# New protocol of Tomato *(Lycopersicon esculentum* Mill.) in vitro propagation

**Nuevo protocolo de propagación invitro del tomate (*Lycopersicon esculentum Mill.*)**


**Author:**

**Gharbia H. Danial**[1]
**Diaa A. Ibrahim**[2]



**SCIENTIFIC RESEARCH**

**How to cite this paper:**
Danial, G.H. & Ibrahim D.A. New protocol of Tomato (*Lycopersicon esculentum* Mill.) in vitro propagation, Kurdistan, Iraq. Innovaciencia. 2018; 6(1): 1-13.
http://dx.doi.org/10.15649/2346075X.459

**Reception date:**
Received: 21 April 2018
Accepted: 20 Septembre 2018
Published: 28 December 2018.

**Keywords:**
in vitro propagation, tomato, BAP, Zeatin.



## ABSTRACT

**Introduction:** A successful *in vitro* propagation system was developed for Sandra and Rocky cultivars of tomato plants (*Lycopersicon esculentum* Mill.) commonly grown in Kurdistan Region of Iraq by *in vitro* culture of shoot tips and node explants. **Methods:** Shoot tip and node explants were excised and cultured on basal MS medium containing several concentrations of BAP and Zeatin. **Results:** Multiple shoots formation of up to 2 shoots were obtained on MS medium supplemented with 2.0 $mgl^{-1}$ Zeatin in node and shoot explants for the two cultivars. Microshoots were tested for root initiation on full MS medium supplemented with different concentrations of IBA, NAA and IAA. The best root formation was observed on a medium containing 1.0 mgl-1 IAA in Sandra cultivar and 0.5 mgl-1 of NAA in rocky cultivar. **Conclusion:** The regenerated plants were successfully acclimatized and transplanted to the open field conditions.



[1] Gharbia H. Danial, College of Science/ Scientific Research Center /Duhok University, gharbia.danial@uod.ac
[2] Diaa A. Ibrahim, College of Science/ Scientific Research Center /Duhok University, diaa.ibrahim@uod.ac.

## INTRODUCTION

Tomato (*Lycopersicone sculentum* Mill.) is one of the most widely grown warm season vegetable fruit. It is grown in almost every country of the world and is the highest quantity of both production and cultivation among vegetable crops [1]. Tomato is source for vitamins A and C and the fruit is rich in lycopene, one of the most powerful natural antioxidants [2]. The genus Lycopersicon includes many species which economically important and contain large numbers of genotypes [3]. According to its nutritional and commercial value, extensive research was reported and use it as a modal plant species for genetic studies, many of fruit development, disease interactions and physiological investigations were carried out due to its low chromosomal no i.e., 2n=2x=24 and a sequenced nuclear tomato genomemics nearly completion which would allow more focused studies in physiological responses [4,5].

Most tomatoes are transplanted to the field from greenhouse-grown plants because direct seeding tomatoes into the field is not recommended due to the high cost of hybrid seed and the specific conditions required for adequate germination. Furthermore, other disadvantages for direct seeding such as weed control is usually much more difficult with direct seeded than with transplanted tomatoes in addition well made seedbeds, specialize planting depth and delayed harvest are required [6].

The most important technique for mass propagation of tomato including shoot tip culture to regenerate multiple shoots [7, 8, 9, 10], direct organogenesis from hypocotyls and cotyledon leaves [11, 12, 13], adventious shoot formation indirectly through the callus formation [14, 15] and somatic embryogenesis [16, 17]. The present work aimed to describe an efficient protocol for micropropagation and root system induction of two tomato cultivars cultivated in Kurdistan Region of Iraq from nodes and shoots explant to become a powerful tool for future studies direct organogenesis and genetic transformation for these two cultivars.

## MATERIALS AND METHODS

This investigation was carried out at Plant Tissue Culture laboratory, Scientific Research Center, University of Duhok, Iraq, during the period from March, 2016 to May, 2017. The two cultivar seeds (cv. Sandra and Rocky) used in this study were obtained from Agriculture research station, Duhok Province, Kurdistan Region of Iraq. The seeds were surface sterilized using1%, 2%, 2.5%, 3% and 4% sodium hypochlorite of commercial bleach (Table 1) for 10 minutes. Seeds were rinsed 3 times with autoclaved Deinonized water, dried on sterilized Whatman filter papers, and then the seeds were germinated in Mason jars containing MS nutrient medium (Murashige and Skoog, 1962) supplemented with 100 $mgl^{-1}$ inositol and 20 $gl^{-1}$sucrose. The pH was adjusted to 5.7±0.1 with 1N HCl or NaOH and solidified with 7 $gl^{-1}$ agar. The cultures were incubated at 25±1°C and kept under 48 h of darkness then transferred into a 16/8 h light/dark photoperiodic regime (1000 lux). After four weeks, the grown seedlings were divided into shoot tips and node segments as explants then transferred and incubated in multiplication media. At multiplication stage, MS medium was supplemented with four different concentrations (0, 1, 2 and 4 $mgl^{-1}$) of either BA or Ziaten. At rooting stage, the experiment was applied to examine the impact of different concentrations (0.0, 0.5, 0.75, and 1.0 $mgl^{-1}$) of NAA, IBA and IAA in explants rooting. A total of 12 replicates were initiated for each treatment and the observations for both multiplication and rooting stages were recorded after 4 weeks of culture period. The experiment was designed as complete randomized design (CRD) and the comparison between

means were estimated according to Duncan's multiple range test ($P < 0.05$) using (SAS) computerized program. At acclimatization stage, numbers of successfully rooted plantlets were removed from culture vessels followed by washing the roots with distilled water and immersed in Benlate fungicide (0.1% for 10 minutes) followed by transferring them to pots containing a steam sterilized soil mixture (peat-moss+ loam+ Styrofoam 1:1:0.5, v:v:v) under tightly controlled atmosphere of the greenhouse.

