ARTICLE / INVESTIGACIÓN

Improved micropropagation and salinity tolerance of strawberry (*Fragaria X ananssa L*) cv. Albion

Raghad Abd Alhamza Juameer[1], Ayad Assi Obaid[2] and Shatha Ayed Yousif[2]*

DOI. 10.21931/RB/2022.07.04.34

[1] University of Diyala, Iraq.
[2] College of Agriculture, University of Diyala, Iraq.
Corresponding author: youssifshatha@yahoo.com

Abstract: Gamma-ray has been used to increase genetic variation to obtain salt-tolerant plants in strawberries.The protocol was established to multiply strawberry cv. Albion from runner segments cultured on multiplication Murashige and Skoog (MS) medium contain 0.5 mg l^{-1} of 6-benzyl adenine (BA) and 0.1 mg l^{-1} of Naphthaleneacetic acid (NAA). Cultures were irradiated with gamma rays at (0, 20, 50, 100) Gy after 30 days, and the irradiated and unirradiated shoots were exposed to different concentrations of Sodium Chloride (NaCl) (6,10,14, 22) dS m^{-1}. The results showed the superiority of doses 20 and 50 Gy in giving the highest rate of the number of shoots reached (9.25 and 8.44) shoot explant^{-1}. The treatment 6 dS m^{-1} NaCl with 20 Gy was superior in giving the highest fresh 4.75 g and dry weight 0.36 g. A significant increase of proline was observed in the shoots irradiated with a dose of 50 Gy and grown on a medium with 22 mg l^{-1} of NaCl, as it reached 34.36 (µm proline g^{-1} fresh weight) compared 6 dS m^{-1} and unirradiated media and the highest enzyme activity of (POD) was)263.50 units g^{-1} FW (when treated with 100 Gy grown on a medium with 22 ds m^{-1} of salt. While the dose exceeded 20 Gy without adding salt, as it gave the highest activity of (CAT) enzyme, reaching)4.042 units g^{-1} FW(. It was observed that multiplication was generally restricted, depending on the increase in salt applications and gamma rays.

Key words: BA, NAA, Fragaria, Micropropagation, mutation gamma ray. Salt tolerance.

Introduction

Strawberry (Fragaria x ananassa) is a hybrid of the genus Fragaria, cultivated worldwide for its fruits. It is distinguished by its red color, juicy texture, aromatic smell and sweet taste[1,2]. Salinity is one of the significant environmental stresses that affect the biological processes of many plants and have different effects on the plant's physiological processes, such as an increase in respiration rate, ion toxicity and a decrease in the amount of net CO_2 assimilation rate[3]. High salinity stimulates ROS (reactive oxygen species) and their accumulation in plant cells[4]. Oxidative stress defenses occur through an enzymatic anti-oxidant mechanism that includes catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD)[5]. based on in various studies, salinity stimulates the plant to produce free radicals reactive oxygen species (ROS)[6], the increase in the production of free radicals in plants encourages the processes of demolition. It develops the strategies that cause necrosis and plant damage[7]. In vitro mutagenesis techniques is an alternative to induce genetic variation[8,9]. Genetic variations resulting from in vitro mutagenesis are free of regulatory limitations and allow the regeneration of genetic variations in a short period and at a low cost. The system provides easy manipulation of explants in confined and controlled spaces under aseptic conditions[10,11]. Mutation with cobalt 60 gamma rays has a high penetration potential and no risk to the environment and can be used to irradiate cells, tissues, organs and whole plants[12,13]. The objective of the present work was to obtain a salt-tolerant clone in strawberries using an in vitro mutation method.

