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Effect of Gamma Irradiation and Mannitol Stress on Physiological and Biochemical Parameters in Callus Derivative from Golden Sunrise Cherry Tomato (*Solanum lycopersicum* var. *cerasiforme*)

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ABSTRACT

The study was performed at the laboratory of tissue culture to evaluate the effect of gamma doses, namely, 0, 20 or 40 Gy and mannitol stress at 0, 40 and 60 g/l on growth parameters on callus produced from Golden Sunrise cherry tomato *in vitro*. The results indicated significant increase in callus fresh and dry weight (232.9 and 33.6 mg) at 20 Gy and 40 g/l mannitol. Although there was a significant increase in the accumulation of CHO at a rate of 5.203 mg/g in the presence of 60/g mannitol, on the contrary, the results displayed a great reduction (1.089 mg/g dry weight) of potassium accumulation in callus exposed to 60 g/l mannitol. Furthermore, 100% of plant regeneration from stressed callus in most of the hormonal combinations in the presence of (0, 40 g/l) mannitol and (0, 20 and 40 Gy) gamma doses. In conclusion, the results of this study recommended low doses of gamma radiation to improve plant regeneration from stressed callus of Golden Sunrise cherry tomato. It was suggested that these dosages could be used as anti-stress factors in tomatoes.

Key words: Cherry tomato, CHO, K^+, mannitol, plant regeneration, potassium

INTRODUCTION

Tomato is one of the most important vegetables in the world. It belongs to the family Solanaceae which contains 2,800 species (Lahoz *et al.*, 2016) such as pepper, eggplants, potato, Cherry tomatoes, etc. For plant description, cherry tomato is a perennial crop (Rambabu and Karunakar, 2019) with tiny fruits size which makes it easy to trade occupying a small space during shipment and transportation. Due to its various bright colouring fruits and delicious tastes, cherry tomatoes are used in many ornamental dishes (Omprasad *et al.*, 2018). The fruit has a high nutritional value, a high quantity of soluble solids and strong market acceptability, all of which ensure quick economic returns for farmers (Maia *et al.*, 2019).

Tomatoes are considered a protective food because of its special nutritional values. It also provides important nutrients such as beta-carotene, lycopene, vitamin C, flavonoids and its derivative hydroxycinnamic acid. Moreover, this crop has a huge success especially in recent years with the discovery of lycopene antioxidant activities and anticancer functions (Distefano *et al.*, 2020; Gómez-Linton *et al.*, 2022). The fruit of cherry tomatoes may be set even at high temperatures. Due to diminutive size, the fruits are popular among customers due to their eye-catching hues, size, delectable flavour and decrease in waste (Venkadeswaran *et al.*, 2018).

Drought is a significant abiotic factor limiting plant growth and output. Tomatoes are a popular vegetable crop, but their output is limited due to lack of irrigation water. Osmotic stress causes a range of morphological, physiological, biochemical and molecular changes in plants that affect their growth, development and production (Litskas *et al.*, 2019). Furthermore, exposing the callus to a biotic stress improves the accumulation of secondary products (Ibrahim and Ameen, 2017). To increase the production of this crop, biotechnology treatments are crucial, which have been used for *in vitro* regeneration and genetic improvement of this crop, as the first step towards genetic transformation of plants, is fundamentally based on the culture of plant cells and tissues. It is impossible to create a fully genetically altered plant without a dependable, repeatable and well-organized

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mechanism to reproduce genetically similar plants from a tiny amount of changed cells. There are several accounts about accidental *in vitro* growing of tomatoes using different explants (Sané *et al.*, 2021; Khaliluev *et al.*, 2022). Moreover; plant biotechnology has the potential to aid plant breeders by generating and modifying genetic diversity. Enhancing crop quality and quantity is one way that plant biotechnology contributes to plant breeding. A plant's tolerance for biotic and abiotic stress may be increased and parasite and pathogen infection can be avoided for improvement in this crop. Breeding tomato cultivars with high resistance to a variety of biotic and abiotic stress conditions requires genetic diversity.