## RESULTS AND DISCUSSION

### Seeds sterilization

Successful results were obtained during surface sterilization of tomato seeds germination by producing healthy seedlings (Table 1). The highest germination percent (100%) and lower contamination percent (zero) were shown after a week in culture when the seeds treated with 2% and 2.5% sodium hypochlorite. On the other hand, the results clarify that seed germination decreased with high concentrations of sodium hypochlorite (3% and 4%) via impress in seeds metabolism hence reduce the germination ability. After 4 weeks, shoot tips and nodes segments were cut with scalpel blade into explants of 1–1.5 cm before cultivation on multiplication medium.

### Effects of cytokinins on shoots and nodes multiplication

The results of Table 2 show the effects of different BAP concentration on shoots multiplication of tomato plant cv. Sandra. The data reveals that adding 2.0 $mgl^{-1}$ BAP recorded significantly highest number of branches per explants (1.4) as compared to the control and 4.0 $mgl^{-1}$ treatments, at the same time, it was insignificant with 1.0 $mgl^{-1}$ BAP (Fig. 1, A) whereas, the addition of different concentrations of BAP shows the highest number of leaves per explants which was insignificantly different among all treatments except the control. The highest length, highest average shoots length (6.4cm) in control treatment was reduced via increasing the BAP levels. Similar responses were found in both roots number and roots length through the reduction of the roots numbers (0.2, 1.9, and 4.1 root/ explant) by increasing the concentration of BAP respectively compare to the higher number of roots in control treatment (18.0), under the same conditions the average of roots length reduce to 0.2, 2.0 and 2.9 cm. compared with control (6.6cm).

On the other hand, the data at Table 3 concerning Rocky cultivar indicate that adding 4.0 $mgl^{-1}$ BAP significantly enhance the branches number / explant (2.3) and leaves number/ explant (7.7) compared to control treatment and insignificantly increase with other BAP treatments (Fig. 1, B). While highest length (5.1cm) and high average shoot length (5.1cm), roots number (8.1) and roots length average (4.4cm) were record in control then started to decrease insignificantly by the increments of BAP concentration (Fig. 1, c).

To evaluate the type of explants that used in this study for shoot proliferation, the results of Table 4 declare that node segments of Sandra cultivar enhance axillary shoot proliferation after 3-4 weeks in MS media supplemented with BAP (Fig. 1, D), however the number of new shoots varied according to BAP concentration which increased from 1.6 in control treatment to 1.8 shoots at the (1.0 and 4.0) $mgl^{-1}$ concentration although of were insignificant increase when compared to all other treatments. The highest shoot length and average shoot length was reduced with the increasing of BAP concentration. The minimal high shoot length (1.8cm) and average shoot length (1.4cm) was attained at the 4.0 $mgl^{-1}$

BAP level and differs significantly with control treatment with slight increase in leaves number/explants, although this increment wasn't always significant. On the other hand, adding of BAP cause significant decrease in the number of roots per explant as compared to the control. The highest number of roots (5.3) and average root length (4.9cm) was recorded in control treatment, whereas the lowest number and average root length was found in 2 $mgl^{-1}$ (1.0), (1.4 cm) respectively.

The effects of different BAP concentrations on nodes multiplication of Rocky cultivar tomato plants are shown in Table 5. It is quite clear that the presence of BAP is significantly affective due to the increase of the shoot numbers per explant as compared to the cytokinin -free medium (control) (Fig. 1, E). The highest number of shoots per explant (1.9) was recorded by the addition of 1.0 $mgl^{-1}$ BAP. At the same time, the presence of ABP reduce the high length of shoots, average shoot length, root number and average root length as compared to the control.

The slight increase of the shoots number with the presence of BAP (tables 2, 3, 4 and 5) which was not always significant compare to control treatment is may be due to the genotype and type of explants that used in this investigation that BAP is highly effective in the regeneration response of tomato [(11)]. The effect of different concentrations of Zeatin on Sandra tomato plants shoots multiplication is shown in Table 6. It is clear that the addition of Zeatin was significantly affective through the increase of the numbers of shoots per explants as compared to control treatment. The highest number of shoots per explants (2.1) was recorded on MS medium supplemented with 2.0 $mgl^{-1}$ Zeatin (Fig. 1, F). On the other hand, significant reduce takes place in the high length of shoots, average shoot length while simultaneously increase the leaves number/explants, however this increase was not always significant when compared to the control. Meanwhile, no root formation was seen by adding zeatin to the medium except 1.0 $mgl^{-1}$ Zeatin that cause slight increase in roots number.
Significant increase was shown in the number of shoots / explant in Rocky cultivar (Table 7). The highest number of shoots per explants (2.6) was recorded in MS medium supplemented with 2.0 $mgl^{-1}$ zeatin (Fig.1, G) and decrease in high shoot length and average shoot length compare with the control treatment, this reduction is may attributable to the competition on nutrient media. Simultaneously leave number/ explants increased and highest leaves number/ explants (7.3) were recorded on MS medium supplemented with 1.0 $mgl^{-1}$ zeatin.

The data on Table 8 clarify the effect of different Zeatin concentrations on shoot multiplication using node segments of Sandra cultivar. Adding 2.0$mgl^{-1}$ Zeatin significantly increase the highest number of branches per explants (2.4) compared with control, but did not show significant differences among the other Zeatin levels (Fig. 1, H). Whereas, the addition of 4.0 $mgl^{-1}$ Zeatin yielded the highest number of leaves (6.5) although of the insignificant increase compare with other treatments. High shoot length, average shoot length of branches, root number and average root length significantly decrease in medium supplement with different concentration of Zeatin.