Materials and methods

The current study was conducted at the Ministry of Science and Technology/ Directorate of Agricultural Research, Genetic Engineering Department, in 2021. Fragaria x ananassa Duch. cv. Albion was used in this experiment. As the seedlings were taken from the Municipality of Baghdad, the runner was separated from the mother plant in the greenhouse and transferred to the laboratory; the top of the runner was cut with a length of approximately 1 cm[14]. Then they were cleaned well with liquid soap and washed with running water for an hour.(to reduce phenolic substances); then, the plant parts were kept in flasks containing an anti-oxidant solution consisting of a mixture of 150 mg.l^{-1} citric acid and 100 mg.l^{-1} of ascorbic acid 15. Plant parts are kept in the refrigerator at a temperature of 4°C for 24 hours before planting it and then transferred inside the laminar air flow cabinet for surface sterilization. Subjected ethyl alcohol (70%) for 1 minute, then treated with mercuric chloride HgCl2 (0.1%) for 7 minutes with a few drops of liquid soap as a diffuser to reduce its surface tension[16]. The explants were washed 3 times with sterile distilled water for 5 minutes. Micropropagation: runners explant were cultured on MS medium[17] supplemented with 0.5 mg l^{-1} of BA and 0.1 mg l^{-1} of NAA. The cultures were incubated in a culture room under 16-h light and 8-h dark photoperiod at 23±1°C.Radiation: To study gamma radiation's effect on plants propagated by tissue culture, the plants were irradiated with different doses (0,20,50,100) Gy of gamma ray (source Co60)at the University of Baghdad / College of Science / Department of Physics. Salinity treatment: The irradiated and unirradiated

Citation: Alhamza Juameer R A, Assi Obaid A, Ayed Yousif S.Improved micropropagation and salinity tolerance of strawberry (Fragaria X ananssa L) cv. Albion. Revis Bionatura 2022;7(4) 34. http://dx.doi.org/10.21931/RB/2022.07.04.34
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

shoots were grown on MS medium containing 0.5 mg l^{-1} of BA and 0.1 mg l^{-1} NAA and supplemented with sodium chloride (NaCl) at concentrations of (6, 10, 14, 22) dS m^{-1}.The culture was incubated at the same conditions previously mentioned above. Thirty days later, shoots numbers, shoot length (cm), vegetative fresh and dry weight (g) were recorded, and amino acid proline was estimated according to 18 as well as the peroxidase activity was evaluated according to the method19 with some modifications and the activity of catalase enzyme was evaluated according to the method of 20 with some changes. The experiment was designed as a factorial experiment based on a Completely Randomized Design (CRD). The factors included gamma irradiation and salt levels with 4 replications for each treatment. DATA analyzed by the Genstat program and means were separated at 5% probability using Duncan's test.

Results and discussion

Effect of salinity levels and radiation dose

Average shoots number

The results in Table 1, figure 1 showed the superiority of the concentration 6 dS m^{-1} and 10 dS m^{-1} in giving the highest average number of shoots, reaching 8.688 and 9.000 shoots, respectively, and no significant differences were found between both treatments. As for the effect of gamma irradiation on the number of projections, the results showed that 20 Gy significantly affected the highest average number of nodes, reaching 9.875 shoots. For the Interaction between gamma radiation and NaCl, results in Table 1 and Figure 1 revealed that the highest number of shoots came at 13.75 shoots at 20 Gy in media stressed with 10 dS m^{-1} NaCl. In contrast, the lowest shoots number 1.250 shoots in 100 Gy interaction with 22 dS m^{-1} NaCl.

Average length of shoots

Table (2) results for shoot length indicated that 6 dS m^{-1} gave the highest average shoot length, reaching 2.311 cm compared to other NaCl treatments. Moreover, (0, 20) Gy were superior in providing the highest average of shoot length (2.053, 1.933) cm, respectively, and no significant differences were found between both treatments. For the Interaction between different radiation doses and saline concentrations, the results showed that 6 dS m^{-1} gave the highest average shoots length, reaching 3.510 cm, which was significantly superior as compared to the other salts treatments, while 22 dS m^{-1} of NaCl with 100 Gy gave the lowest length of shoots reached 0.150 cm. The effect of salts on inhibiting plant growth is due to the increased concentration of salts with many physiological and biochemical processes, causing problems in ionic imbalance, lack of nutrient absorption, osmotic stress, ion toxicity and oxidative stress. These processes interfere with cellular components, including DNA, proteins, lipids, and pigments, and thus affect the overall operations of growth and development[21].