Ionizing radiation from gamma ray generates biological consequences such as inhibition, stimulation, mutation and cell death, furthermore affects plant growth and development by triggering cytological, biochemical, physiological and morphological changes in cells and tissues by producing free radicals (Aly *et al.*, 2022). Researchers from all around the world have documented the impact of gamma radiation on agricultural productivity (Ali *et al.*, 2015; Annon and Abdulrasool, 2020; Shanab and Ali, 2022; Taher *et al.*, 2022). 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. According to Hussain *et al.* (2017), radical length, plumule length, number of roots, seedling fresh weight, seedling dry weight, germination percentage and period of germination all had a beneficial effect on gamma radiation doses. Irradiations at low doses improved morphological features such as plant height, shoot number, panicle length and seeds per panicle. It investigated the effect of irradiations on improving drought stress induced by mannitol on some morphological characteristics and active compounds in sorghum (Yang *et al.*, 2020). The current investigation aimed at evaluating the ability of Golden Sunrise cherry tomato for *in vitro* plant regeneration under gamma radiation and mannitol stress conditions.

MATERIALS AND METHODS

Cherry tomato seeds cv. (Golden Sunrise) were irradiated with gamma cobalt 60 source (CO^{60}) at the doses (0, 20 or 40 Gy) and then germinating on Murashige and Skoog (MS) basal medium supplemented with organic components, namely, pyridoxine, nicotinc acid, thiamine- HCl, myo-insitol, glycine and sucrose at concentrations 4400, 0.5, 0.5, 0.1, 100, 2.0 and 30000/mg/l, respectively. Medium components adjusted to 5.72 pH and solidified with 7000 mg/l of agar. For callus induction, a combination of 2.0 kin+2.0 IAA mg/l proved best for callus induction from cotyledon leaves explants (data not shown). One month later, a constant 100 mg of callus fresh weight (FW) were cultured on MS medium supplemented with mannitol at concentration of 0, 40 and 60 g/l.

Two hundred and fifty mg of callus fresh weight from each sample exposed to stress was washed well with double distilled water to remove remains of the agar. It was crashed with 2 ml of water and centrifuged at 1500/ rpm for 10 min. 25 µl of phenol reagent (5% concentration) was added. Three hundred and fifty µl of each sample were transferred and 30 µl of phenol reagent (5% concentration) was added, with 200 µl of sulfuric acid. The mixture was incubated in water bath at 25-35°C for 20 min. For statistical analysis, each sample was repeated thrice for each treatment. Total CHO was estimated at 488 nm using spectrophotometer and total CHO was expressed as mg glucose/g fresh weight following:

mg glucose/g FW=(device reading/dilution factor) × volume used for reading.

A constant 200 mg of callus fresh weight from each sample was dried in thermal oven at 70°C and digested by using pots filled with mixture of nitric acid perchloric acid, and sulfuric acid at a ratio of 10:4:1, respectively. The pots were placed in a sand bath at a temperature of 80°C. Atomic absorption spectrophotometer (Shimadzu AA- 670 Kyoto, Japan) was used to determine K^+.

Thirty days after exposing to mannitol stress, callus was transferred to regeneration medium containing different combinations as listed in Table 1. The experiment included three factors, namely, gamma doses, media combination and mannitol concentrations. The experiment design was completely randomized (C.R.D). Each treatment was

Table 1. Plant combination hormones for regeneration of stressed callus

Hormones combinations mg/l	References
(2.0) BAP+(0.5) IAA	Azami *et al.* (2010)
(2.0) BAP+(0.2) IAA	Arulananthu *et al.* (2019)
(1.0) Zea+(0.1) IAA	Kumari *et al.* (2017)

BAP = 6-Benzylaminopurine, IAA = Indole acetic acid and Zea = Zeatin.

replicated five times and each replicate glass jar contained three pieces of callus.