The results on Table 9 announce that using the nodes of Rocky cultivar in different concentration of Zeatin caused significant increase in the number of shoots per explants as compared to the control treatment. The highest number of shoots per explants (2.0) was recorded by adding (1.0 or 4.0)

$mgl^{-1}$ Zeatin (Fig.1, J). Meanwhile, Zeatin reduce significantly the high length of shoots, average shoot length, root number and average root length as compared to the control.

**Effects of auxins on rooting**

Table 10 shows the effects of the three types of auxins investigated in this study at different concentrations on root formation in Sandra cultivar. In general, the obtained results confirm the need of auxins to increase the numbers of adventitious root formation in tomato plant. It is clear that all treatments, including the control, record the highest rooting percentage 100%, meanwhile, the presence of auxins had positive influences via increasing the rhizogenesis in tomato plant in vitro. Moreover, IAA was the most effective auxin in the rooting (Fig 2, A) followed by NAA (Fig 2, B) then IBA (Fig 2, C). The highest number of roots per explant (24.8, 23.5 and 20.7) was recorded, by adding 1.0 $mgl^{-1}$ IAA, NAA and IBA respectively to the nutrient medium and differs significantly from the control treatment as well as slight increase in the average root length for IAA treatments though of insignificant increase when compared to the control treatments. Such differences in the potency of auxin induce rooting might attributed to the structure of the auxins under study, the endogenous hormone level, as well as the genetic makeup of species under consideration (Maheswaran et al., 2000).

Regarding Rocky cultivar, the results (Table 1) revealed that the three tested auxins (NAA, IAA, and IBA) stimulate root initiation in *Lycopersicon esculentum* by increasing the rooting ratio 100% when compared with a control treatment (75 and 50 %) However, roots elongation varied according to auxin type and its concentration. NAA was the most effective auxin (Fig 2, D), followed by IAA (Fig 2, E) and IBA (Fig 2, F). Significant increase in roots number was enroll at the level 0.5 $mgl^{-1}$ of NAA and IAA which records 24.8 and 22.5 roots / explant in NAA and IAA respectively, while this increment wasn't significant by the inclusion of IBA through the rooting medium compared to the control treatments. The average length of root in control plants (7.3cm) was significantly higher than treated one, followed by significant reduction in all NAA treatment, while IAA rise insignificantly the average root length. Meanwhile, insignificant effect was observed by IBA in the average root length.

The role of auxins in rooting induction is widely known [13]. In this investigation, the best rooting was attained in the presence of IBA. This result is in agreement with others who worked on tomato [11, 18]. Other investigators suggested the inclusion of IAA for rooting [19]. In general the role of auxins in root inductions is by stimulation cell elongation upon rising cell wall elasticity followed by adventitious root formation [20].

The results in Table 10 and 11 clarify that exogenous application of auxins was not efficient for in vitro rhizogenesis of tomato plants in MS basal medium in both genotypes Sandra and Rocky due to the presence of high levels of endogenous auxins in this crop [21]. Plantlets were successfully transplanted in pots after adequate roots development, (Fig. 2, G and H), followed by transfer them to grow under field condition (Fig. 2, J).

