# Employment of in vitro and Gamma mutation for micropropagation of Golden Sunrise cherry tomato (Solanum lycopersicum var cerasiforme)

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## Abstract

An experiment was performed at the laboratory of tissue culture to study the effect of different plant growth hormones on callus induction from different seedling explants of Golden Sunrise cherry tomato. Seeds were irradiated with (0, 20 or 40 Gy) of gamma ray, and germinated on MS medium. Two weeks later, shoot tips, hypocotyls, cotyledon leaves and true leaves were separated from seedlings and cultured on MS medium supplemented with different combinations of plant growth hormones. Factorial completely randomized design (C.R.D) experiments were setup. Seeds germination%, seedling height (cm), day to germinate, callus induction from different explants% beside callus fresh weight (mg) were recorded. Results for all parameters were significantly influenced by different factors. 88% and 100 % of cherry tomato seeds germinated at 20 and 40 Gy respectively. Beside, significant decreasing pattern with seedling height (3.32 cm) was recorded at 40 Gy. However, when callus induced from different explants, variance responses were found and among all growth hormones combinations media containing (2 BAP + 1.75 kin and 2.0 kin + 2.0 IAA mg. l-1) with all radiation doses (0, 20 and 40) were superior in giving the highest response rate of 100% for all explants excised from cherry tomato seedling also results indicated the superiority of the growth hormonal combinations (2.0 kin + 2.0 IAA mg. l-1) with 20 Gy in producing highest callus FW (0.393 mg) for cotyledon leaves and hypocotyls explants beside (0.384 mg) at 40 Gy for callus produced from shoot tips and no significant differences were found among those treatments.

**Keyword:** *Cherry tomato, In Vitro, Gamma, Callus, Kin, IAA, BAP, Explants, FW.*

## INTRODUCTION

Tomatoes (Solanum lycopersicum L.) were imported from the Andes to Europe in the 16th century. At Nowadays, this plant is popular all over the world, and it has become an economically important crop. (Gerszberg et al. 2015). Tomatoes belong to the Solanaceae family, and are a perennial crop (FAOSTAT,2019) with 2,800 species (Lahoz, 2016). They contain 2.5 % total sugars, 94 % water, 2 % total fiber, and 1% protein, as well as other nutrients such as fats, amino acids, and carotenoids (Koh et al, 2012, Razdan and Mattoo, 2007) .Tomatoes also include bioactive components such as phenols and vitamins C, which are anti-cancer (Vinha et al,

2014). B-carotene and lycopene are mostly found in tomatoes (Rao and Rao, 2007).

Plant tissue culture is regarded as a valuable biotechnological technique for scientific research, particularly for the production of undifferentiated callus cells and regeneration via suspension or static media.( Mutasher and Attiya,2019).

There are evidences that the use of low doses of gamma radiation can stimulate germination and plant production. (Franco,et al,2015). Ionizing radiation from a gamma ray generates biological consequences such as inhibition, stimulation, mutation, and cell death. Researchers from all around the world have documented the impact of gamma radiation on agricultural productivity (Ali, et al., 2015; Mokobia and Anomohanran, 2005; Alwan et al,2016).

A little dosage of gamma radiation can improve seedling germination and development. Beyaz et al. (2016) reported on the stimulatory effect of modest gamma doses on seedling germination and growth. Wiendl et al.2013) (discovered evidence that modest doses of gamma radiation can boost tomato plant germination and production.

The purpose of this project is to improve a possible method for increasing tomato germination using gamma radiation and study the effect of different plant growth regulators on callus induction from different tomato seedling explants in vitro.

**Materials and methods:**

The study was performance at the laboratory of tissue culture/ Department of Genetic Engineering/ Food and Biotechnology Center/ Ministry of Sciences and Technology/ Baghdad/ Iraq, during the years 2021-2022.

Plant materials:

Mature seeds of cherry tomato (Solanum lycopersicum var cerasiforme) cv. (Golden Sunrise) were bought from a local market and irradiated with three doses of Gamma radiation (0, 20 and 40) Gy, using cobalt -60 source (CO60 ) dosage rate of 2Gy/sec, at the physics department/college of science/Baghdad university.