RESULTS AND DISCUSSION

A considerable increase was obtained in callus FW and DW in response to 40 g/l mannitol reaching (203.1 and 28.57 mg) as compared to a progressive decline at 60 g/l reaching (110.5 and 0.08 mg), respectively (Tables 2 and 3). Furthermore, 20 and 40 GY of gamma radiation had a substantial effect and resulted in the greatest callus F.W. 166.4 and 152.0 mg, respectively as well as (16.38 and 15.42 mg) for callus dry weight with no significant differences between both the doses, respectively. For the interaction between mannitol concentrations and gamma radiation; 40 g/l mannitol in combination with 20 and 0 Gy was significantly affected and recorded (232.9 and 204 mg) for callus FW and no significant differences were found between them. While highest DW (33.6 mg) was achieved at combination of 40 g/l and 20 Gy with a notice that 60 g/l mannitol in combination with all gamma doses recorded significant decrease in callus FW and DW.

Highest K^+ concentration was at 20 Gy of gamma doses (3.703 mg/g dry weight) compared to low concentrations (3.233 mg/g dry weight) at 40 Gy, while a great reduction (1.089 mg/g dry weight) was recorded in media containing 60 g/l mannitol compared to highest K^+ accumulation (5.989 mg/g) in media free (0) mannitol (Table 4). For the interaction between gamma doses and mannitol concentrations, the results clarified that highest accumulation (6.553 mg/g dry weight) was recorded at control (0) treatment.

Table 2. Effect of Gamma ray and mannitol stress on callus F.W. (mg) in cherry tomato callus

Gamma doses (Gy)	Mannitol (g/l)			Mean
	0	40	60	
0	133.6de	204.6ab	107.3e	148.5b
20	154.2cd	232.9a	112.2e	166.4a
40	172bc	172bc	112e	152.0a
Mean	153.3b	203.1a	110.5c	

Different letters significantly differed from each other at P=0.05 level.

Table 3. Effect of Gamma ray and mannitol stress on callus D. W. (mg) in cherry tomato callus

Gamma doses (Gy)	Mannitol (g/l)			Mean
	0	40	60	
0	12.36f	24.51c	0.09g	12.32b
20	15.49e	33.6a	0.05g	16.38a
40	18.6d	27.6b	0.08g	15.42a
Mean	15.48b	28.57a	0.08c	

Different letters significantly differed from each other at P=0.05 level.

Table 4. Effect of Gamma ray and mannitol stress on K+ accumulation (mg/g)

Gamma doses (Gy)	Mannitol (g/l)			Mean
	0	40	60	
0	6.553a	2.900f	1.110g	3.521b
20	6.177b	3.803d	1.130g	3.703a
40	5.237c	3.437e	1.027h	3.233c
Mean	5.989a	3.38b	1.089c	

Different letters significantly differed from each other at P=0.05 level.

A significant increase in CHO accumulation at the control treatment (0) without gamma, reached (3.902 mg/g), compared to a lower value (3.277 mg/g) at 40 Gy (Table 5). Unlike gamma dosages effects, mannitol stress resulted in a greater accumulation of CHO (5.203 mg/g) at (60/g) compared to a lower value (1.489) at control treatment (0). Furthermore, the interaction between gamma treatments and mannitol concentration revealed that in the absence of gamma (0) treatment, 60 g/l of mannitol induced a

Table 5. Effect of Gamma ray and mannitol stress on CHO (mg/g) accumulation

Gamma doses (Gy)	Mannitol (g/l)			Mean
	0	40	60	
0	1.833f	3.897d	5.977a	3.902a
20	1.377g	3.780e	4.973b	3.377b
40	1.257h	3.913d	4.660c	3.277c
Mean	1.489c	3.863b	5.203a	

Different letters significantly differed from each other at P=0.05 level.

greater rise in CHO 5.977 mg/g with superiority to all other aspects of interaction. For individual effect of hormone combinations, mannitol stress and gamma doses revealed significant differences among plant hormone combinations with 71.11% superiority for (2.0 BAP + 0.2 IAA mg/l) compared with 1.33% at media free hormones (Table 6A). Mannitol and gamma effect referred that higher regeneration reached 76% at control treatment (media without mannitol) compared to 5.67% in media containing 60 g/l mannitol. In contrast the effect of radiation doses, the highest regeneration reached 45.67 and 43.33% at 20 and 40 Gy compared with lowest ratio 37.67% to the control treatment (Table 6B).

