Morphological and Molecular characterization of Physical and Chemical Mutations on Durado Plum Cultivar

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ABSTRACT

Improving the marketability of plum cultivars; requires the production of large and high fruits. Breeding by amutation on plums can be used to generate genetic diversity that allows the breeder to screen the mutants for superior quality and quantity fruits. The study was conducted over two-years (2021-2022) in a private farm. It aimed to investigate the impact of various doses of gamma irradiation acting as physical mutagens (20, 30, 40, and 50 Gy) of gamma irradiation from a cobalt (^{60}CO) source, as well asthe effects of (ethyl methane sulphonate EMS) as chemical mutagens with different concentrations at (0.05, 0.1 and 0.2%)on inducing mutations in the Durado plum cultivar. The study on survival percentage after grafting, morphology and molecular characteristics, identifies new mutated genotypes at the molecular level and finally evaluate some of the new genotyping. Utilizing the ISSR technique of compare and relationships between control with Mutagenic plants based on a dendrogram. The results of control gave the highest value on survival percentage after grafting. 40 Gy and EMS 0.2% given the best results in morphology characteristics of the tree in compare with the control. Additionally, the study of relationships and correlation coefficient based on a dendrogram revealed that the relationships between 20 Gy and EMS 0.05% and the control are similar. While 40 Gy and EMS 0.2% is very far in relationship with control.

Keywords: *Prunussalicina*, physical mutagen, chemical mutagen, molecular genetic

INTRODUCTION

The plum cultivars, which belong to the *Prunoideae* subfamily, are defined by their one-carpelled, drupaceous fruit and simple leaves. The absence of a terminal blossom, the existence of a suture and a waxy bloom on the fruit, and the pit's flatter shape distinguish plums from cherries. In order to distinguish plums from peaches, almonds, and cherries, they are placed in the *Prunophora* subgenus. As a result of the sutured fruit, waxy bloom, lone axillary buds, and absence of terminal buds that are present in plums, this is done (Topp et al., 2012). Plums have a hard pit; they are grouped with other stone fruits in the Rosaceae genus *Prunus* (Milatovi´ et al, 2019). The hexaploid (2n = 6x = 48) European plums (*Prunus domestica L.*) and the diploid (2n = 2x = 16) Japanese plums (*Prunus salicinaL indl.*) are the only two varieties that have x=8. Both P. *domestica*and P. *salicina* are widely cultivated across the world have a long history of cultivation (4000–6000 years) they have a lengthy history of cultivation, are the most widely cultivated plum species worldwide (Topp et al, 2012). Plums are One of the deciduous fruit trees cultivated in Egypt is the plum (*Prunus salicina L.*). The total cultivated area of plums in Egypt is about 115 hectares; occupying rank No.44 all over the world with a total production of about 14775 tons (FAO, 2019).

The various techniques used in plum breeding programmers include: (1) conventional techniques (selection, hybridization, polyploidy, induced mutation); (2) biotechnological techniques (*in vitro* plant cell culture and regeneration of plants from cultured cells; *in vitro* selection and somaclonal variation; somatic hybrid plants);

and (3) genetic engineering techniques (restriction fragment length polymorphism (RFLP), gene transfer, transgene expression, selection, and plant regeneration) (Minev and Balev, 2002; Blazek, 2007). 170 new plum cultivars have been registered on the basis of traditional breeding techniques such as controlled hybridization, open pollination, selection from the wild population of *Prunus spp.,* and mutagenesis utilizing X-ray technology (Butac*et al*., 2013). Kamile and Ayse, (2015) Physical mutagens like gamma rays are frequently utilized to diversify and produce new varieties of many plant species; the compilation looked at various gamma-ray practices on numerous plant types. Rawat and Singh, (2021) gamma irradiation is the most widely utilized mutagen among all of them due to its great efficacy and penetrating power. Gamma rays are also non-toxic to humans and the environment when utilized as a mutagen in crop development, Gamma induction has created plant varieties that are improving global food security, nutritional stability, and standard of living. Mutants resistant to this disease were created as a result of gamma radiation. It was discovered that chemical mutagens were quite efficient at causing real gene changes, and the specificity of action could be explored by examining how they interacted with various DNA bases. The most effective alkylating agent for mutation is ethyl methane sulphonate (EMS), which is one of several chemical mutagens used to cause mutation in fruit crops (Luan et al., 2007).

The method amplifies primarily the ISSR sequences of various sizes by using microsatellites, which are typically 16–25 bp long, as primers in a single primer PCR reaction that targets several genomic loci (Gupta et al., 2000). Di-nucleotide, tri-nucleotide, tetranucleotide, or penta-nucleotide microsatellite repeats can be employed as primers. The primers might be either anchored or unanchored. Using five primers, the ISSR analysis identified polymorphic bands. With 1 to 4 degenerate bases extending into the neighbouring sequences, gamma-irradiation or more is typically anchored at the 3' or 5' end. (Zietkiewicz et al., 1994).

