*. (2010) who showed that the explant source had shown significantly different results for various hormones. The best shoot formation was found for cotyledon explant callus induced and sub cultured on MS medium containing TDZ at 2.0 and 3.0 $mgl^{-1}$. The requirement of cytokinin for shoot initiation is well established (Beck and Coponetti, 1983; Evans *et al.,* 1984). In this investigate; TDZ presented the greatest result for shoot organogenesis than BA. TDZ, a synthetic phenylurea, is considered to be one of the most active cytokinins for shoot induction in plant tissue culture (Huetteman and Preece, 1993). Many reports confirmed that TDZ produces shoot regeneration better than other cytokinins (Thomas, 2003; Thomas and Puthur, 2004; Husain *et al*., 2007). In other study, Mok *et al.* (1982), confirmed that TDZ is involved in cytokinin metabolism. TDZ has been exposed to stimulate accumulation of endogenous cytokinins (Murthy *et al*., 1995; Hutchinson *et al.,* 1996). Furthermore to the cytokinin-like activity, Hutchinson *et al*. (1996) detected that, TDZ stimulated auxin accumulation. Other studies showed that TDZ affected auxin transport in hypocotyl tissues of *Pelargonium hortorum* (Murch and Saxena, 2001) and promoted the regeneration frequency by altering the levels of abscisic acid (Li and Yang, 1988), ethylene (Yip and Yang, 1986) and proline (Murch and Saxena, 1997). On the other hand, the mechanism of TDZ action in plant regeneration in vitro is not clear.

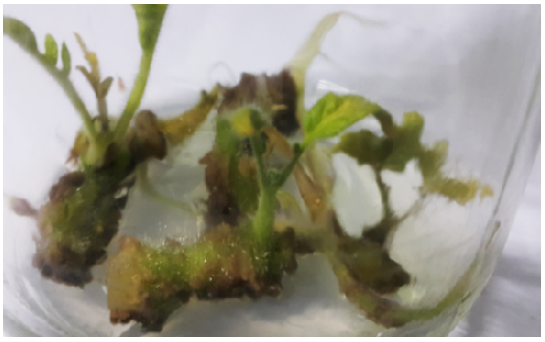
In this experiment, the best callus induction was achieved on hypocotyl explant. However, the leaf disc followed by cotyledon had better performance in shoot regeneration through callus than hypocotyl explant, indicating that, the cotyledonary and leaf disc tissue of tomato is an excellent explant for plant regeneration. Previous studies demonstrated that cotyledons and leaf disc of tomato were better to other sources of explants, including hypocotyls and stems, for promoting shoot organogenesis of tomato (Hamza and Chupeau, 1993; Van Roekel *et al.,* 1993; Ling *et al.,* 1998).

Induction of roots on regenerated shoots is important for effective establishment of the plantlet on the soil. Half strength MS was the best tomato rooting medium supplemented with any of the synthetic hormones (Table 3). In uniformity with our result, Devi *et al.* (2008) reported that the best tomato rooting was obtained on half strength MS basal medium. The highest mean number of $rootsshoot^{-1}$ (22.5) was noticed on half strength MS supplemented with IAA at 1.0 $mgl^{-1}$ (Fig. 5a,b), and longest root (11 cm) seen in the same medium containing IBA at 2.0 mg/l. Our results indicated that 90% rooting was found on half strength MS basal medium without growth regulator, indicating that this tomato genotype possessed sufficient level of endogenous auxin.