As for the effect of radiation levels on the studied traits, the increase in the number of shoots at a dose of 20 Gy

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	8.750 c	12.00 ab	11.25 b	2.750 efgh	8.688 A
10	1.750 gh	13.75 a	10.25 bc	10.25 bc	9.000 A
14	4.750 de	10.00 bc	4.000 def	5.750 d	6.125 B
22	1.250 h	3.750 defg	2.000 fgh	1.250 h	2.062 C
mean	4.125 C	9.875 A	6.875 B	5.000 C	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly diferent (P<0.05).

Table 1. Effect of gamma rays and salinity on shoots number of strawberry cv. Albion after four weeks from cultured on MS medium.

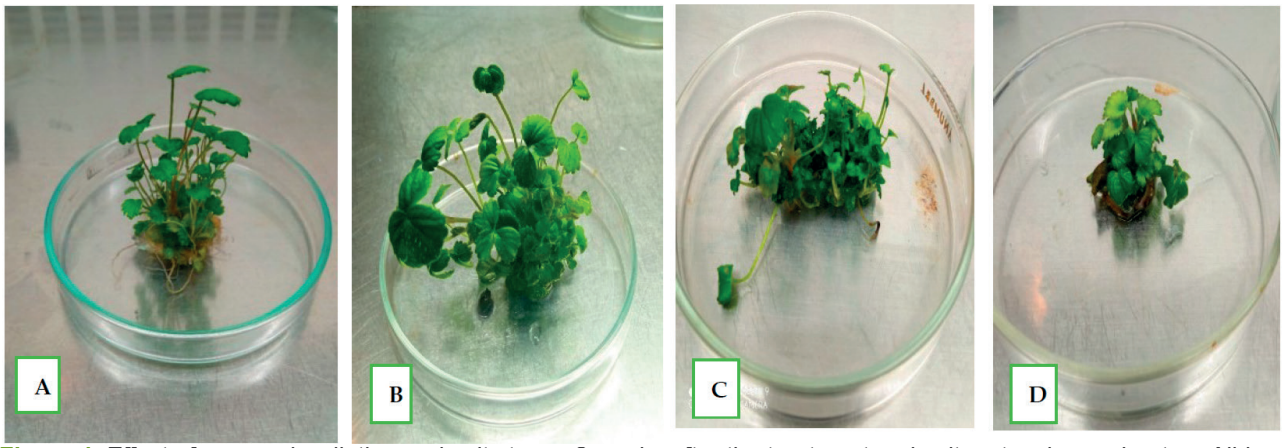

Figure 1. Effect of gamma irradiation and salt stress, 8 weeks after the treatment on in vitro strawberry shoot cv. Albion. A-6 dS m^{-1} .B- 20 Gy and 10 dS m^{-1} NaCl. C- 50 Gy and 10 dS m^{-1} NaCl D- 100 Gy and 10 dS m^{-1}.

could be associated with a hormetic result. According to 22 the hormetic effect is characterized by beneficial or stimulation of development at low doses; and toxicity and inhibition at high doses. According to 23, In this study, doses higher than 100 Gy caused a reduction in the number of shoots per explant and shoot length. The reduced development and increased mortality rate at high doses could be associated with prolonged exposure to 60Co. The high dosage of gamma-ray causes the production and accumulation of ROS, which are toxic to plant tissues[24,25].

Average of fresh weight (g)

The results shown in Table (3) revealed that 6 dS m^{-1} gave the highest average of fresh weight, reaching (2.36 g), which was significantly superior as compared to the other salts treatments, while 22 dS m^{-1} of NaCl gave the lowest fresh weight got 0.37 g. Regarding the gamma effect, the dose 20 Gy showed the highest new importance, reaching 2.82 g, which differed significantly from the other doses, and 100 Gy gave the lowest fresh weight of 0.74 g. Concerning the Interaction between salt levels and gamma doses, 20 Gy with 6 dS m^{-1} NaCl treatment showed the highest fresh weight, reaching 4.75 g compared to the lowest new weight of 0.12 g in 22 dS m^{-1} of NaCl with 100 Gy treatment.