MATERIALS AND METHODS

This experiment was carried out during two consecutive seasons (2021- 2022). The experiment was done on a private farm in Sadat City, Menoufia Governorate. The shoots were irradiated using gamma rays at Radiation in Atomic Energy Commission, Nasr City. Biochemical analysis was carried out at Ain Shams Center for Biotechnology and Genetic Engineering.

Plant materials:

The Japanese plum *Prunussaliciana* (Durado) varieties are the subject of the current inquiry, which used a few from a mature bearing, 20cm long shoots of the plum cvs. Durado variety having 5-7 buds was chosen as one-year-old shoots. The terminal apex was removed and immediately wrapped in foil paper, to prevent branches from becoming dehydrated during treatment. Prudencio et al., (2022) agenotype with the mutant phenotype can be created by vegetative propagating mutations in somatic cells.

Grafting of Durado mutated scion:

After exposing the offshoots to the mutagens, the shoots are grafted using the budding grafting method on the Maryana rootstock in the nursery one-year-old in the previous summer and planted in the field at 4× 2m distances. The following observations and measurements were carried out the survival percentage after grafting and some morphology and vegetative characteristics (Ban and Jung, 2023).

Physical mutation via Gamma- ray radiation:

The irradiation procedure was conducted according to the protocol described by (Jain, 2005; Riviello-Flores et al., 2022), at the Atomic Energy Commission located in Nasr City, Cairo. A total of 30 buds were selected for each dose, divided into four groups, and subjected to gamma-ray exposure a duration of 2 minutes at doses of 20, 30, 40, and 50 Gy.

Chemical mutation via Ethyl methanesulphonate:

Ethyl methane sulphonate (EMS) was used as a chemical mutagen for inducing mutations in Durado cultivar buds. EMS was purchased from Sigma Aldrich. Induction of mutations using EMS was conducted following the protocol described by (Kishor et al., 2017). The buds were soaked in different concentrations of EMS, three concentrations were used (0.05%, 0.1%, and 0.2%), a duration of 24 hours.

Survival percentage after grafting mutated scions:

Calculated the number of total germination determined as follows:
Survival percentage = {N.of survival (30 days after grafting) × 100}/ Total number of grafted plant

Assessment of vegetative characteristics:

Different vegetative characteristics including Tree height (cm) point of the plant up to the crown surface, Trunk diameter (cm) above the soil surface by 20 cm measured, Number of shoots branches on the main stem, Distance between internodes (cm) measure the distance between internodes on plant branches, Leaf length (cm) measured from leaf tip to the point of petiole intersection along the midrib and Leaf width (cm) was measured at the widest part of the leaf, were recorded for the mutant plants and control as mentioned by (Hartman, 1997). All of these records were taken for plants after one year of grafting.

Total chlorophyll content:

Where chlorophyll is measured using a device chlorophyll meter (SPAD – 502).For scale calibration the measuring head is pressed closed with a paper insert and the measurement sheets. Leaves were taken from the middle of the branches.

Phonological measurements characterization:

Were taken from each treatment after one year of grafting; expressed as the number of days during March and April Leaf bud starts (days), and leaf bud end (days).Ganji Moghaddam et al, (2010) the following measurements and counts were made on fruits of the plum tree produced through bud mutation, phonological findings.

Molecular genetics study

DNA sample collection:

To collect DNA samples, vegetative superior genotypes were selected to characterize and identify them at the molecular level; therefore, three genotypes that represent the most superior vegetative parameter were subjected to DNA investigation. As follows; half – gram of young, fresh leaves from each treatment and control were collected, stored in an ice chest, and then quickly transported to the lab

Genomic DNA Extraction:

DNA extraction was done according to (Doyle and Doyle 1990). These samples were required to be devoid of any infection or pathogenic indications. Plant tissues were ground to a fine powder in a pre-chilled mortar and pestle and then submerged in liquid nitrogen. Using QIAGEN's DNeasy plant Mini Kit for DNA extraction.

ISSR analysis:atotal of 5 random primers were used, (Table 1).

Table (1): Characteristics and identification of ISSR primers used in this investigation. The information includes primer names and sequences 5’-3’.

Primer No.	Primer name	Primer sequence
1	HB-08	5’GAGAGAGAGAGAGG3’
2	HB-10	5’GAGAGAGAGAGACG3’
3	HB-11	5’GTGTGTGTGTGTGCG3’
4	HB-13	5’GAGGAGGAGGC3’
5	HB-15	5’GTGGTGGTGGC3’

Data scoring and statistical analysis:

Discrete variables were used to record the data, with 1 denoting the presence of a similar band and 0 denoting its absence. On the gel, only distinct and repeatable bands will be scored. The Bio-Rad manufacturer's software was used to analyze the band scoring. Utilizing diversity database software from Bio-Rad manufacturing, genetic relatedness among genotypes was investigated using UPGMAM (Unweight Pair Group Method with Averages Mean) according to (Lynch, 1990).

Statistical Analysis:

According to **(Snedecor and Cochran 1982),**an analysis of variance (ANOVA) was performed on the collected data. Duncan's multiple range test was used to differentiate between means at probability of 5% level of significance according to (Waller and Duncan 1969). While qualitative and quantitative traits were subjected to cluster analysis to draw the relationship among the cultivar analyzed (dendrogram) by GelAnalyzer 4 program.