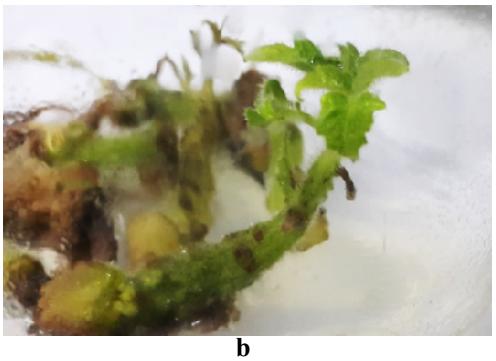
**Table (2):** Regeneration %, number of shoots regenerated explant-1 and shoot length from different explant of cherry tomato on MS medium supplemented with different concentrations of TDZ and BAP

| PGR concentration ($mgl^{-1}$) | Regeneration % | | | | Shoots No. explant-$^{1}$ | | | | Shoot length cm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of explant | | | | | | | | | | | |
| **TDZ** | **Hypocotyls** | **Cotyledon** | **Leaf disc** | **Mean** | **Hypocotyls** | **Cotyledon** | **Leaf disc** | **Mean** | **Hypocotyls** | **Cotyledon** | **Leaf disc** | **Mean** |
| **0.5** | 40 | 80 | 80 | **66.67** | 1.6d | 10.3a | 2.9d | **4.9c** | 5.0c | 5.2c | 6.0C | **5.4c** |
| **1.0** | 50 | 80 | 85 | **71.67** | 1.9d | 11.7a | 2.8c | **5.5c** | 6.1b | 6.4b | 7.6a | **6.7b** |
| **2.0** | 55 | 100 | 100 | **85.00** | 4.8b | 13.4a | 12.0a | **10.1a** | 7.5a | 8.0a | 7.9a | **7.8a** |
| **3.0** | 65 | 100 | 100 | **88.33** | 5.0b | 12.1a | 12.8a | **10.0a** | 6.3b | 6.8b | 6.5b | **6.5b** |
| **4.0** | 30 | 90 | 100 | **73.33** | 2.2d | 9.2a | 11.2a | **7.5b** | 6.6b | 7.6a | 7.8a | **7.3a** |
| **Mean** | **48** | **90** | **93** | | **3.1c** | **11.3a** | **8.3b** | | **6.3b** | **6.8b** | **7.16a** | |
| **BAP** | | | | | | | | | | | | |
| **0.5** | 30 | 75 | 80 | **61.67** | 0.7d | 7.2bc | 4.6c | **4.2c** | 4.2c | 3.7c | 4.1c | **4.0c** |
| **1.0** | 45 | 85 | 80 | **70.00** | 1.5d | 8.1ab | 6.2bc | **5.3c** | 5.8b | 6.5b | 6.6b | **6.3b** |
| **2.0** | 65 | 85 | 100 | **83.33** | 2.5d | 11.0a | 6.8bc | **6.8bc** | 7.0a | 7.2a | 7.1a | **7.1a** |
| **3.0** | 66 | 90 | 92 | **82.67** | 4.4c | 11.9a | 8.7ab | **8.3a** | 4.1c | 3.7c | 3.8c | **3.8c** |
| **4.0** | 60 | 95 | 96 | **83.67** | 4.6c | 10.9a | 7.2bc | **7.6ab** | 2.2d | 2.6d | 3.0c | **2.6d** |
| **Mean** | **53.2** | **86** | **89.6** | | **2.74c** | **9.82a** | **6.7b** | | **4.66a** | **4.74a** | **4.92a** | |

Data represent the mean of four replicates with six explants for each treatment. Means followed by same letter do not differ statistically at p=0.05 according to the Duncan's multiple range test



a



b

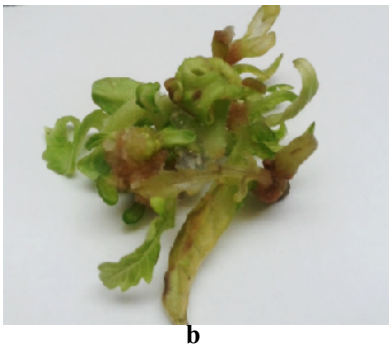
**Fig (2):** Callus formation and shoot regeneration from Hypoctyle explant in MS media with 3.0 $mgl^{-1}$BA. (a). and MS media with 3.0 $mgl^{-1}$ TDZ (b)

a

b

**Fig (3):** Shoot regeneration from cotyledon explant on (a) MS containing 3.0 $mgl^{-1}$BA. and on (b) MS containing 3.0 $mgl^{-1}$TDZ

a



b

**Fig (4):** Shoot regeneration from leaf disc explant on (a) MS containing 3.0 $mgl^{-1}$BA. and on (b) MS containing 3.0 $mgl^{-1}$ TDZ