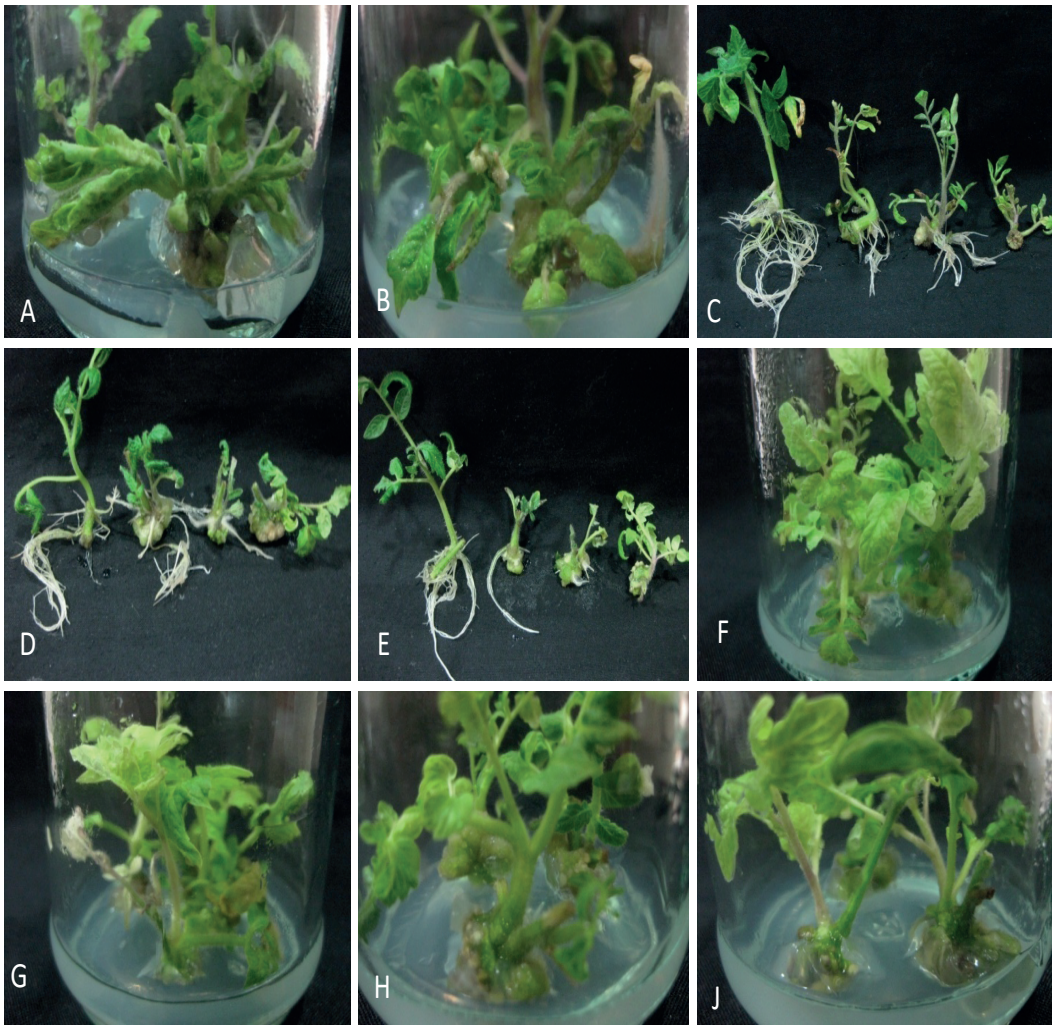


**Figure 1.** The effects of different cytokinins concentrations in tomato micropropagation.

**A.** Multiple shoots differentiation from shoot tips cv. Sandra culture in MS medium supplemented with 2mgl$^{-1}$ BA after four weeks culture.

**B.** Multiple shoots differentiation from shoot tips culture cv. Rocky in MS medium supplemented with 4mgl$^{-1}$ BA after four weeks culture.

**C.** The effects of different BA concentrations from left to right (0.0, 1.0, 2.0 and 4.0 mgl$^{-1}$) on root formation cv. Rocky shoot explants after four weeks culture.

**D.** The effects of different BA concentrations from left to right (0.0, 1.0, 2.0 and 4.0 mgl$^{-1}$) on multiplication cv. Sandra node explants after four weeks culture.

**E**. The effects of different BA concentrations from left to right (0.0, 1.0, 2.0 and 4.0 mgl$^{-1}$) on multiplication cv. Rocky node explants after four weeks culture.

**F.** Multiple shoots differentiation from shoot tips cv. Sandra culture in MS medium supplemented with 2mgl$^{-1}$ ziaten after four weeks culture.

**G.** Multiple shoots differentiation from shoot tips cv. Rocky culture in MS medium supplemented with 2mgl$^{-1}$ ziaten after four weeks culture.

**H.** Multiple shoots differentiation from node explants cv. Sandra culture in MS medium supplemented with 2mgl$^{-1}$ ziaten after four weeks culture.

**J.** Multiple shoots differentiation from node explants cv. Rocky culture in MS medium supplemented with 1mgl$^{-1}$ ziaten after four weeks culture.