RESULTSAND DISCUSSIONS

Effect of gamma rays doses on survival ofgrafted plum:

Data in Figure (1) illustrated the effect of gamma ray doses on the survival percentage of grafted plant which ranged from 0 to 90%. A significant survival percentage (90%) was observed by control; while 50 Gytreatment was a lethal dose and no survival plants obtained after grafting with 50 Gy. This finding aligns with the observations were obtained (Briggs, and Constantin, 1977). Concept of LD50 (lethal dose 50 %) is the dose that causes 50 % lethality in the organism used for irradiation in a defined time. Generally, irradiated populations are generated by using an LD50 dose treatment and with a dose lower than LD50. To obtain a mutant, the mutagen dose must be strong enough to increase the likelihood of triggering a mutation to produce a mutant. In terms of the specifics, the harmful effect of greater radiation doses, as previously discovered, may be responsible for the decline in survival percentage with increasing radiation doses. On the other hand, 20 Gytreatment recorded (79%) survival followed by 30 Gy (64%) and finally 40 Gy (39%). In this respect, (Predieri and Gatti, 2000) reported that radiated scions (20Gy gamma rays) which were best, the Rough lemon rootstock was grafted with the irradiation bud scions using the side-graft method.

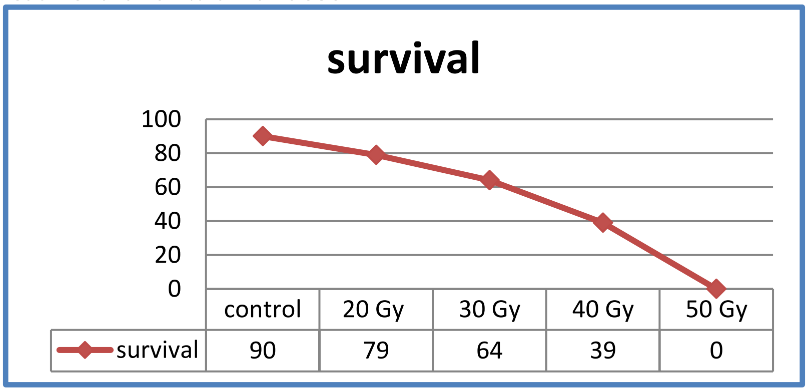


Fig 1 Effect of Gamma- ray radiation on the survival of grafted plum plants.

Effect of different concentrations of EMS on survival percentage of grafted plants:

The data presented in Figure (2) demonstrated a range of survival percentages after grafting, ranging from 90% to 66.5%. The control group exhibited the highest and statistically significant survival percentage (90%) after grafting, while the treatment with EMS 0.2% resulted in the lowest significant survival percentage (66.5%).

These results align with previous findings for EMS treatments, which showed a significant decrease in explant survival with longer durations of EMS treatment (Abd El-Latifi et al., 2018).

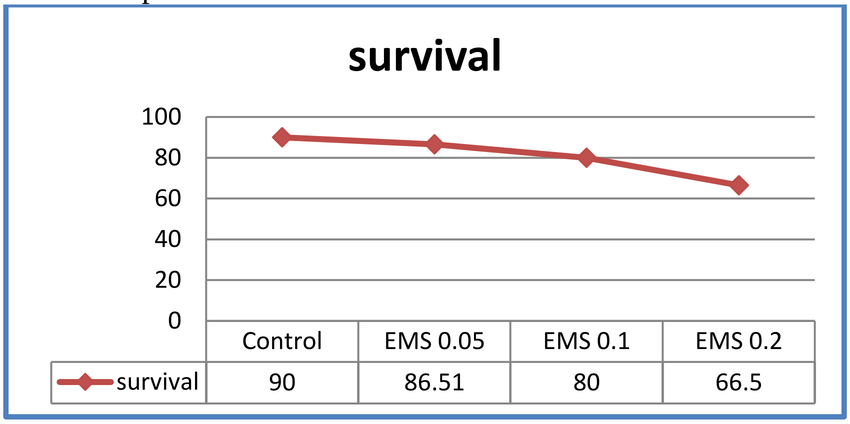


Fig (2). Effect of different concentrations of EMS on survival percentage of grafted plum plants.

Effect of difference doses of gamma-irradiation and EMS on tree morphology measurements.

Tree height (cm)

The data presented in Table (2) exhibited the impact of gamma-irradiation doses on tree height compared with the control group. There were significant differences among the doses. The highest significant average tree height was observed with a gamma-irradiation mutagen at 40 Gy, measuring was 60.20 cm and 165 cm in both seasons, respectively. On the other hand, the control group had the lowest significant tree height, measuring was 47.75 cm and 49.5 cm in both seasons, respectively. The measurements were taken at the end of each season. These results are confirmed by the findings of (Jain, 2005) gamma radiation has provided a high number of useful mutants and is still showing an elevated potential for improving vegetative propagated plants.

Data in Tables (3) illustrated the highest significant average tree height was found with treatment EMS 0.2% (64.60 cm and 100.67 cm) in both seasons respectively. While the lowest significant tree height obtained by the control was 47.75 cm and 49.5 cm in both seasons respectively. These results are similar to those obtained by (Yogesh et al., 2014).