**Fig (5. a):** Rooting of elongated shoots on MS medium with 1.0 $mgl^{-1}$ IAA

**Fig (5.b):** Rooting of elongated shoots on MS medium with 1.0 $mgl^{-1}$ IBA

**Table (3):** Effect of different concentrations of auxins and MS salt strength on rooting percentage, number of root per shoot and root length in tomato (*Lycopersicon esculentum* Mill., c.v. *Summer Cherry*)

| PGR | | | Medium | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Full MS | | | ½ MS | | |
| IAA | IBA | NAA | Response (%) | Average no. of roots | Average length of roots cm. | Response (%) | Average no. of roots | Average length of roots cm. |
| 0.0 | | | 55.3 | 6.8ghi | 6.6fgh | 92 | 8.5fg | 7.4ef |
| 0.1 | | | 96 | 11.5ef | 5.6ij | 100 | 8.5fg | 8.5dc |
| 0.5 | | | 100 | 12.5e | 4.8jk | 100 | 12.5e | 7.5ef |
| 1.0 | | | 100 | 15.5cd | 3.9kl | 100 | 22.5a | 8.0de |
| 2.0 | | | 100 | 15.0d | 3.0 lm | 100 | 20.5ab | 8.0de |
| | 0.1 | | 96 | 5.3hi | 10.0 b | 100 | 6.5ghi | 9.0c |
| | 0.5 | | 98 | 7.5ghi | 6.5ghi | 100 | 7.5ghi | 10.2b |
| | 1.0 | | 100 | 17.0cd | 6.6fghi | 100 | 8.0gh | 10.0 b |
| | 2.0 | | 90 | 11.5ef | 5.9hi | 100 | 8.8fg | 11.0 a |
| | | 0.1 | 100 | 17.8cd | 3.7 l | 100 | 18.5bc | 6.0 hi |
| | | 0.5 | 96 | 16.5cd | 2.9lm | 100 | 21.0a | 3 .0 lm |
| | | 1.0 | 80 | 9.0fg | 1.0 n | 100 | 18.0cd | 2.6m |
| | | 2.0 | 65 | 4.5 i | 0.7n | 100 | 9.0fg | 1.3n |

Data represent the mean of four replicates with six explants for each treatment. Means followed by same letter do not differ statistically at p=0.05 according the Duncan's multiple range test