Seeds sterilization in vitro:

Irradiated and non irradiated cherry tomato 's seeds were surface sterilized with 90% ethanol for 30 sec followed by 4.2 % v/v of sodium hypochlorite (6% NaOCl) whereas diluted in sterilization double-distilled deionized water (D.D.W) to obtain 4.2 %) volume.

Seeds culture conditions:

Sterilization seeds were cultured on (Murashige and Skoog, 1962) MS basal medium supplemented with vitamins and organic compounds listed in (table 1). The pH medium was adjusted to 5.72 before autoclaving. 10 seeds (Fig1A) were culture in glass jars with three replicates for each treatment. The cultures were kept in a controlled growth chamber at 25±2°C with a light photoperiod of 18/6 darkness for two weeks,seeds germination % , seedling height (cm) and day to germinate were recorded.

**Table1: Media MS basal with organic components (mg.l$^{-1}$)**

| | |
|---|---|
| MS salts | 4400 |
| Pyridoxine | 0.5 |
| Nicotinc acid | 0.5 |
| Thiamine- HCl | 0.1 |
| Myo-insitol | 100 |
| Glycine | 2.0 |
| Sucrose | 30000 |
| Agar | 7000 |

In vitro Effect of plant hormones on callus induction from different seedling explants:

An experiment was conducted to test the effectiveness of plant growth hormones depending on previous citation (table 2). In this experiment, callus induction from different explants namely(Shoot tips, hypocotyls, cotyledons and true leaves) which were excised from 2 weeks old seedling of cherry tomato and cultured horizontally in Petri dishes (Fig 1B,C,D,E,F)filled with MS medium. One month later, callus induction% and callus fresh weight was recorded. The experiment was designed as a factorial in completely randomized (C.R.D). In the presence of an interaction among three factors: the three levels of radiation doses with 5 hormonal combinations, in the presence of the 4 different explants resulting from cherry tomato seedlings grown from mutagenic and non-mutagenic seeds. After 30 days of culture, callus induction % was recorded based on the following formula (Abd El-Hammeid et al, 2016). Data were analyzed statically using GenStat softwere (12ed) and means were compared based on Duncan's multiple range test at 5% level.

$$\text{Callus induction \%} = \frac{\text{Number of explants induced callus}}{\text{Total number of explants cultured}} \times 100$$

**Table 2: Different plant growth hormones concentrations mg.l$^{-1}$ for callus induction from different explants**

| References | Plant growth hormones mg.l$^{-1}$ | Type of explants |
|---|---|---|
| Abdelraheem *et al*,2007 | 2 BAP + 0.2 NAA | Cotyledon leaves, hypocotyls |
| ElKaaby *et al*, 2015 | 2.0 kin + 2.0 IAA | Cotyledon leaves, hypocotyls, epicotyls |
| Hasan *et al*, 2021 | 1 BAP+ 1 NAA | leaves, stemes |
| Hanur and Krishnareddy,2016 | 2 BAP + 1.75 kin | Cotyledon leaves, hypocotyls |

BAP= Benzyl amino purine , Kin= Kinetin IAA= Indole acetic acid, NAA= Naphthalene acetic acid