Trunk diameter (cm)

Table (2) illustrated the effect of gamma-irradiation doses and the control on average trunk diameter. As for the specific effect of the trunk diameter compare between different doses and control, high significant average trunk diameter development was with 40Gy (0.59 cm and1.38 cm) in both seasons respectively. While20 Gy gives the lowest significant average trunk diameter (0.41 cm) in the first season. Whereas, the lowest significant value of trunk diameter was recorded with the control (0.86 cm) in the second season.

Moreover, In Table (3) displays the effect of EMS on average trunk diameter. The treatment with EMS 0.2% resulted in a significant increase in average trunk diameter, measuring 0.62 cm in the first season. Additionally, the highest significant average trunk diameter was achieved with EMS 0.1%, measuring 1.41 cm in the second season. Conversely, the control group exhibited the lowest significant value of average trunk diameter, measuring was 0.42 cm and 0.86 cm in both seasons, respectively. Anil Kumar et al., (2013) reported that concentrations of 0.1% and 0.3% EMS treatment significantly affected morphological characteristics such as trunk diameter.

Number of shoots

As illustrated in Table (2), the highest significant number of shoots was observed with a dose of 40 Gy, reaching the highest significant values of 4.80 and 5.83 in both seasons, respectively. Conversely, the lowest significant average number of shoots was recorded with the 30 Gy treatment, measuring 3.0 in the first season. The control group exhibited the lowest significant number of shoots, with a value of 4 in the second season.

In Table (3) the impact of EMS mutagen on the obtained average number of shoots was observed at which the highest significant numbers of shoots were recorded with EMS 0.2% treatment, reaching values of 4.6 and 7.5 in both seasons, respectively. Conversely, the control group exhibited the lowest significant value of the number of shoots, measuring was 3.2 and 4 in both seasons. These results align with the findings of Lemo et al., (2017).

Table (2). Effect of different doses of gamma rays on tree characterization during theyears 2021 and 2022.

Treatments	Tree characteristics					
Radiation doses	Tree height (cm)		Trunk diameter (cm)		No. of shoots	
	2021	2022	2021	2022	2021	2022
Control	47.75 C	49.50 D	0.42 C	0.86 D	3.20 C	4.00 B
20 Gy	54.28 B	136.7 C	0.41 C	1.04 C	3.85 B	4.50 B
30 Gy	60.05 A	143.0 B	0.49 B	1.07 B	3.00 C	5.75 A
40 Gy	61.20 A	165.0 A	0.59 A	1.38 A	4.80 A	5.83 A

Means followed by the same letter (s) in each row, column or interaction are not significantly different from each other at 5% level.

Table (3). Effect of different EMS concentrations on tree characteristics during years 2021 and 2022.

Treatments	Tree characteristics					
	Tree height (cm)		Trunk diameter (cm)		No. of shoots	
Chemical of mutagens	2021	2022	2021	2022	2021	2022
Control	47.75 C	49.50 D	0.42 C	0.86 D	3.20 C	4.00 B
EMS 0.05	56.57 B	90.33 B	0.51 D	1.28 B	3.50 B	6.71 A
EMS 0.10	61.28 A	95.00 B	0.60 B	1.41 A	4.20 A	7.25 A
EMS 0.20	64.60 A	100.7 A	0.62 A	1.09 C	4.60 A	7.50 A

Means followed by same letter (s) in each row, column or interaction are not significantly different from each other at 5% level

Total chlorophyll contents:

Tables (4 and 5) Total chlorophyll content almost showed a constant trend in both of the study seasons regarding radiation and/or chemical mutagenesis treatments. The control revealed the highest significant value of chlorophyll content in comparison to both radiation and EMS in both of the studied seasons except for the second season with EMS treatments, as a significant value (44.48) of chlorophyll content was observed by EMS0.1 % in the second season.(Salih, 2018) indicated that loss of chlorophyll due to plastid mutations typically produce albino plants or variegated plants with both green and albino sectors

Leaf length (cm):

Table (4) gamma ray treatment at 40 Gy recorded the highest significant value of leaf length in both of the studied seasons (7.07 cm and 7.23 cm, respectively).However, the lowest value was observed by the control in both seasons (5.6 cm and 5.77 cm, respectively).Naotoshiet al., (1998); Kaufmaneet al., (2002) wild plum cultivars showed a high level of fruit and stone features and substantial variation in leaf-associated morphological factors.

On the other hand, EMS chemical mutagen enhanced leaf length; EMS 0.2% recorded the highest significant value in both of the studied seasons (6.35 cm and 6.90 cm, respectively). Meanwhile, the control exhibit the same trend in radiation treatments, the control showed the lowest value of leaf length in both seasons (5.22 cm and 5.3 cm, respectively.)

Leaf width (cm):

Data in Table (4) explained the effect of gamma irradiation on leaf width, the highest significant value (2.22 cm and 2.25 cm) of average leaf width was achieved by treatment 40 Gy during both seasons. While the lowest significant value of average leaf width was obtained by control (1.75 cm and 1.43 cm) in both seasons.