**Acclimatization**

Meanwhile in vitro rooted plantlet are raised in the most congenial environmental conditions, hardening is necessary to ensure survival of the micro propagated plants upon transfer to soil under natural conditions. Therefore, in vitro rooted plantlets with 3 to 4 fully expanded leaves and well developed roots derived from different explant and different concentration of TDZ and BA were transferred to pots filled with peat moss and vermiculite. The percentage of survived plantlets after shifting to peat moss was 100%. These grew well and were transferred to the greenhouse condition. Seed-derived plants of the same genotype were also grown in the same time under the same condition to compare the ex vitro growth performance of in vitro propagated plants with those of seed derived plants. No abnormal phenotype was observed during the test period which terminated after 12 weeks from transferring those plants from culture jars to the greenhouse. Data on growth performance ex vitro are presented in (Table 4) Results presented that cherry tomato derived from different explant sources under different concentration of TDZ and BA were not significantly different in their vegetative characters (leaf number plant-1, plant height cm. and number of nodes to first flower) to those obtained from seed, as shown in (Table 4). However Results showed that cherry tomato derived from different explant sources under different concentration of TDZ and BA were significantly different in number of (raceme plant$^{-1}$, flower raceme-1, fruit raceme-1, fruit plant$^{-1}$) when compared to seed-derived plants. From the result in (Table 4), data presented that number of (raceme plant$^{-1}$, flower raceme$^{-1}$, fruit raceme$^{-1}$, fruit plant$^{-1}$) which produced by seed-derived plants was significantly less than those derived from *in vitro* propagated plants. In the present study, tissue culture propagated tomatoes plants showed normal growth performance and continued their growth until flowering and fruiting. This stability in growth behavior may be due to the short growth cycle under the *in vitro* condition of this study as regenerates were exposed only to one subculture. The obtained results are in agreement with (Ali *et al.,* 2012; Wayase and Shitole, 2014), which showed that the increase in the number of subcultures enhances the rate of off-type plants. The present investigation demonstrated that a dedifferentiated propagation route via de novo shoots development in *Solanum lycopersicum* L. var. *cerasiforme* could be used for large scale multiplication and future genetic transformation techniques.

**Table (4):** Ex vitro reproductive growth performance of cherry tomato derived from in vitro regenerated hypocotyl, cotyledon and leaf disc or seedlings (after 12 weeks from transferring those plants from culture jars to the greenhouse)

| Growth Performance | Plantlet derived from Hypocotyls | | | | | | | | | | Mean | Seed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TDZ $mgl^{-1}$ | | | | | BA $mgl^{-1}$ | | | | | | |
| | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | | |
| **Leaf number $plant^{-1}$** | 35.8b | 36.1b | 35.0b | 35.5b | 43.8a | 36.0b | 36.2b | 36.1b | 35.9b | 35.4b | 36.6a | 36.7a |
| **Plant height cm.** | 160.0b | 164.0ab | 160.0b | 164.8ab | 166.0a | 167.0a | 166.0a | 166.1a | 165.8a | 163.0b | 164.3a | 165.0a |
| **No. of nodes to first flower** | 9.0ab | 10.6a | 10.0a | 10.0a | 10.0a | 9.5a | 9.8a | 9.9.0a | 10.0a | 10.0a | 10.0a | 10.0a |
| **No. of raceme $plant^{-}$** | 20.1ab | **20.8a** | 20.0ab | **21.0a** | **20.7a** | 20.0ab | 20.0ab | **20.6a** | 19.9ab | 20.0ab | 20.31a | 18.0b |
| **No. of flower $raceme^{-1}$** | 31.0b | 33.0a | 32.0ab | 33.4a | 34.8a | 32.0ab | 33.2a | 32.0ab | 32.0ab | 33.7a | 32.7a | 32.0ab |
| **No. of fruit $raceme^{-1}$** | 10.0ab | **11.0a** | **11.2a** | 10.0ab | 9.6ab | 8.7b | 10.0ab | 9.2b | 10.0ab | 10.0ab | 9.97a | 8.0b |
| **Total number of fruit $plant^{-1}$** | 139.0a | 139.0a | **140.0a** | 139.0a | 136.0ab | 134.0b | 139.0a | 138.0a | 137.0ab | **140.0a** | 138.1a | 130.0b |