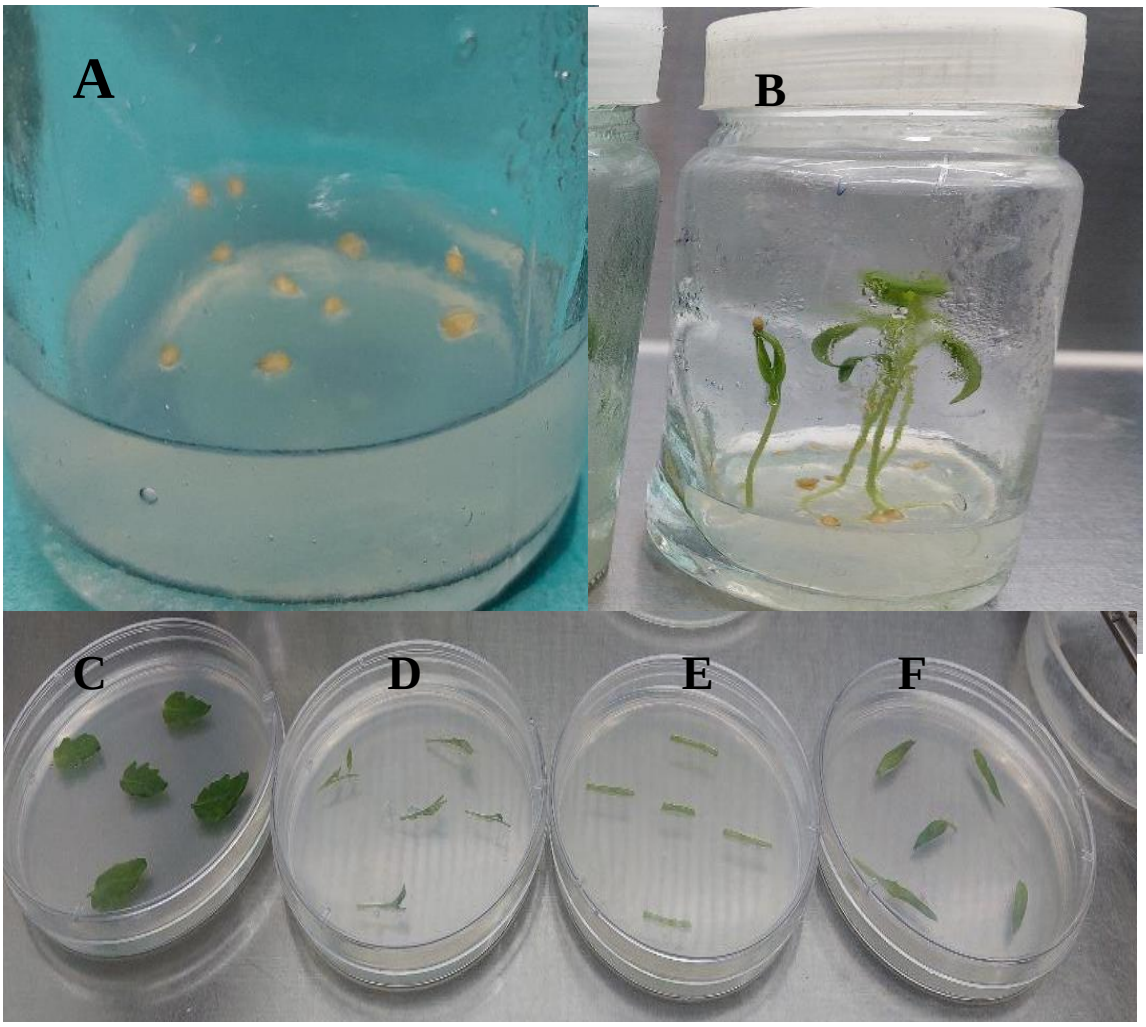



**Fig 1. A: Cherry tomato seeds germinated on MS medium (B) seedling 14 day age (C) true leaves (D) shoot tips (E) hypocotyls (F) cotyledon leaves explants separated from seedlings**

## Results and discussion

Effect of gamma doses on seeds germination % , seedling height (cm) and day to germinate is shown in (table 3). After two weeks, 88% and 100 % of cherry tomato seeds germinated at 20 and 40 Gy respectively and no significant differences were found between both doses comparing to 68% of germination for untreated seeds (0). Concerning to seedling height, data in (table 3) revealed significant increasing pattern with highest seedling height (5.38 cm) at control treatment compare to (4.18 cm, 3.32) cm at 20 and 40 Gy respectively. Furthermore, gamma radiation had a positive effect on day to germination whereas seeds irradiated with 40 Gy germinated in 5.20 day while 20 and 0 Gy germinated more than 8.20 and 9.80 days respectively.