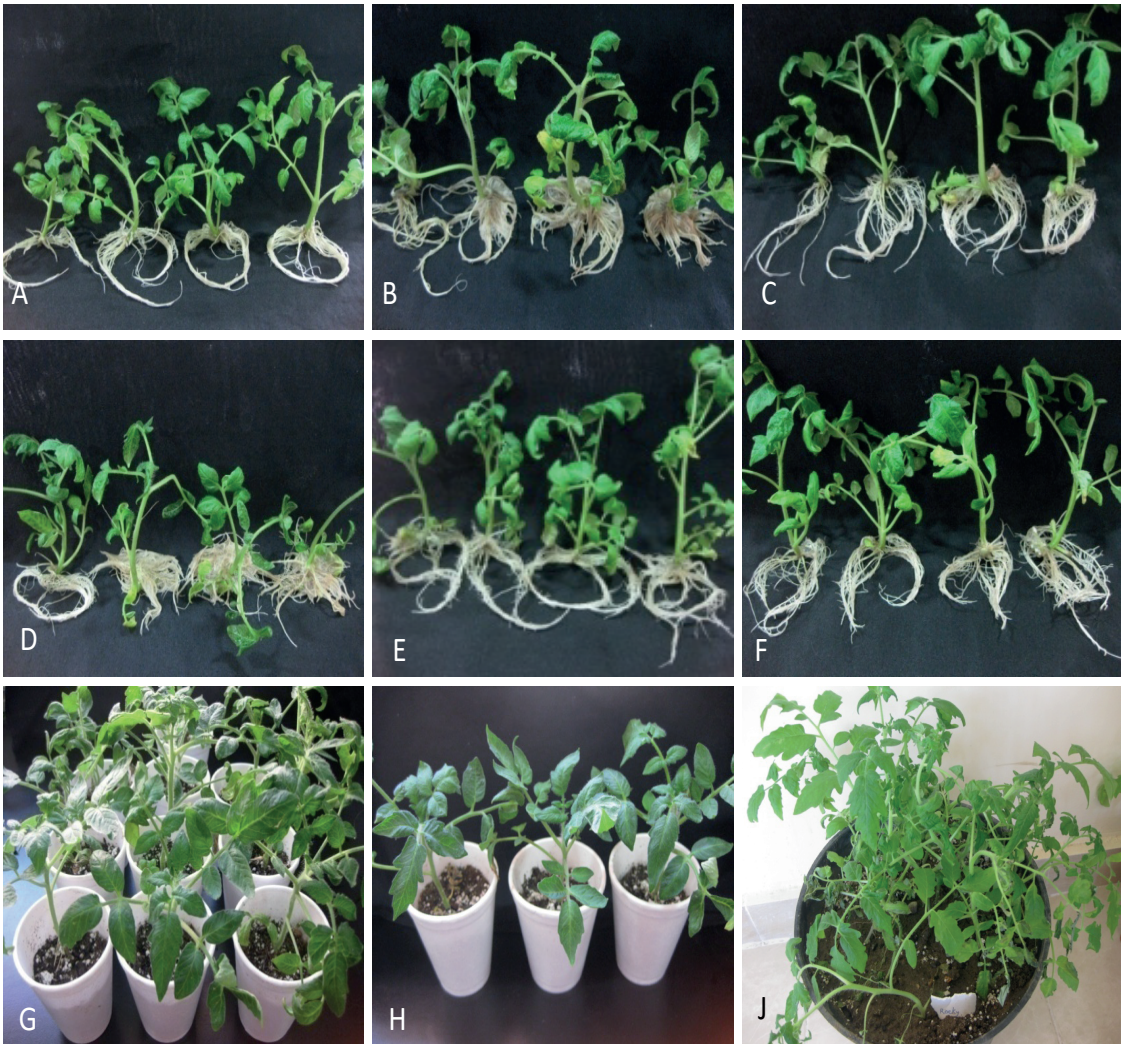


**Figure 2.** The effects of different auxins concentrations in tomato plants rooting and acclimatization.

**A**. The effects of different IAA concentrations from left to right (0.0, 0.5, 0.75 and 1.0 $mgl^{-1}$) on rooting Sandra cultivar plant shoots.
**B**. The effects of different NAA concentrations from left to right (0.0, 0.5, 0.75 and 1.0 $mgl^{-1}$) on rooting Sandra cultivar plant shoots.
**C**. The effects of different IBA concentrations from left to right (0.0, 0.5, 0.75 and 1.0 $mgl^{-1}$) on rooting Sandra cultivar plant shoots.
**D**. The effects of different NAA concentrations from left to right (0.0, 0.5, 0.75 and 1.0 $mgl^{-1}$) on rooting Rocky cultivar plant shoots.
**E**. The effects of different IAA concentrations from left to right (0.0, 0.5, 0.75 and 1.0 $mgl^{-1}$) on rooting Rocky cultivar plant shoots.
**F.** The effects of different IBA concentrations from left to right (0.0, 0.5, 0.75 and 1.0 $mgl^{-1}$) on rooting Rocky cultivar plant shoots.
**G**. Rocky cultivar acclimatized and well established plants.
**H.** Sandra cultivar acclimatized and well established plants.
**J.** Acclimated plants grown under field conditions.

**Table 1.** Effect of different concentrations of sodium hypochlorite on seeds sterilization of tomato plants after one week.

| Cultivars | 1% sodium hypochlorite | | 2% sodium hypochlorite | | 2.5% sodium hypochlorite | | 3% sodium hypochlorite | | 4% sodium hypochlorite | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cont. | Germ. | Cont. | Germ. | Cont. | Germ. | Cont. | Germ. | Cont. | Germ. |
| | % | % | % | % | % | % | % | % | % | % |
| Sandra | 0.0 | 80% | 0.0 | 100% | 0.0 | 90% | 0.0 | 100% | 0.0 | 100% |
| | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 90% |
| | 0.0 | 90% | 0.0 | 100% | 0.0 | 100% | 0.0 | 90% | 0.0 | 100% |
| | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% |
| Rocky | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 90% | 0.0 | 100% |
| | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 90% |
| | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 80% |
| | 0.0 | 90% | 0.0 | 100% | 0.0 | 100% | 0.0 | 100% | 0.0 | 90% |

Cont. = Contaminated percentage; Germ. = Germinated percentage

**Table 2.** Effect of BAP on shoots multiplication in tomato plants after four weeks in culture of Sandra cultivar.

| BA conc. mgl-1 | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 6.4 a ±0.2 | 1.0 a ±0.0 | 6.4 a ±0.2 | 4.7 a ±0.2 | 18.00 a ±0.5 | 6.6 a ±0.4 |
| **1.0** | 2.9 b ±0.3 | 1.3 ab ± 0.1 | 2.5 b ±0.1 | 6.9 b ±0.6 | 4.1 b ±1.7 | 2.9 b ±0.9 |
| **2.0** | 1.9 c ±0.08 | 1.4 b ±0.1 | 1.6 c ±0.1 | 6.6 b ±0.3 | 1.9 b ±0.7 | 2.00 bc ±0.7 |
| **4.0** | 1.8 c ±0.1 | 1.0 a ± 0.0 | 1.8 c ±0.1 | 6.4 b ±0.4 | 0.2 b ±0.2 | 0.2 c ±0.1 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 3.** Effect of BAP on shoot multiplication in Tomato after four weeks in culture on Rocky cultivar.

| BA conc. mgl-1 | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 5.1 a ±0.2 | 1.00 a ±0.0 | 5.1 a ±0.2 | 4.4 a ±0.4 | 8.1 a ±0.9 | 4.4 a ±0.7 |
| **1.0** | 1.7 b ±0.3 | 1.9 ab ±0.1 | 1.5 b ±0.4 | 6.3 ab ±0.7 | 1.4 b ±0.5 | 2.6 ab ±1.2 |
| **2.0** | 1.7 b ±0.1 | 1.7 ab ±0.2 | 1.5 b ±0.1 | 6.3 ab ±0.2 | 1.2 b ±0.4 | 1.3 ab ±0.6 |
| **4.0** | 1.6 b ±0.3 | 2.3 b ±0.9 | 1.3 b ±0.3 | 7.7 b ±1.00 | 0.1 b ±0.1 | 0.3 b ±0.2 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 4.** Effect of BAP on nodes segments multiplication in Tomato after four weeks in Sandra cultivar.