Data Table (5) shows the effect of different EMS concentrations the highest significant value (2.30 cm and 2.37 cm) of average leaf width was detected by EMS 0.2% during both seasons. While the lowest significant value of average leaf width was obtained by control (1.43 cm and 1.75 cm) in both seasons.

Internodes length (cm)

Data in Table (4) deal with the effect of different gamma irradiation doses; the highest significant value (1.18 cm and 1.20 cm) of average internodes length was influenced by treatment 20 Gy during both seasons. While the lowest significant value with control (0.88 cm and 0.92 cm) in both seasons.

Data in Table (5) show the highest significant value (0.97 cm) and (0.99 cm) of average internodes length was detected by EMS 0.05% during both seasons. While the lowest significant value with control (0.88 cmand0.92 cm) in both seasons.

Table (4). Effect of different doses of gamma rays on vegetative characteristics during the years 2021 and 2022.

Treatments	Tree characteristics							
Radiation Doses	Total chlorophyll		Leaf length (cm)		Leaf width (cm)		Internodes length (cm)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	45.28 A	44.39 A	5.60 C	5.77 C	1.43 C	1.75 C	0.88 B	0.92 C
20 Gy	41.38 B	41.23 C	6.37 B	6.48 B	1.97 B	1.98 B	1.18 A	1.20 A
30 Gy	41.35 B	41.20 C	6.90 B	6.62 B	2.10 A	1.92 B	1.03AB	1.13 AB
40 Gy	43.79 A	42.33 B	7.07 A	7.23 A	2.22 A	2.25 A	0.92 B	1.00 BC

Means followed by the same letter (s) in each row, column or interaction are not significantly different from each other at 5% level

Table (5). Effect of different EMS concentrations on vegetative characteristics during the years 2021 and 2022

Treatments	Tree characteristics							
Chemical of Mutagens	Total chlorophyll		Leaf length (cm)		Leaf width (cm)		Internodes length (cm)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	45.28 A	44.39 A	5.22 C	5.30 C	1.43 C	1.75 C	0.88 B	0.92 C
EMS 0.05	41.12 C	43.45 B	5.37 C	5.42 B	1.66 B	1.78 B	0.97 A	0.99 A
EMS 0.10	43.03 BC	44.48 A	5.42 B	5.51 B	2.12 A	2.30 A	0.92 A	0.97 A
EMS 0.20	43.70 AB	43.92 AB	6.35 A	6.90 A	2.30 A	2.37 A	0.85 B	0.88 B

Means followed by the same letter (s) in each row, column or interaction are not significantly different from each other at 5% level

Effect of different treatments of gamma rays and EMS on leaf bud at the beginning and end date of 2021 season.

It is clear that, the first date of the leaf bud beginning was recorded by 40 Gy (19 March) while the end date of the leaf bud end was 20 Gy and control (30 March) in the first season. These results are in agreement with those obtained by (Marti et al., 2018).

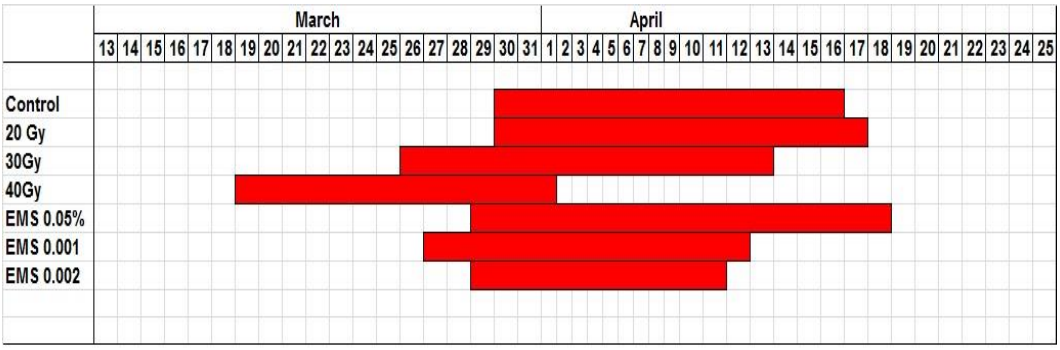


Fig. (4). Effect of different treatments of gamma rays and EMS on the beginning and date of leaf differentiation 2021 season.

Effect of different treatments of gamma rays and EMS on leaf bud at the beginning and end date of 2022 season.

The data depicted in Figure 5 demonstrate the impact of various treatments involving gamma rays and EMS on the initiation and completion of leaf bud development. The data specifically highlight the effects of different mutagen treatments and the control group on the starting and ending dates of bud formation in plant leaves during the 2022 season. According to the results, the earliest leaf bud start date was observed in the 40 Gy treatment group on March 14th, while the latest leaf bud end date was recorded in the EMS 0.05% treatment group on March 25th. These findings provide valuable insights into the temporal dynamics of leaf bud development under different mutagenic treatments in the second season. These results are in agreement with those obtained by (Ruiz, 2019) a somatic mutation may underlay the early-flowering phenotype.