**Table 3: Effect of gamma doses on seeds germination % , seedling height (cm) and day to germinate**

| Gamma doses Gy | Seeds germination % | seedling height (cm) | Day to germinate |
|---|---|---|---|
| 0 | 68 b | 5.38 a | 9.80 c |
| 20 | 88 a | 4.18 b | 8.20 b |
| 40 | 100 a | 3.32 c | 5.20 a |

* Means followed by the different letters are significantly differed from each other within the same column at 5% level according to Duncan's multiple range test.

Effect of plant growth hormones on callus induction % from different seedling explants:

Based on data recorded on the effect of different plant hormones on callus induction % (table 4A) , (2 BAP + 1.75 kin and 2.0 kin + 2.0 IAA mg. l-1 ) produced 100% for callus induction while media free hormones (0) failed to produce callus. For interaction between different plant hormones combinations and response of different explants, it was noted in the same table cotyledon, hypocotyls, leaves and shoot tips were superior in giving 100% of callus induction in media contain (2 BAP + 1.75 kin and 2.0 kin + 2.0 IAA mg. l-1 ) and shoot tips in the presence of (1 BAP + 1 NAA mg. l-1 ) respectively. Regarding the influence of individual factors, the results in (Table 4B) indicated that 40 Gy had a significant increase on callus induction with average 73.67 % compare to 69.67 % and 61% at 20 and 0 Gy respectively with superiority for shoot tips with average 74.67 % as compare with other explants. While the results of the interaction between radiation doses and the response of different explants, table (4B) showed the superiority of 20 Gy for shoot tips with 76% and 40 Gy with 73.33 % for cotyledon, hypocotyls and shoot tips respectively. As for the interaction between radiation doses and different hormonal

**Table (4A): effect of hormones treatments and type of explants on callus induction% from cherry tomato**

| hormones Treatments mg. $l^{-1}$ | hormones Treatments X Explants | | | | Treatments Mean |
|---|---|---|---|---|---|
| | cotyledon | hypocotyls | leaves | shoot tip | |
| 0 | 0 e | 0 e | 0 e | 0 e | 0 d |
| 1 BAP + 1 NAA | 62.22 c | 53.33 d | 60 c | 100 a | 68.89 c |
| 2 BAP + 0.2 NAA | 75.56 b | 75.56 b | 62.22 c | 73.33 b | 71.67 b |
| 2 BAP + 1.75 kin | 100 a | 100 a | 100 a | 100 a | 100 a |
| 2.0 kin + 2.0 IAA | 100 a | 100 a | 100 a | 100 a | 100 a |
| 4B | Gamma doses X Explants | | | | |
| Gamma doses Gy | cotyledon | hypocotyls | leaves | shoot tip | Gamma Mean |

| | | | | | |
|---|---|---|---|---|---|
| 0 | 60 d | 57.33 d | 56 d | 70.67 bc | 61 c |
| 20 | 69.33 bc | 66.67 c | 66.67 c | 76 a | 69.67 b |
| 40 | 73.33 ab | 73.33 ab | 70.67 bc | 77.33 a | 73.67 a |
| Explants Mean | 67.56 b | 65.78 bc | 64.44 c | 74.67 a | |
| 4 C Gamma doses Gy | Gamma doses X hormones Treatments | | | | |
| | 0 | 1 BAP + 1 NAA | 2 BAP + 0.2 NAA | 2 BAP + 1.75 kin | 2.0 kin + 2.0 IAA |
| 0 | 0 g | 46.67 f | 58.33 e | 100 a | 100 a |
| 20 | 0 g | 68.33 d | 80 c | 100 a | 100 a |
| 40 | 0 g | 91.67 b | 76.67 c | 100 a | 100 a |

* Means followed by the different letters within single or two variants are significantly differed from each other 5% level according to Duncan's multiple range test