| BA conc. $mgl^{-1}$ | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 5.3 a ±0.6 | 1.6 a ±0.1 | 4.5 a ±0.1 | 4.6 a ±0.2 | 5.3 a ±1.4 | 4.9 a ±1.0 |
| **1.0** | 2.8 b ±0.3 | 1.8 a ±0.1 | 2.1 b ±0.2 | 4.4 a ±0.3 | 2.9 ab ±0.8 | 2.4 bc ±0.3 |
| **2.0** | 2.1 b ±0.3 | 1.7 a ±0.1 | 1.7 bc ±0.1 | 4.0 a ±0.3 | 1.00 b± 0.8 | 1.4 c ±0.7 |
| **4.0** | 1.8 b ±0.1 | 1.8 a ±0.3 | 1.4 c ±0.1 | 3.6 a ±0.3 | 0.0 b ±0.0 | 0.0 c ±0.0 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 5.** Effect of BAP on nods segments multiplication in Tomato after four weeks in Rocky cultivar.

| BA conc. $mgl^{-1}$ | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 7.0 a ±0.05 | 1.00 a ±0.0 | 7.1 a ±0.05 | 3.2 a ±0.4 | 8.9 a ±0.3 | 5.4 a ±0.5 |
| **1.0** | 3.1 a ±0.4 | 1.9 b ±0.1 | 2.5 b ±0.5 | 4.00 a ±0.2 | 3.1 b ±0.8 | 4.1 a ±0.5 |
| **2.0** | 2.1 b ±0.4 | 1.6 ab ±0.3 | 1.6 b ±0.2 | 3.22 a±0.6 | 2.2 bc ±1.1 | 1.3b ±0.6 |
| **4.0** | 1.6 b ±0.1 | 1.4 ab ±0.1 | 1.3 b ±0.1 | 2.9 a ±0.6 | 0.0 c ±0.0 | 0.0 b ±0.0 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 6.** Effect of Zeatin on shoots multiplication after four weeks in culture in Sandra cultivar.

| BA conc. $mgl^{-1}$ | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 6.4 a ±0.2 | 1.00 a ±0.0 | 6.4 a ±0.2 | 4.6 a ±0.1 | 17.9 a ±0.4 | 6.6 a ±0.5 |
| **1.0** | 3.4 b ±0.5 | 1.3 ac ±0.0 | 3.1 b ±0.4 | 5.5 a ±0.5 | 0.1 bc ±0.1 | 0.1 bc ±0.1 |
| **2.0** | 2.1 c ±0.5 | 2.1 bc ±0.1 | 1.5 c ±0.3 | 6.6 a ±0.6 | 0.0 b±0.0 | 0.0 b ±0.0 |
| **4.0** | 1.7 c ±0.1 | 1.7 c ±0.3 | 1.4 c ±0.1 | 5.3 a ±0.5 | 0.0 bc ±0.0 | 0.0 bc ±0.0 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 7.** Effect of Zeatin on shoots multiplication after four weeks in culture in Rocky cultivar.

| BA conc. $mgl^{-1}$ | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 5.1 a ±0.2 | 1.00 a ±0.0 | 5.1 a ±0.2 | 4.7 a ±0.1 | 8.1 a ±0.9 | 4.4 a ±0.7 |
| **1.0** | 3.6 b ±0.4 | 1.9 ab ±0.2 | 2.3 b ±0.4 | 7.3 b ±0.3 | 0.2 b ±0.2 | 0.2 b ±0.2 |
| **2.0** | 2.3 c ±0.1 | 2.6 b ±0.5 | 1.4 b ±0.1 | 6.8 b ±0.7 | 0.0 b ±0.0 | 0.0 b ±0.0 |
| **4.0** | 2.1 c ±0.1 | 2.1 ab ±0.1 | 1.3 b ±0.1 | 6.3 b ±0.3 | 0.0 b ±0.0 | 0.0 b ±0.0 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 8.** Effect of Zeatin on nodes segments multiplication after four weeks in culture in Sandra cultivar.

| BA conc. $mgl^{-1}$ | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 5.3 a ±0.6 | 1.6 a ±0.05 | 4.5 a ±0.1 | 4.6 a ±0.2 | 5.3 a 1.4 | 4.9 a ±1.0 |
| **1.0** | 2.6 b ±0.3 | 2.1 ab ±0.2 | 1.7 b ±0.1 | 5.4 a ±0.2 | 1.6 b 1.1 | 0.6 b ±0.3 |
| **2.0** | 1.6 b ±0.05 | 2.4 b ±0.1 | 1.1 c ±0.08 | 5.3 a ±0.3 | 0.3 c 0.3 | 0.1 c ±0.1 |
| **4.0** | 1.5 b± 0.07 | 2.1 ab ±0.1 | 1.1 c ±0.02 | 6.5 a ±1.0 | 0.1 c 0.1 | 0.1 bc ±0.1 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 9.** Effect of Zeatin on nods segments multiplication after four weeks in culture in Rocky cultivar.

| BA conc. $mgl^{-1}$ | High length(cm) | Numbers of branches /explant | Average shoot length (cm) | Numbers of leaves /explant | Numbers of roots /explant | Average root length (cm) |
|---|---|---|---|---|---|---|
| **0.0** | 7.1 a ±0.05 | 1.00 a ±0.0 | 7.1 a ±0.05 | 3.2 a ±0.4 | 8.9 a ±0.3 | 5.6 a ±0.6 |
| **1.0** | 1.8 b ±0.2 | 2.00 b ±0.2 | 1.2 b ±0.2 | 4.3 ab ±0.5 | 0.4 b ±0.4 | 0.1 b ±0.1 |
| **2.0** | 1.8 b ±0.2 | 1.8 b ±0.1 | 1.4 b ±0.2 | 5.2 b ±0.2 | 0.4 b ±0.3 | 0.2 b ±0.09 |
| **4.0** | 1.5 b ±0.1 | 2.00 b ±0.0 | 1.3 b ±0.2 | 5.7 b ±0.3 | 0.0 b ±0.0 | 0.0 b ±0.0 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 10.** Effect different concentrations of Auxins on rooting of Sandra cultivar after four weeks in culture.