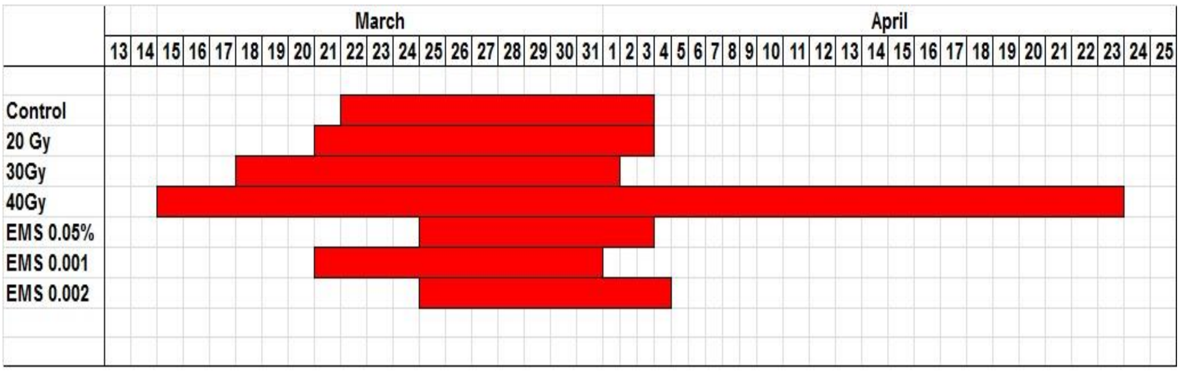


Fig (5). Effect of different treatments of gamma rays and EMS on the beginning and end date of leaf bud differentiation 2022 season.

Table (6). Polymorphic bands detected in the ISSR analysis using five primers for Gamma rays treatments.

	Primers HB- 08	Primers HB -10	Primers HB-11	Primers HB- 13	Primers HB - 15
Monomorphic bands	0	0	1	2	0
Polymorphic (without Unique	17	18	11	8	12
Unique bands	1	2	1	2	1
Polymorphic (with Unique)	18	20	12	10	13
Total number of bands	18	20	13	12	13
Polymorphism (%)	100.00%	100.00%	92. 00%	80.00%	100.00%

The dendrogram was constructed to assess the genetic relationships among ten plum plant varieties using variation data obtained from five primers. The dendrogram was generated using the neighborhood joining method of the UPGMA method, and it revealed three distinct clusters (see Fig 7). The genotypes were classified into four main groups, exhibiting variations in tree characteristics, vegetative characteristics, and the timing of leaf bud opening. Notably, there was a noticeable difference between the 40 Gy treatment group and the control group.

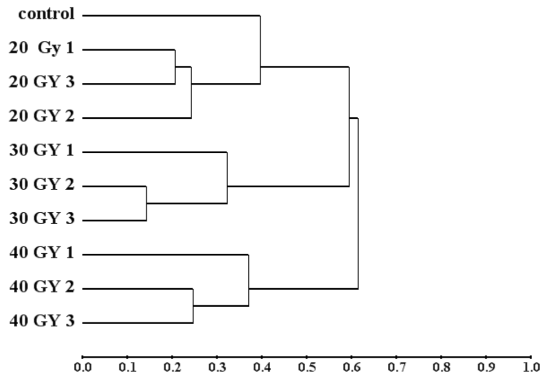


Fig (7). UPGMA dendrogram based on ISSR markers showing similarity between different concentrations of Gamma-ray used on Durado cultivars.

Table (7). Polymorphic bands detected in the ISSR analysis using five primers for EMS treatments.

	Primers	Primers	Primers	Primers	Primers
	HB- 08	**HB -10**	**HB-11**	**HB- 13**	**HB – 15**
Monomorphic bands	0	0	0	1	0
Polymorphic (without Unique	17	22	12	11	12
Unique bands	4	1	1	0	2
Polymorphic (with Unique)	21	23	13	11	14
Total number of bands	21	23	13	12	14
Polymorphism (%)	100.00%	100.00%	100.00%	92. 00%	100.00%

Fig (8) illustrating the dendrogram construed to analyze the genetic variation among the ten plum plants across the five primers employed, the generated dendrogram revealed the presence of three distinct clusters. The genotypes were classified into four primary groups, exhibiting variations in tree characteristics, vegetative characteristics, and the timing of leaf bud opening. Notably, a notable difference was observed between the treatment involving EMS 0.2% and the control group.