**Table 4 D: In vitro effect of interaction among Gamma radiation, hormones treatments and Type of explants on callus induction % from cherry tomato**

| Gamma (Gy) | hormons Treatments $mg.l^{-1}$ | **Type of explants** | | | |
|---|---|---|---|---|---|
| | | **cotyledon** | **hypocotyls** | **leaves** | **shoot tip** |
| 0 | 0 | 0 i | 0 i | 0 i | 0 i |
| | 1 BAP + 1 NAA | 26.67 h | 20 h | 40 g | 100 a |
| | 2 BAP + 0.2 NAA | 73.33 de | 66.67 e | 40 g | 53.33 f |
| | 2 BAP + 1.75 kin | 100 a | 100 a | 100 a | 100 a |
| | 2.0 kin + 2.0 IAA | 100 a | 100 a | 100 a | 100 a |
| 20 | 0 | 0 i | 0 i | 0 i | 0 i |
| | 1 BAP + 1 NAA | 66.67 e | 53.33 f | 53.33 f | 100 a |
| | 2 BAP + 0.2 NAA | 80 cd | 80 cd | 80 cd | 80 cd |
| | 2 BAP + 1.75 kin | 100 a | 100 a | 100 a | 100 a |
| | 2.0 kin + 2.0 IAA | 100 a | 100 a | 100 a | 100 a |
| 40 | 0 | 0 i | 0 i | 0 i | 0 i |
| | 1 BAP + 1 NAA | 93.33 ab | 86.67 bc | 86.67 bc | 100 a |
| | 2 BAP + 0.2 NAA | 73.33 de | 80 cd | 66.67 e | 86.67 bc |
| | 2 BAP + 1.75 kin | 100 a | 100 a | 100 a | 100 a |
| | 2.0 kin + 2.0 IAA | 100 a | 100 a | 100 a | 100 a |

* Means followed by the different letters are significantly differed from each other within the three interactions at 5% level according to Duncan's multiple range test

Effect of plant growth hormones on callus fresh weight (mg) from different seedling explants

Results in (table 5A) showed that combination of (2.0 kin + 2.0 IAA mg. l-1 ) was superior to produced maximum callus FW reach (0.29 mg). For interaction between plant hormones combinations and response of different explants, data revealed that leaves, cotyledons and hypocotyls were superior in

giving (0.30, 0.29 and 0.29 mg) callus FW respectively in media contain (2.0 kin + 2.0 IAA mg. l-1 ). Regarding the influence of individual factors, the results in (Table 5B) indicated that 40 Gy had a significant increase in callus FW with average 0.21 mg compare to 0.20 and 0.11 mg at 20 and 0 Gy respectively with superiority of 0.18 mg for callus produced from cotyledons and hypocotyls and no significant differences was found between both explants compare with minimum average of callus FW (0.17 mg) which produced from shoot tips explants. Interaction between gamma doses and the response of different explants, data in table (5B) showed the superiority of 40 Gy in producing (0.226, 0.223, 0.217mg) callus FW for cotyledon leaves, hypocotyls and shoot tips respectively and (0.220 mg) callus FW which produced from cotyledon leaves at 20 Gy and no significant differences were fond among these treatments. As for the interaction between radiation doses and different hormonal combinations, the results in (table 5C) showed superiority of ( 2.0 kin + 2.0 IAA mg. l-1 ) in giving 0.362 mg of callus FW at 20 Gy . Concerning to the interaction among gamma doses, hormones treatments and type of explants. Results in (table 5D) indicated the superiority of the hormonal combinations (2.0 kin + 2.0 IAA mg. l-1) with 20 Gy in producing highest callus FW (0.393 mg) for cotyledon leaves and hypocotyls explants in beside (0.384mg) at 40 Gy for callus produced from shoot tips and no significant differences were found among those treatments.