The combination of 2.0 BAP+ 0.2 IAA mg/l and 2.0 BAP +0.5 IAA mg/l significantly affected the plant regeneration with average of 69.33, 72.0 and 72.0% at 0, 20 and 40 Gy in addition to 68.0% at 40 Gy, respectively. Concerning to the interaction between gamma doses and mannitol stress, revealed that higher regeneration reached (75, 76 and 77%) at 0, 20 and 40 Gy of gamma doses and control treatment (media free mannitol). Whereas callus cultured in medium containing 60 g/l mannitol, produced less regeneration in the presence of 0, 20 and 40 Gy. For the interaction between hormone combinations and mannitol stress, the results of the interaction between hormone combinations and different concentrations of mannitol showed the superiority for plant regeneration with 100% for all the hormonal combinations used in this experiment as well as the superiority of 2.0 BAP + 0.2 IAA mg/l in the presence of 40 Gy, which also gave a significant superiority with 100% for plant regeneration (Table 6C). The different hormonal combinations indicated the superiority for plant regeneration with 100% of most of the hormonal combinations in the presence of (0, 40 g/l) mannitol and (0, 20 and 40 Gy) gamma doses (Table 6D).

Table 6. Plant regeneration (%) from callus subjected to mannitol osmotic stress

Hormone combinations (mg/l)	Gamma doses (Gy)			Mean
	0	20	40	
6A				
0	0d	1.33d	2.67d	1.33d
2.0 BAP + 0.2 IAA	69.33a	72.0a	72.0a	71.11a
2.0 BAP + 0.5 IAA	41.33c	58.67b	68.0a	56.0b
1.0 Zea + 0.1 IAA	40.0c	41.33c	40.0c	40.44c
6B				
Mannitol (g/l)	0	20	40	Mean
0	75a	76a	77a	76a
40	35c	49b	51b	45b
60	3d	5d	9d	5.67c
Mean	37.67b	43.33a	45.67a	
6C		Mannitol concentration (g/l)		
Hormone combinations (mg/l)	0	40	60	
0	4ef	0f	0f	
2.0 BAP+0.2 IAA	100a	100a	13.33d	
2.0 BAP+0.5 IAA	100a	58.67b	9.33de	
1.0 Zea+0.1 IAA	100a	21.33c	0f	
6D		Gamma doses (Gy)		
Hormone combinations (mg/l)	Mannitol	0	20	40
0	0	0f	4ef	8def
	40	0f	0f	0f
	60	0f	0f	0f
2.0 BAP+0.2 IAA	0	100a	100a	100a
	40	100a	100a	100a
	60	8def	16cde	16cde
2.0 BAP+0.5 IAA	0	100a	100a	100a
	40	20cd	72b	84b
	60	4ef	4ef	20cd
1.0 Zea+0.1 IAA	0	100a	100a	100a
	40	20cd	24c	20cd
	60	0f	0f	0f

Different letters within individual factors or among two or three interactions were significantly different at P=0.05 level.

The current findings demonstrated that the interaction impact of both gamma radiation rays and mannitol concentrations on callus FW and DW (mg) was greatest when subjected to 20 Gy. The results of our study are supported by Agisimanto *et al.* (2016) who reported that gamma radiation at low doses stimulated cell division, growth and development in various organisms.

Regarding the effect of mannitol concentrations, a significant increase in fresh and dry weight was observed at a concentration of 40 g/l accompanied by a decrease at a concentration of 60 g/l. Low concentration of mannitol increased the fresh weight of *Datura inoxia* callus (Twaij *et al.*, 2022) and *Dianthus caryophyllus* (Matter *et al.*, 2017) where growth rate was reduced at high concentrations. The reduction in callus fresh weight was caused by a decrease in water content, which resulted in a decrease in cell turgor pressure and, as a result, a decrease in callus development (Al-Taha and Mazine, 2020).