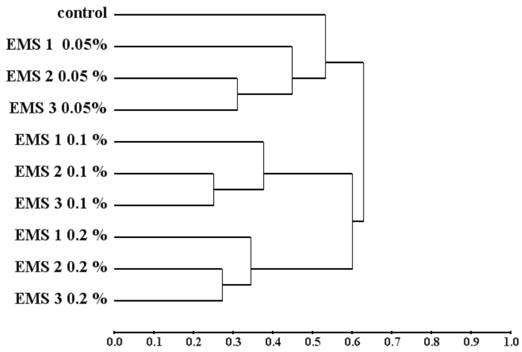
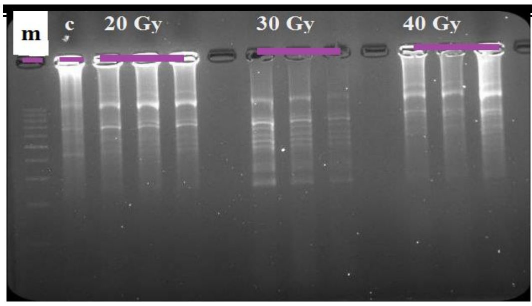
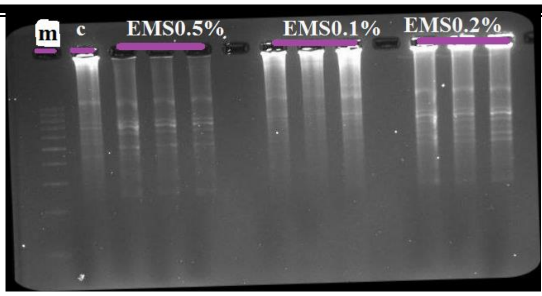


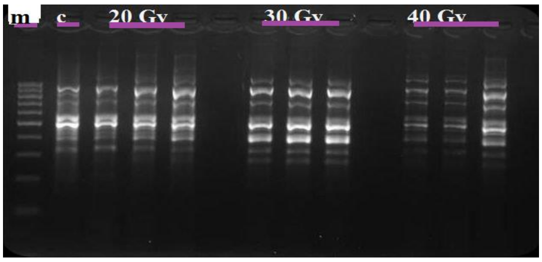
Fig (8). UPGMA dendrogram based on ISSR markers showing similarity between different concentrations of EMS used on Durado cultivars.



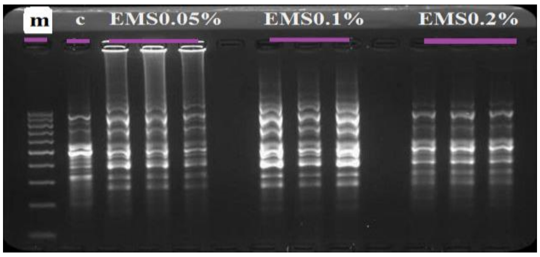
Primer HB -08 (Radiation)



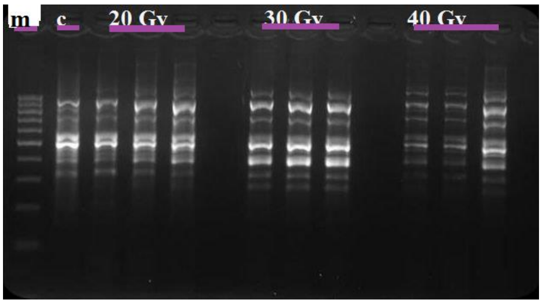
Primer HB -08 (EMS)



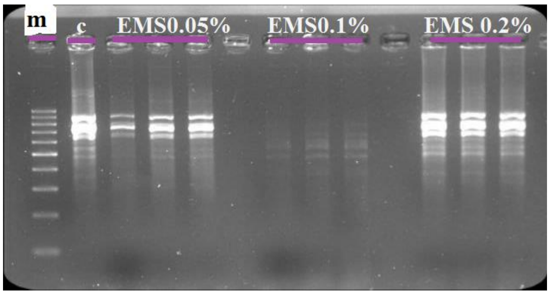
Primer HB -10 (Radiation)



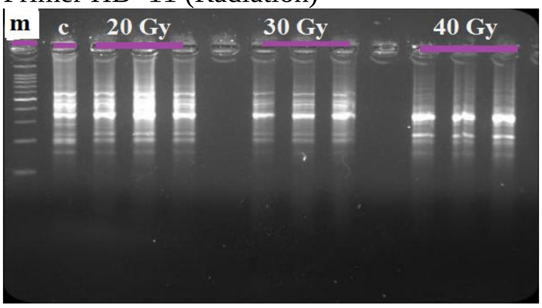
Primer HB -10 (EMS)



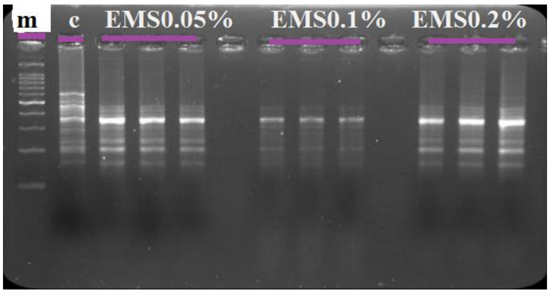
Primer HB -11 (Radiation)



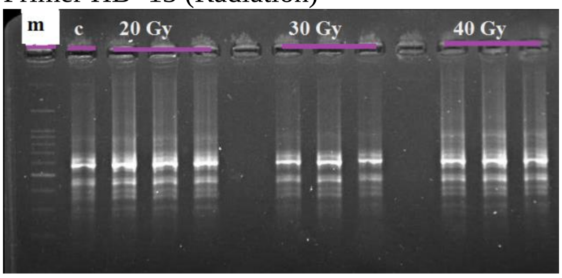
Primer HB -11 (EMS)