**Table (5A) : effect of hormones treatments and type of explants on callus fresh weight (mg) from cherry tomato**

| **hormones Treatments mg. $l^{-1}$** | **hormones Treatments X Explants** | | | | **Treatments Mean** |
|---|---|---|---|---|---|
| | **cotyledon** | **hypocotyls** | **Leaves** | **shoot tip** | |
| 0 | 0 i | 0 i | 0 i | 0 i | 0 e |
| 1 BAP + 1 NAA | 0.214 e | 0.222 de | 0.156 h | 0.200 f | 0.198 c |
| 2 BAP + 0.2 NAA | 0.246c | 0.234 d | 0.248 c | 0.213 e | 0.235 b |
| 2 BAP + 1.75 kin | 0.171g | 0.166 gh | 0.156h | 0.163 gh | 0.164 d |
| 2.0 kin + 2.0 IAA | 0.293 a | 0.297 a | 0.303 a | 0.272 b | 0.291 a |

| **5B** | Gamma doses X Explants | | | | |
|---|---|---|---|---|---|
| Gamma doses Gy | cotyledon | hypocotyls | leaves | shoot tip | Gamma Mean |
| 0 | 0.108 f | 0.115 f | 0.130 e | 0.096 g | 0.112 c |
| 20 | 0.220 ab | 0.212 b | 0.185 d | 0.194 cd | 0.203 b |
| 40 | 0.226 a | 0.223 a | 0.202 c | 0.217 ab | 0.217 a |
| Explants Mean | 0.185 a | 0.183 a | 0.172 b | 0.169 b | |

| **5 C** | Gamma doses X hormones Treatments | | | | |
|---|---|---|---|---|---|
| Gamma doses | 0 | 1 BAP + 1 | 2 BAP + 0.2 | 2 BAP + 1.75 | 2.0 kin + 2.0 |

| Gy | | NAA | NAA | kin | IAA |
|---|---|---|---|---|---|
| 0 | 0 i | 0.127 h | 0.133 h | 0.135 h | 0.166 f |
| 20 | 0 i | 0.254 d | 0.250 d | 0.149 g | 0.362 a |
| 40 | 0 i | 0.211 e | 0.322 c | 0.208 e | 0.346 b |

* Means followed by the different letters within single or two variants are significantly differed from each other 5% level according to Duncan's multiple range test

**Table (5 D): In vitro effect of interaction among Gamma radiation, hormones treatments and Type of explants on callus fresh weight (mg) from cherry tomato**

| Gamma (Gy) | hormons Treatments mg.l$^{-1}$ | Type of explants | | | |
|---|---|---|---|---|---|
| | | cotyledon | hypocotyls | leaves | shoot tip |
| 0 | 0 | 0 w | 0 w | 0 w | 0 w |
| | 1 BAP + 1 NAA | 0.123 stuv | 0.156 pqr | 0.121 tuv | 0.111 v |
| | 2 BAP + 0.2 NAA | 0.148 qr | 0.115 uv | 0.148 qr | 0.122 stuv |
| | 2 BAP + 1.75 kin | 0.139 rstu | 0.135 rstuv | 0.135 rstuv | 0.132 rstuv |
| | 2.0 kin + 2.0 IAA | 0.130 rstuv | 0.171 opq | 0.245 ijk | 0.119 tuv |
| 20 | 0 | 0 w | 0 w | 0 w | 0 w |
| | 1 BAP + 1 NAA | 0.293 gh | 0.265 hi | 0.146 rs | 0.314 def |
| | 2 BAP + 0.2 NAA | 0.266 hi | 0.254 i | 0.279 gh | 0.201 mn |
| | 2 BAP + 1.75 kin | 0.150 qr | 0.150 qr | 0.154 pqr | 0.141 rst |
| | 2.0 kin + 2.0 IAA | 0.393 a | 0.393 a | 0.348 bc | 0.314 de |
| 40 | 0 | 0 w | 0 w | 0 w | 0 w |
| | 1 BAP + 1 NAA | 0.225 jkl | 0.246 ij | 0.200 mn | 0.174 op |
| | 2 BAP + 0.2 NAA | 0.324 d | 0.333 cd | 0.316 de | 0.315 de |
| | 2 BAP + 1.75 kin | 0.223 klm | 0.213 lm | 0.180 no | 0.215 lm |
| | 2.0 kin + 2.0 IAA | 0.357 b | 0.326 cd | 0.317 d | 0.384 a |