| Growth Performance | Plantlet derived from Cotyledon | | | | | | | | | | Mean | Seed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TDZ $mgl^{-1}$ | | | | | BA $mgl^{-1}$ | | | | | | |
| | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | | |
| **Leaf number $plant^{-1}$** | 35.8a | 36.1a | 35.0a | 35.5a | 34.8ab | 36.0a | 36.2a | 36.1a | 35.9a | 35.4a | 35.7a | 36.7a |
| **Plant height cm.** | 166.0a | 164.0ab | 164.2ab | 164.8ab | 166.0a | 167.0a | 166.0a | 166.1a | 165.8a | 163.0ab | 165.3a | 165.0a |
| **No. of nodes to first flower** | 9.6a | 10.6a | 10.0a | 10.0a | 10.0a | 9.5a | 9.8a | 9.9a | 10.0a | 10.0a | 9.9a | 10.0a |
| **No. of raceme $plant^{-}$** | 19.1ab | 19.8a | 19.0ab | **20.0a** | 19.7ab | 19.0ab | 19.0ab | 19.6ab | 18.9ab | 19.0ab | 19.3a | 18.0b |
| **No. of flower $raceme^{-1}$** | 32.0ab | **34.0a** | 32.0ab | 33.4a | 34.8a | 32.0ab | 33.2a | 32.0ab | 33.0ab | 33.7a | 33.0a | 32.0ab |
| **No. of fruit $raceme^{-1}$** | 9.3ab | **10.5a** | **10.6a** | 9.5ab | 8.4ab | 7.9b | 9.9a | 9.2ab | **10.0a** | 9.0ab | 9.4a | 8.0b |
| **Total number of fruit $plant^{-1}$** | **140.0a** | **140.2a** | **140.0a** | 139.3a | 136.8ab | 134.9b | 139.5a | 138.7a | 137.5ab | **140.0a** | 140.0a | 130.0b |

| Growth Performance | Plantlet derived from Leaf disc | | | | | | | | | | Mean | Seed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TDZ $mgl^{-1}$ | | | | | BA $mgl^{-1}$ | | | | | | |
| | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | | |
| **Leaf number $plant^{-1}$** | 35.8a | 36.1a | 36.0a | 35.5a | 34.8ab | 36.0a | 36.2a | 36.1a | 36.9a | 35.4a | 35.9a | 36.7a |
| **Plant height cm.** | 165.0a | 164.8ab | 164.5ab | 164.9a | 165.5a | 166.0a | 166.1a | 165.8a | 166.0a | 164.0ab | 165.3a | 165.0a |
| **No. of nodes to first flower** | 10.0a | 10.2a | 10.0a | 10.0a | 10.0a | 10.0a | 9.9a | 9.7a | 10.0a | 10.0a | 10.0a | 10.0a |
| **No. of raceme $plant^{-}$** | 19.1ab | 19.8a | 19.0ab | **20.0a** | 19.7ab | 19.0ab | 19.0ab | 19.6ab | 18.9ab | 19.0ab | 19.3a | 18.0b |
| **No. of flower $raceme^{-1}$** | 32.0ab | **34.0a** | 32.0ab | 33.4a | 34.8a | 32.0ab | 33.2a | 32.0ab | 33.0ab | 33.7a | 33.0a | 32.0ab |
| **No. of fruit $raceme^{-1}$** | 9.3ab | **10.6a** | **10.0a** | 9.3ab | 7.4b | 8.9ab | **10.0a** | 9.2ab | **10.1a** | **10.4a** | 9.5a | 8.0b |
| **Total number of fruit $plant^{-1}$** | **140.0a** | **140.5a** | **140.7a** | 139.9a | 137.8ab | 134.5b | 138.5a | 139.7a | 140.5a | **142.0a** | 139.4a | 130.0b |

Values represent the mean SE. Means followed by the same letter within columns are not significantly different, according to Duncan's multiple range test ($P<0.05$). Best results are indicated in bold

## CONCLUSION

In conclusion, the results verified during the present study clearly suggest that cotyledon explant achieved from 10 - 12 day-old seedlings of cherry tomato are very important for efficient shoot regeneration. Also, the present study underlines the importance of inclusion of TDZ in cherry tomato regeneration media. Furthermore, the present callus induction regeneration system would be valuable for genetic transformation and also has considerable potential to study somaclonal variation as an alternative mean of conventional hybridization.