Average of dry weight (g)

The result shown in table (4) revealed 6 dS m^{-1} NaCl gave the highest dry weight average, reaching 0.21 g, which was significantly superior to the other salts treatments, while 22 dS m^{-1} of NaCl gave the lowest dry weight, going 0.04 g. Regarding the gamma effect, the dose 20 Gy gave the highest dry weight, getting 0.19 g, which differed significantly from the other doses, and 50 and 100 Gy gave the lowest dry weight (0.08, 0.07)g respectively, and no significant differences were found between both treatment Concerning to the Interaction between salt levels and gamma doses, 20 Gy with 6 dS m^{-1} gave the highest dry weight reached 0.36 g compared to lowest dry weight 0.02 g in media supplemented with 22 dS m^{-1} of NaCl interaction with 100 Gy.

It is noted from the results that salinity caused a decrease in the fresh and dry weight of the shoots may be due to an increase in salt levels resulting in a reduction of the storage of carbohydrates[26], which consequently decreases the growth. As for the inhibitory effect of irradiation, whether as a single factor or its Interaction with salt levels, it has been indicated by several studies[27]. In another study, it was found that the culture media containing l.0 mg l-1 Kin + 1.0 mg l-1 2,4-D gave the highest average fresh weight was 1.0.2 [28].

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	3.510 a	2.700 b	1.243 def	1.790 cd	2.311 A
10	2.698 b	2.223 bc	0.703 fghi	1.050 efg	1.668 B
14	1.460 de	2.095 bc	1.018 efg	0.850 efgh	1.356 C
22	0.542 ghi	0.713 fghi	0.300 hi	0.150 i	0.426 D
mean	2.053 A	1.933 A	0.816 B	0.960 B	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 2. Effect of gamma rays and salinity on strawberry cv shoots length (cm). Albion after 4 weeks from cultured on MS medium.

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	2.80 c	4.75 a	1.40 e	0.50 ij	2.36 A
10	1.14 f	4.22 b	0.94 g	1.59 e	1.97 B
14	0.56 ij	1.82 d	0.64 hi	0.77 gh	0.95 C
22	0.39 j	0.49 ij	0.47 ij	0.12 k	0.37 D
mean	1.22 B	2.82 A	0.86 C	0.74 D	

According to Ducan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 3. Effect of gamma rays and salinity on shoots fresh weight of strawberry cv. Albion after 4 weeks from cultured on MS medium.

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	0.27 b	0.36 a	0.14 d	0.05 fgh	0.21 A
10	0.10 e	0.22 c	0.07 f	0.14 d	1.13 B
14	0.06 fg	0.14 d	0.05 fghi	0.07 f	0.08 C
22	0.03 ghi	0.03 hi	0.06 fg	0.02 i	0.04 D
mean	0.12 B	0.19 A	0.08 C	0.07 C	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 4. Effect of gamma rays and salinity on shoots dry weight (g) of strawberry cv. Albion after 4 weeks from cultured on MS medium.

Proline content in shoots

Data in table (5) clarified that 22 dS m^{-1} positively affected the highest accumulation of amino acid proline, reaching 21.18 µm. g^{-1} FW, compared to other treatments. As for gamma effects, data in the same table revealed 50 Gy gave the highest proline accumulation, reaching 18.02 µm. g^{-1} FW. Interaction between irradiation and salt levels showed a significant increase of proline came 34.36 µm. g^{-1} FW at the dose 50 Gy in the presence 22 dS m^{-1} of NaCl.