The control treatment demonstrated that the K^+ concentration decreased significantly under mannitol stress. The results are in agreement with Ramadan and Shalaby (2018) and Abdelsalam *et al.* (2021) who detected a decrease in the K^+ content as mannitol concentrations increased. Potassium is one of two basic nutrients, along with nitrogen, that plays an important role in many biochemical processes such as photosynthetic activity and the movement of photosynthates from the lower part of the plant to the shoots and leaves, protein synthesis, stomatal closure and opening, water-use effectiveness and enzyme activity control. K^+ accumulations as a result of their critical roles in osmotic adjustment in stressed cells, on the other hand, ion tended to accumulate in excess in the cytoplasm because cells could continue to compartmentalize ions in their vacuoles until their capacity to contain ions was exhausted. This caused acute ion imbalances and conformational changes in the plasma membranes' electrical potential. However, the majority of the K^+ ions would primarily affect bulk solution properties, such as the system's electrical charge (Delgado and Gómez, 2016). Furthermore, the increase in K^+ ion helped to remove ionic confounding in the callus for well growth and regenration. K^+ ion increased its content in callus tissues as an osmoprotectant to maintain sufficient water for growth and regeneration processes (Alzahrani *et al.*, 2018; Ur rehman *et al.*, 2018; Obaid and Reddy, 2019; Rady *et al.*, 2019). In terms of CHO accumulation in callus the results revealed that higher accumulation occurred at 60 g/l of mannitol, which agreed with Ramadan and Shalaby (2018).

The effect of radiation on carbohydrate concentration was consistent with the findings of Kebeish *et al.* (2015) who investigated that total carbohydrate contents decreased as radiation doses increased in garlic plants. These results support the hypothesis that gamma radiation stress reduced plant growth and, as a result, total carbohydrates biosynthesis. Total carbohydrate contents decreased as irradiation dosage was increased due to its effect on enhancing the metabolic activities of hydrolyzing enzymes in germinating bulbs. According to previous research, the total protein and carbohydrate contents decreased with increasing irradiation dosage due to higher metabolic activities and hydrolyzing enzyme activity in germinating seed (Hasan, 2022).

Although a good response rate was recorded for all the used hormone combinations. However, shoots developed better when callus cultured in media containing the combination of 2.0 BAP + 0.2 IAA mg/l (Fig. 1). Similarly, for

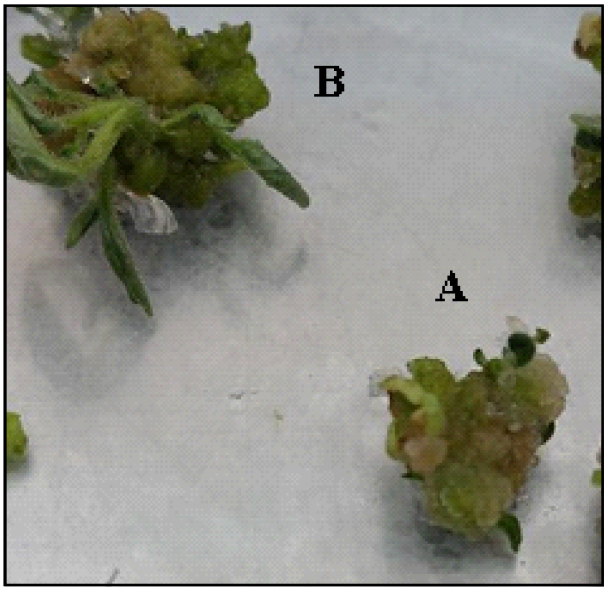

Fig. 1. (A). Shoots induced from stressed callus after 15 days of culturing in media containing 2.0 BAP + 0.2 IAA mg/l and (B) Developing shoots after 30 days.

Fig. 2. Rooting shoots on MS medium supplemented with 1.5 mg/l IBA.

rooting, regenerated plants separated from callus and transferred into MS medium (Fig. 2) supplemented with 1.5 mg/l IBA.

CONCLUSION

The results of this study concluded that the low doses of gamma radiation were useful for improvement of plant structural and physiological regeneration from stressed callus of Golden Sunrise cherry tomato.

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