Cherry tomato plants derived from rooted plantlets of callus regenerated in vitro were morphologically true to type. This indicate the possibility of using this system for secure micropropagation of valuable genotypes or breeding lines and hybrids in short time which might accelerate the breeding efforts of tomato plants.

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# تأثير كل من مركب Thidiazuron والمنفصل النباتي على كفاءة الإكثار المعملي الكثيف لنباتات الطماطم الشيري

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تعتبر الطماطم الشيري من أهم محاصيل الخضر التي تزرع بغرض التصدير في مصر. تم تقييم قدرة الطماطم الشيري للإكثار المعملي وذلك باستخدام صنف Summer Cherry لقياس قدرتها على تخليق callus وكذلك على إنتاج النبتات معمليا". بعد ١٢ يوم من الزراعة لوحظ ظهور callus مباشرة في أماكن القطع للمنفصلات النباتية المستخدمة وهي السويقة الجنينية والأوراق الفلقيه وكذلك أجزاء الأوراق والتي تمت زراعتها قي بيئة MS تحتوي على تركيزات مختلفة من TDZ وكذلك BA في صور منفردة، لكن لم يتم زراعة المنفصلات النباتية على بيئة خاليه من منظمات النمو PGR. أظهرت النتائج أن أنسجة السويقة الجنينية أعطت أعلى دليل للـ callus ٣.٩ و٣.٧ وذلك عند الزراعة على بيئة MS تحتوي على تركيز TDZ(1.0 &2.0 $mgl^{-1}$) و كذلك قيمة دليل callus (٣.٥) على نفس المنفصل النباتي عند زراعته على بيئة MS تحتوي على تركيز BA(0.5 $mgl^{-1}$). بالرغم من ذلك كان أعلى نسبة callus لوحظ على أجزاء الأوراق (3.1) على بيئة MS تحتوي على BA (2.0 $mgl^{-1}$) . بعد ٨ أسابيع من زراعة المنفصلات النباتية ظهر التبرعم فقط على المنفصلات النباتية المزروعة في بيئة MS تحتوي على تركيزات مختلفة من BA ، TDZ . كانت أفضل نسبة سيقان خضرية تكونت (93%)من callus الناتج من أجزاء الأوراق والمنزرعة في بيئة تحتوي على TDZ. حيث كان أعلى عدد من السيقان المنبته لكل منفصل نباتي (13.4) ظهر من الـ callus (الأوراق الفلقيه) و التي كانت منزرعة على ببيئة تحتوي على TDZ(2.0 $mgl^{-1}$) . تعتبر البيئة النصف سائله أفضل بيئة لمرحلة التجذير بالإضافه إلى أن IAA بتركيز (1.0 $mgl^{-1}$) و IBA بتركيز(2.0 $mgl^{-1}$) لهم أهمية كبيرة في إنتاج اكبر عدد من الجذور (22.5) و كذلك اطول جذر(11.0 cm) بالترتيب. عملية الأقلمة للنباتات المستخرجة من المعمل بعد تكوين الجذور من أهم العمليات التي تجرى لدراسة قدرة النبات على النمو طبيعيا بعد خروجه من المعمل. لوحظ عدم وجود اختلافات مظهرية واضحة بين نباتات الطماطم الشيري المتكاثرة معمليا" من أ كثر من منفصل نباتي. كذلك تحت تأثير التركيزات المختلفة من TDZ و BA) عن النباتات الناتجة طبيعيا من البذرة. وبالرغم من ذلك وجد أن النباتات المنبته من البذور كانت اقل نسبيا" من حيث (عدد العناقيد الزهرية لكل نبات – عدد الأزهار لكل عنقود زهري - عدد الثمار لكل عنقود وكذلك عدد الثمار لكل نبات) بالمقارنة بالنباتات المستخرجة معمليا". يعتبر هذا البروتوكول مفيدا في برامج تحسين الصفات الوراثية وكذلك تطوير إنتاج الطماطم الشيري.