The proline content in leaves generally increases with the salinity level; proline has essential functions in regulating and reducing the undesirable effect of reactive oxygen species (ROS)under saline stress. Therefore, high proline accumulation in plants can induce high tolerance under saline conditions[29,30]. This may be due to the osmotic regulation at different salinity levels. Several studies have suggested that the production of these osmotic alterations may be a common occurrence in response plants to salinity conditions. The role of proline is to increase the adaptation and survival of plants[31,32]. The results of the study showed an increase in the content of proline in irradiated plants; these results are consistent with what was found by 33, who concluded that different doses of gamma rays had other effects on plant biochemical properties such as increasing proline, chlorophyll content and stimulating germination and growth. This technique can be used to produce mutant plants that have the capability of withstanding environmental stresses, it was also found that spraying proline at a concentration of 200 ppm and interfering with irrigation water salinity 2 dS/ m gave the highest total chlorophyll[34].

Activity of peroxidase

The results in table (6) revealed the superiority of the concentration of 22 dS m^{-1} NaCl in giving the highest activity of POD enzyme reached 255.6 units g^{-1} FW, compared to the other salt treatments. Regarding the gamma effect, the dose 100 Gy gave the most increased activities of POD reaching 85.79 units g^{-1} FW, and the control treatment (0 Gy) gave the lowest activities of POD, running 78.81 units g^{-1} FW. Interaction between irradiation and salt levels showed a significant increase of activities of POD got 263.50 units g^{-1} FW compared to the lowest activities of POD 2.92 units g^{-1} FW in 10 dS m^{-1} of NaCl with 20 Gy.

Activity of catalase

The results in a table (7) revealed that 6 dS m^{-1} of NaCl gave the highest average of activities of CAT reaching 3.222 units g^{-1} FW, which was significantly superior as com-

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	4.89 f	7.11 ef	4.83 f	7.96 ef	6.20 C
10	5.40 f	6.76 ef	8.25 ef	11.57 de	8.00 C
14	8.48 ef	6.89 ef	24.64 b	7.08 ef	11.77 B
22	20.95 bc	13.14 d	34.36 a	16.27 cd	21.18 A
mean	9.93 B	8.48 B	18.02 A	10.72 B	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 5. Effect of gamma rays and salinity on proline content(µm. g^{-1} FW) in strawberry CV. Albion after 4 weeks from cultured on MS medium..

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	14.08 h	9.58 hi	9.00 hij	4.75 ij	9.35 C
10	25.67 g	2.92 j	8.00 hij	11.58 h	12.04 C
14	29.17 g	75.25 d	53.75 f	63.33 e	55.38 B
22	246.33 c	252.3 b	260.4 a	263.5 a	255.6 A
mean	78.81 C	85.00 AB	82.79 B	85.79 A	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 6. Effect of salinity levels and radiation doses on the activity of peroxidase (POD) (unit g^{-1} FW) of strawberry, CV Albion after 4 weeks from cultured on MS medium.

NaCl levels (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	2.788 cd	4.042 a	3.143 c	2.913 c	3.222 A
10	1.611 i	2.742 de	2.151 g	1.834 h	2.085 B
14	2.624 e	1.217 j	1.577 i	2.315 f	1.933 C
22	1.258 j	0.647 l	0.937 k	1.217 j	1.015 D
mean	2.070 B	2.162 A	1.952 C	2.070 B	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 7. Effect of salinity levels and radiation doses on the activity of Catalase (CAT) (unit g^{-1} FW) of strawberry, CV Albion after 4 weeks from cultured on MS medium.