* Means followed by the different letters are significantly differed from each other within the three interactions at 5% level according to Duncan's multiple range test

**Discussion**

Gamma rays had stimulatory effects on seeds germination which may be explained as the activation of RNA and protein synthesis during early stage of germination after seeds irradiated which in turn enhanced respiration rate or auxin metabolism in seedlings(Jan. et al, 2012). In our study, an increase in seeds germination was recorded when cherry seeds irradiated with 20 and 40 Gy. Using gamma rays had been also reported to improve shoots growth of potato plantlets (Annon and Abdulrasool, 2020) as well as accelerate seeds germination (Prabhat and Leena, 2020;Taher et al,2022). Low doses of gamma rays also reported to increase seeds germination, cell proliferation, cell growth as well as enzyme activity ( Beyaz. et al, 2016). In contrast, another study found that gamma rays have no effect on seed germination (Abd,2019).

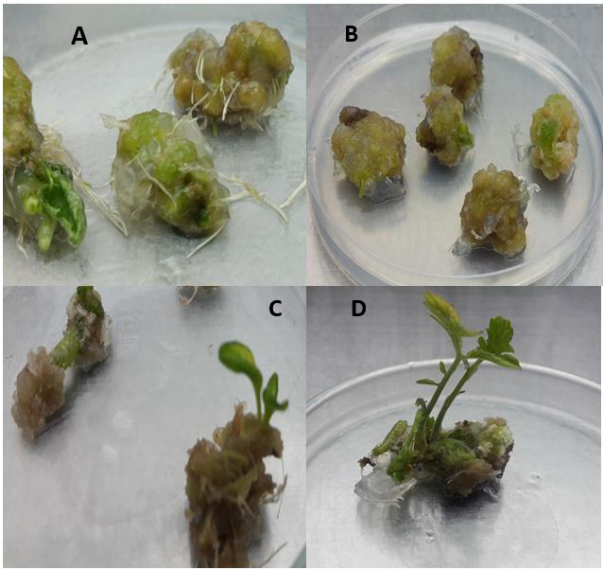
For callus induction, most of combinations hormones improved high efficiency for callus induction with remarkable notice, for example enhancing media with equimolar concentration of auxin and cytokinins (2.0 kin + 2.0 IAA mg. l-1) produced a good callus (Fig2 A,B,C,D) accompanied by roots in fig A. or shootlets in C as well as rooted shoot in fig D. Using equimolar concentration of auxin and cytokinins (2.0 kin + 2.0 IAA mg. l-1) had

improved its efficiency in most of Solanaceae family such as tomato and pepper (Al-Dabbagh ,2011.; Elkaaby et al, 2015 a; Elkaaby et al, 2015 b). While The best media combinations for callus induction were 1 BAP + 1 2, 4-D, 1 BAP + 1 NAA, and 1 BAP + 2 2, 4-D mg.l -1 , which gave 100% callus induction from cotyledons, hypocotyls, leaves and shoots from Different Explants of Cabbage Brassica Oleracea(Hasan et al,2021) and medium supplement with (2.0 mg. L-1 NAA with 0.5 mg. L-1 BA) was good for callus induction on stevia plant(Rahim and Jawad,2021)Also our results agree with Jaafar et al (2017) in related to using equimolar concentration (4.0 kin + 4.0 IAA mg. l-1) for callus induction from eggplants improved positive results. On the other side, gamma ray found to be effective also on callus fresh weight especially at 40 Gy which reached 0.217 mg. Our results are disagree with (AL-Hussaini et al,2010) who recorded gradual reductions in callus fresh weight of soybean at 40 Gy of gamma doses.

## Conclusion:

Our results clarified that Gamma rays and In Vitro biotechnology has been considered as reliable approaches for improving seeds germination and micropropagation of cherry tomato.

**Fig 2. Callus induction from explants of cherry tomato in media contain (2.0 kin + 2.0 IAA mg. l-1) . (A) True leaves (B) cotyledon leaves (C) hypocotyls (D) shoot tips**

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