| Auxin | NAA | | | IAA | | | IBA | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. mgl-1 | % Rooting | Root s number | Average root length (cm) | % Rooting | Root s number | Average root length (cm) | % Rooting | Root s number | Average root length (cm) |
| **0.0** | 100 | 12.2 a ± 1.7 | 5.8 a±0.9 | 100 | 12.83 a ±0.4 | 6.8 a ±0.2 | 100 | 12.8 a ± 0.2 | 7.0 a ± 0.3 |
| **0.5** | 100 | 20.7 b ±0.3 | 5.5 a ±0.1 | 100 | 20.67 b ±0.9 | 7.3 a ± 0.6 | 100 | 15.8 a ± 0.6 | 6.5 a ± 0.5 |
| **0.75** | 100 | 21.7b ±0.7 | 5.0 a ±0.5 | 100 | 20.3 b ±0.3 | 6.4 a ± 0.2 | 100 | 15.3 a ±0.7 | 7.0 a ± 0.0 |
| **1.0** | 100 | 23.5 b ±0.0 | 5.1 a ±0.2 | 100 | 24.8 c ±1.8 | 7.4 a ± 0.1 | 100 | 20.7 b ± 1.4 | 6.8 a ± 0.9 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

**Table 11.** Effect of different types and concentration of Auxins in Rocky cultivar rooting

| Auxin | NAA | | | IAA | | | IBA | | |
|---|---|---|---|---|---|---|---|---|---|
| Con. mgl-1 | % Rooting | Root No. | Average root length(cm) | % Rooting | Root No. | Average root length(cm) | % Rooting | Root No. | Average root length(cm) |
| 0.0 | 75 | 13.7 a± 1.4 | 7.3a±0.4 | 50 | 8.3 a±0.6 | 5.5 a±1.2 | 75 | 14.5 a ±1.0 | 7.3 ab±0.1 |
| 0.5 | 100 | 24.8 b ±1.8 | 4.8b±0.4 | 100 | 22.5c ±0.6 | 6.8 a ±0.0 | 100 | 17.5 a ±1.5 | 8.0 b ±0.3 |
| 0.75 | 100 | 19.3 ab±3 | 4.2b ±0.2 | 100 | 19.5bc±0.0 | 7.7 a ±0.5 | 100 | 18.5 a±1.8 | 6.1 a ± 0.1 |
| 1.0 | 100 | 23.5b ±0.5 | 4.8b ±0.08 | 100 | 20.83c ± 0.8 | 7.08 a±0.3 | 100 | 18.83 a±1.0 | 7.0 ab ±0.5 |

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

## DISCUSSION

The reason of using sodium hypochlorite in this study as one of the compound that widely used for seeds surface sterilizing in plant tissue culture is due to its high efficiency for completely remove of fungi and bacteria from the seeds [22].

According to the previous results of Tables (6, 7, 8, and 9) it is clear that zeatin stimulated and induced shoot proliferation in *Lycopersicon esculentum* Mill. of Sandra and Rocky cultivars using both types of explants as compared with BAP treatments. Similar observations has been reported for the role zeatin in direct regeneration for different explants and cultivars in tomato plants [23, 24, 25]. This effect is may due to the Zeatin role in cell division in plant tissue culture in addition of some physiological activates such as RNA and protein synthesis and some enzymes activates [26, 27].

The role of auxins in rooting induction is widely known [13]. In this investigation, the best rooting was attained in the presence of IBA. This result is in agreement with others who worked on tomato [11, 18].

Other investigators suggested the inclusion of IAA for rooting [19]. In general the role of auxins in root inductions is by stimulation cell elongation upon rising cell wall elasticity followed by adventitious root formation [20].

The results in Table (10) and (11) clarify that exogenous application of auxins was not efficient for in vitro rhizogenesis of tomato plants in MS basal medium in both genotypes Sandra and Rocky due to the presence of high levels of endogenous auxins in this crop [21]. Plantlets were successfully transplanted in pots after adequate roots development, (Fig. 2, G and H), followed by transfer them to grow under field condition (Fig. 2, J).

## CONCLUSIONS

A successful *in vitro* propagation system was developed for Sandra and Rocky cultivars of tomato plants (*Lycopersicon esculentum* Mill.) commonly grown in Kurdistan Region of Iraq by *in vitro* culture of shoot tips and node explants. Multiple shoots formation were obtained on MS medium supplemented with 2.0 $mgl^{-1}$ Zeatin in node and shoot explants for the two cultivars. Microshoots were rooted on full

MS medium supplemented with different concentrations of IBA, NAA and IAA. The regenerated plants were successfully acclimatized and transplanted to the open field conditions.