pared to the other salts treatments, while 22 dS m^{-1} of NaCl gave the lowest activities of CAT reached 1.015 units g^{-1} FW. At the same time, the effect of irradiation was superior to the treatment 20 Gy, which gave 2.16 units g^{-1} FW, which was significantly superior compared to the other treatments. Concerning the Interaction between irradiation and salinity levels, the dose 20 Gy with 6 dS m^{-1} of NaCl gave the highest activity CAT reaching 4.042 units g^{-1} FW. In contrast, the dose 20 Gy with 22 dS m^{-1} NaCl delivered the lowest activities reaching 0.647 units g^{-1} FW. The increase in the activity of the peroxidase enzyme POD with an increase in salinity level may be due to the increase in anti-oxidants due to iron deficiency, as well as the disruption of the electron transport chain in chloroplast[35] due to the increase in sodium or chloride ions, which increases the effectiveness of some enzymes, including POD[36]. While the results of table (7) showed that salinity caused a decrease in the activity of the CAT enzyme by increasing the salt levels, it may be due to the highly dynamic system of anti-oxidants, where the enzyme activity varies in different species and also depends on the intensity or duration of the stress. The activity of the CAT enzyme was inhibited under exposure to salt stress for a long time, which leads to the inhibition of the primary product of stress for the expression of the corresponding gene, which is consistent with what (37) found. Still, a decrease in the activity of CAT enzyme may occur due to exposure to less time to stress, as shown in other experiments[38]. The mechanism of the effect of gamma rays lies in its stimulation of many genetic changes[39], phenotypic and biochemical changes[40] and physiological changes in plant cells and tisues[41].

Conclusions

Gamma radiation 20, 50 and 100Gy were chosen to induce variations in vitro. The result proved that 20 Gy had a good efficiency for strawberry improvement, giving the highest shoots number, fresh and dry weight and activity of peroxidase and catalase. The increased salinity increased proline and peroxidase activity.

Funding

This research received no external funding

Acknowledgments

We would like to thank Laboratory of Plant Physiology and Plant Tissue Culture of the Universidad de Antioquia. A special acknowledgment to Universidad de Antioquia's

Research Development Committee (CODI) and Granja Yariguíes – Compañia Nacional de Chocolates.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Bibliographic references

1. Giampieri, F., Tulipani S.; Alvarez-Suarez J.M.; Quiles J.L.; Mezzetti B.; Battino M. The strawberry: Composition, nutritional quality, and impact on human health. Nutrition. 2012. 28: 9–19.
2. Ulrich, D.; Kecke S.; Olbricht K. What do we know about the chemistry of strawberry aroma; J. Agric. Food Chem. 2018. 66: 3291–3301.
3. Hajlaoui, H., M. Denden and M. Bouslama. Effet du chlorure de sodium sur les critères morpho-physiologiques et productifs du pois chiche (Cicer arietinum L.). Institut National de Recherches en Génie Rural; Eaux et Forêts, 2006; 8: 171-187.
4. Chawla, S., S. Jain and V. Jain. Salinity induced oxidative stress and anti-oxidant system in salt-tolerant and saltsensitive cultivars of rice (Oryza sativa L.). Journal of Plant Biochemistry and Biotechnology, 2013; 22: 27-34.
5. Alharby, H.F.; E.M.R. Metwali; M.P. Fuller and A.Y. Aldhebiani. Impact of zinc oxide nano particle application on callus induction, plant regeneration, element content and anti-oxidant enzymes activity in tomato (Solanum lycopercicum L.) under salt stress. Archives of Biological Sciences; 2016; 68(4): 723-735
6. Yaghobi, K.H.; Ghaderi, N.; Vafaee; Y. and Javadi, T. Potassium silicate alleviates deleterious effects of salinity on two strawberry cultivars grown under soilless pot culture.Sci.Hortic., 2016; 213: 87–95.
7. Tanou, G.; Job, C.; Rajjou, L.; Arc, E.; Belghazi, M.; Diamantidis, G.; Molassiotis, A. and Job, D. Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. The Plant J., 2009. 60 (5): 795–804.
8. Li,F.; Shimizu, A.; Nishio, T.; Tsutsumi, N.; Kato, H.Comparison and Characterization of Mutations Induced by Gamma-Ray and Carbon-Ion Irradiation in Rice (Oryza sativa L.) Using Whole-Genome Resequencing. G3 Genes Genom. Genet. 2019. 9, 3743–3751.
9. IAEA.Organismo Internacional de Energía Atómica. Inducción de Mutaciones. Available online: https://www.iaea.org/es/ temas/induccion-de-mutaciones.2021.
10. Waugh, R.; Leader, D.J.; Mc Callum, N.; Cadwell, D. Harvesting the potential of induced biological diversity. Trends Plant Sci; 2006. 11, 71–79.
11. Salava, H.; Thula, S.; Mohan, V.; Kumar, R.; Maghuly, F. Application of Genome Editing in Tomato Breeding: Mechanisms, Advances, and Prospects. Int. J. Mol. 2021. Sci. 22, 682.
12. Oladosu, Y.; Rafii, M.Y.; Abdullah, N.; Hussin, G.; Ramli, A.; Rahim, H.A.; Miah, G.; Usman, M. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnol. Biotechnol. Equip., 2016. 30, 1–16.
13. Spinoso-Castillo, J.L.; Escamilla-Prado, E.; Aguilar-Rincón, C.H.; Morales-Ramos, V.; García-de los Santos, G.; Corona-Torres, T. Physiological response of seeds of three coffee varieties to gamma rays (60Co). Rev. Chapingo Ser. Hortic., 2021. 27, 101–112.
14. Ashrafuzzaman , M.; Faisal, S. M.; ⁋Yadav, D.; Khanam, D. and Raihan, F.Micropropagation of Strawberry (Fragaria ananassa) through runner culture . Bangladesh .J. Agril. Res.,2013; 38(3):467-472.
15. Zaid, A. and Tisserat, B. ⁋In vitro shoot tip differentiation in [date Palm] Phoenix dactylifera L.[growth stimulators]. Date – Palm- Journal,1983; 2(2):163-182.
16. Jan, A. , Bhat K. M., Bhat, S. J. A., Mir M. A., Bhat M. A., Imtiyaz A. Wani and Rather J. A. Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for in vitro culture. Afr. J. Biotechnol.2013; Vol. 12(39), pp. 5749-5753.
17. Murashige T. and Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant.,1962; 15:473–497.
18. Bates ,L.S.; Waldren, R.P.and Teare, I.D. Rapid determination of free proline for water-stress studies. Plant Soil,1973; 39:205–207.
19. Kim, Y., Chung, T. and Choi, W. Increased regeneration from ⁋NaCl ⁋tolerant callus in rice. Euphytica,1988; 39: 207 – 212.⁋
20. Aebi, H. Catalase in vitro. Methods in enzymology,1984; 105: 121-126.
21. Al-Sumaidai, Kazem Muhammad Ibrahim. Applications in plant biotechnology. C1. The two rivers university . Baghdad . Iraq.2017.
22. Calabrese, E.J. Hormesis: Path and progression to significance. Int. J. Mol. Sci, 2018; 19, 2871.
23. Jalal, A.; Oliveira, J.J.C.; Ribeiro, J.S.; Fernandes, G.C.; Mariano, G.G.; Trindade, V.D.R.; Reis, A.R.Hormesis in plants: Physiological and biochemical responses. Ecotoxicol. Environ, 2021. Saf. 207, 111225.
24. Oliveira, N.M.; de Medeiros, A.D.; de Lima Nogueira, M.; Arthur, V.; de Araújo Mastrangelo, T.; da Silva, C.B.,. Hormetic effects of low-dose gamma rays in soybean seeds and seedlings: A detection technique using optical sensors. Comput. Electron. Agric;2021;187, 106251.
25. Liu, H.;Li, H.;Yang, G.;Yuan, G.;Ma, Y.;Zhang, T.Mechanism of early germination inhibition of fresh walnuts (Juglans regia) with gamma radiation uncovered by transcriptomic profiling of embryos during storage. Postharvest Biol. Technol.2021; 172, 111380.
26. Cushman, J.C., DeRocher, E.J. and Bohnet, H. J. Gene expression during adaptation to salt stress. Pp:173-203.In: Katterman, F.(ed.). Enviromental Injury to Plant .1990; Academic Press Inc.,San Diego.
27. Yaycili, O. and Alikamanoglu, S. Induction of salt –tolerant potato (Solanum tuberosum L.) mutants with gamma irradiation and characterization of genetic variations via RAPF-PCR analysis .Turk J Biol.,2012; 36:405-412.
28. Obaid, A.A. , Dawood. W.M., Al-Azawy, E. KEfect of plant growth regulators and type of explant on phenolic production from strawberry plant in vivo and in vitro. Diyala Journal of Agricultural Sciences.2018; 10(2):114-128.
29. Akbari, M. , Mahna, N., Ramesh, K., Bandehagh, A. , Mazzuca , S. Ion homeostasis, osmoregulation, and physiological changes in the roots and leaves of pistachio rootstocks in response to salinity.Protoplasma.2018;255(5),1349-1362
30. Alizadeh, M. , Singh, S.K., Patel, V.B., Bhattacharya , R.C., Yadav, B.P.In vitro responses of grape rootstocks to NaCl .Biol. Plant 2010.54(2),381-385.
31. Watanabe, S., Kojima K., Ide Y. and Satohiko, S. ,. Effects of saline and osmotic stress on proline and sugar accumulation in Populus euphratica in vitro. Plant Cell, Tissue and Organ Culture, 2000, 63(3): 199-206.
32. Saruhan, N., Turgut-Terzi, R., Kadioglu, A. The effects of exogenous polyamines on some biochemical changes during drought stress in Ctenanthe setosa (Rosc.) Eichler. Acta Biologica Hungarica.,2006; 57(2): 221-229.
33. Borzouei, A.,Kafi, M., Khazaei H., Naseriyani B. and Majdabadi A. Effects of gamma radiation on germination and physiological aspects of wheat (Triticum aestivum l.) seedlings. Pak. J. Bot.,2010; 42(4): 2281-2290.
34. Rashid, Z. S. , Mohammed, M. S. and Al-Gawoshe, S.A. I.. effect of levels salinity, irrigation water and spraying amino acids (proline, arginine) in the growth and holds potato Solanum tuberosum L. Diyala Journal of Agricultural Sciences,2017; 9(2):169-178.