## REFERENCES


1. Food and Agricultural Organization of the United Nations (FAO), 2008, http://faostat.fao.org.
2. Bhatia P, Ashwath N, Senaratna T, Midmore D. Tissue culture studies of tomato (Lycopersicon escuelentum). *Plant Cell Tissue Organ Cult* 2004; 78:1-21. https://doi.org/10.1023/B:TICU.0000020430.08558.6e
3. Gunay A L and Rao P S. In vitro Propagation of Hybrid Tomato Plants (*Lycopersicon esculentum* L.) Using Hypocotyl and Cotyledon Expiants. *Ann.Bot.* 1980; 45, 205-207.
4. Porter J E. Analysis of Tomato Synthetic Seeds for the Development of an Optimized Encapsulation System. Ph.D Dissertation in Horticulture. West Virginia University. Davis College of Agriculture, Forestry and Consumer Sciences .U.S.A. 2008.
5. Chaudhry Z, Abbas A Y, Rashid H, Ahmed H, and Anjum M A. Tissue culture studies in tomato (Lycopersicon esculentum) var. moneymaker. *Pak. J. Bot.*, 2010; 42(1): 155-163.
6. Kelley W T and Boyhan G. Commercial tomato production handbook, Published by the University of Georgia in cooperation with Fort Valley State University, the U.S. Department of Agriculture and counties of the state, 2017.
7. Padliskikh V L and Yarmishin A P. Features of microclonal propagation in tomato. Vestsi Akademii Navuk BSS, Reryya Biyalagichnykh Navuk 1990; 6: 52–54.
8. Fari M, Szasz A, Mityko J, Nagy I, Csanyi M, and Andrasfalvy A. Induced rganogenesis via the seedling decapitation method (SDM) in three solanaceous vegetable species. *Capsicum Newsletter* 1992; 243–248.
9. Izadpanah M and Khosh-Khui M. Comparisons of in vitro propagation of tomato cultivars. *Iran Agric. Res.* 1992; 8: 37-47.
10. Jehan S and Hassanein A M. Hormonal requirements trigger different organogenic pathways on tomato nodal explants, *American Journal of Plant Sciences* 2013; 4: 2118-2125. https://doi.org/10.4236/ajps.2013.411263
11. Wayase U R and Shitole M G. Effect of plant growth regulators on organogenesis in tomato (*Lycopersicon esculentum* Mill.) cv.Dhanashri. Int. J. *Pure Appl. Sci. Technol.*, 2014; 20(2): 65-71.
12. Kalyani B G and Rao S. Zeatin induced direct plant regeneration from cotyledon explants of cultivated tomato (*Lycopersicon esculentum* Mill.). *World Journal of Pharmacy and Pharmaceutical Sciences* 2014; 3: 1034-1040.
13. Kauser N, Khan S, Mohammadi A, Ghareyazil B, Uliaie E D, and Darvishrohani B. Agrobactrium mediated transformation and direct shoot regeneration in Iranian Tomato (Solanum lycopersicum L.) cultivar Falat-CH.Pak. *J. Bot.,* 2016; 48(6): 2489-2498.
14. Rashid R., Bhat J A, Bhat Z A, Dar W A, and Shafi W. Callus formation and organogenesis of tomato (*Solanum lycopersicum* L.). *Vegetos-An International Journal of Plant Research*, 2012; 25(2):243-248.
15. Hanur V S and Krishnareddy B. In vitro organogenesis in tomato (*Solanum lycopersicum*) using kinetin. *Advances in Plants and Agriculture Research*, 2016; 4(6):00158. https://doi.org/10.15406/apar.2016.04.00158
16. Rzepka-Plevneš D, Kulpa D and Kurek J. Somatic embryogenesis in callus cultures of tomato Lycopersicon peruvianum (L.) MILL. *Zeszyty Problemowe Postepow Nauk Rolniczych z.* 2007; 523: 185-193.
17. Ammara U, Al-Maskri A Y, Jamal Khan A, and Al-Sadi A M. Enhanced Somatic Embryogenesis and *Agrobacterium*-Mediated Transformation of three Cultivars of Tomato by Exogenous Application of Putrescine. *Int. J. Agric. Biol.,* 2014; 16 (1): 81-88.
18. Manawadu I, Nilanthi D, and Senanayake S. Callus formation and organogenesis of Tomato (*Lycopersicom esculentum* Mill. Variety Thilina). *Tropical Agriculture Research and Extension* 2014; 17(2): 86-94. https://doi.org/10.4038/tare.v17i2.5311
19. Gerszberg A, Hnatuszko- Konka K., Kowalczyk T, and Kononowicz A K. Efficient In vitro callus induction and plant regeneration protocol for different Polish tomato cultivars. *Not. Bot. Horti. Agrobo* 2016; 44 (2):452-458. https://doi.org/10.15835/nbha44210530
20. Damiano C, Arias P., La starza S R, and Frattarelli A. Temporary immersion system for temperate fruit trees. *Acta Hortic.* 2007; 748:87-90. https://doi.org/10.17660/ActaHortic.2007.748.7
21. Rashid R and Bal S. Effect of hormones on direct regeneration in hypocotyls explants of Tomato. *Not. Sci. Biol.* 2010; 2(1):70-73. https://doi.org/10.15835/nsb213556

22. Abdul-Baki A A. Short communication hypochlorite and tissue sterilization. *Planta* 1974; 115: 373-376. https://doi.org/10.1007/BF00388620
23. Gubi J, Lajchovál Z, Faragó J, and Jureková Z. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). *Biologia Bratislava* 2004; 59(3): 405- 408.
24. Sarwarkhan M, Usman M, and Ilya Slilla M. Facile plant regeneration from tomato leaves induced with spectinomycin. *Pak. J. Bot.*, 2006; 38(4): 947-952.
25. Pawer B D, Jadav A S, Kale A A, Chimote V P, and Pawer S V. Zeatin induced direct in vitro shoot regeneration in tomato (*Solanum lycopersicum* L.). *NEA The biosean* 2012; 7(2): 247-250.
26. Genkov T, Ivanov I, and Ivanova I. Analysis of cytokinins by immune assay and high performance liquid chromatography of in vitro cultivated Dianthus caryophyllus. *Bulg. J. Plant Physiol.*, 1996; 22(3–4), 95–104.
27. Debergh P C, Zimmerman R H. Micropropagation technology and application: Kluwer Academic Publishers. Dordecht the Netherlands; 1991.W https://doi.org/10.1007/978-94-009-2075-0