35. Kaya C., N. A. Akram and M. Ashraf.,. Influence of exogenously applied nitric oxide on strawberry (Fragaria× ananassa) plants grown under iron deficiency and/or saline stress. Physiologia plantarum, 2019; 165(2): 247-263.
36. Alhasnawi, A. N, A. A. Kadhimi, A. R. Ibrahim, A. Isahak, A. Mohamad, F.Doni, W. M. W. Yusoff and C. R. C. M. Zain. Salinity tolerant enhancement, tissue culture in vitro biochemical procedures. Journal of Plant Biology Research,2014; 3(2): 51-64.
37. Hussein, A. E., A. Y. El-Kerdany and K. M. Afifi.,. Effect of drought and salinity stresses on two strawberry cultivars during their regeneration in vitro. International Journal of Innovative Science Engineering and Technology,2017; 4(8):83-93.
38. Kukreja, S., Nandwal, A.S., Kumar, N., Sharma, S.K., Sharma, S.K., Unvi, V. and Sharma P.K. Plant water status, H2O2 scavenging enzymes, ethylene evolution and membrane integrity of Cicer arietinum roots as affected by salinity. Biologia Plantarum, 2005; 49:305–308.
39. Akshatha, Chandrashekar, K. R., Somashekarappa, H .M. and Souframanien, J .. Effect of gamma irradiation on germination, growth, and biochemical parameters of Terminalia arjuna Roxb. Radiat. Prot. Environ. 2013, 36(1) 38–44.
40. Haris, A. Abdullah, Bakhtiar, Subaedah, Aminah and Jusoff K. Gamma Ray Radiation Mutant Rice on Local aged Dwarf Middle East J. of Sci. Res.,2013; 15(8): 1160-1164.
41. Rahimi, M. M. and Bahrani, A . Effect of gamma irradiation on qualitative and quantitative characteristics of canola (Brassica napus L.). Middle-East J. of Sci. Res. 2011. 8 (2): 519-525.