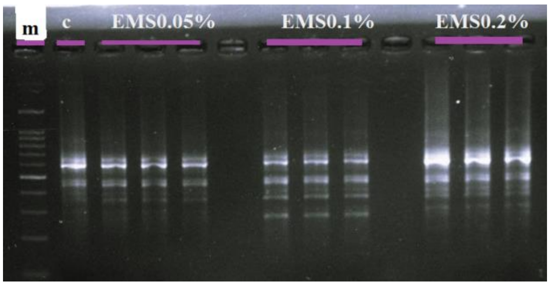
Primer HB -13 (Radiation)



Primer HB -13 (EMS)



Primer HB -15 (Radiation)



Primer HB -15 (EMS)

Figure (6): ISSR profiles of mutated grafted plants.
1-m (marker) 2- c (control) 3- Gy: represents 3 replicates of Gamma rays at 20, 30 and 40 doses
EMS: represents 3 replicates of Ethyl methane sulphonate at 0.05%, 0.1% and 0.2% concentrations

Conclusion

A crucial breeding method for producing variety in fruit crops is mutation. It provides an opportunity for the improvement of plant, earliness, within a short period time; mutant identification at the genotypic level using new technologies, to produce commercial varieties and fulfill the objective of nutritional security.

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التوصيف المورفولوجي والجزيئي للطفرات الفيزيائية والكيميائية لصنف البرقوق دورادو

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أجريت هذه الدراسه خلال موسمين متتالين 2021 – 2022 وذلك بهدف دراسه تأثير المطفرات على البرقوق صنف درادو، وذلك بأخذ عقل حديثة النمو في الصيف السابق بطول 20سم من نبات درادو وتم تعريضها إلي:

أولا: المطفرات الفيزيائية: وتتمثل في أشعه جاما على جرعات مختلفة (20 – 30 – 40 – 50 جراي) لمدة دقيقتين.

ثانيا: المطفرات الكيميائية: حيث تم إستخدام مادة ايثيل ميثان سلفونيت من خلال نقع العقل لمدة 24 ساعة في تركيزات مختلفة (0.05% - 0.1% - 0.2%) وبعد ذلك تم تطعيم البراعم المطفرة على أصل ماريانا في المشتل ثم نقلها إلي الحقل المستديم في شهر فبراير2021، وتم أخذ القياسات المورفولوجيه في موسمين متتاليين وهما موسم اولى 2021 وموسم ثانى 2022. لتحقيق أهداف الدراسة التي تتمثل في:

1- دراسة نسبة البقاء بعد التطعيم.

2- دراسة تأثير أشعة جاما وتأثير ايثيل ميثان سلفونيت علي الصفات المظهريه للطرز الجينيه الناتجة بعد التطفير بالمقارنه بالأصل.

3- عمل بصمه وراثية لدراسه درجه القرابه بين نواتج التشعيع والاصل والمقارنه بأستخدام تكنيك ISSR.

أوضحت النتائج المتحصل عليها ما يلي:

أولا نسبة البقاء بعد التطعيم: وجد ان الكنترول أعطي أعلى نسبة بقاء للنباتات (90 %) بينما الجرعه 50 جراي أظهرت انها جرعه مميته قاتلة للطعم والجرعه 20 جراي أعطت (79 %) أعلى نتائج بين جرعات الجاما بينما أعطي تركيز 0.2 % من إيثيل ميثان سلفونيت أقل النتائج (66.5 %) وأعطي تركيز 0.05 % أعلى نتائج (86.51 %) بين تركيزات إيثيل ميثان سلفونيت.

ثانيا الخصائص المورفولوجيه:

أرتفاع النبات: أعطت أعلى النتائج مع الجرعة 40 جراي (165 سم) بين جرعات التشعيع، بينما أعطي تركيز 0.2% من إيثيل ميثان سلفونيت أعلى النتائج (100.67 سم) بين تركيزات إيثيل ميثان سلفونيت وذلك مقارنة بالاصل.

قطر جذع النبات: أظهرت النتائج المتحصل عليها أن الجرعه 40 جراي كانت أعلى النتائج (1.38 سم) بين جرعات التشعيع، بينما النتيجه الأعلى بين تركيزات إيثيل ميثان سلفونيت لتركيز 0.1% (1.41 سم) مقارنة بالأصل.

عدد الأفرع: وجد أن تركيز 0.2% أعطت أعلى النتائج (7.5) في عدد الأفرع على النبات بين تركيزات إيثيل ميثان سلفونيت، بينما أعطت الجرعه 40 جراي أعلى النتائج (5.83) بين جرعات التشعيع وذلك مقارنة بالأصل.

ثالثا ميعاد تكشف البراعم الورقيه: أوضحت النتائج أن الجرعه 40 جراي مبكره خلال الموسمين في ميعاد الظهور وذلك خلال النصف الاول من مارس، بينما الكنترول متأخرا في النصف الثاني من مارس.

رابعا دراسه درجة القرابه بين نواتج المطفرات والكنترول: أعطت النتائج تقارب الجرعه 20 جراي مع الكنترول وتباعد الجرعه 40 عن الكنترول، بينما أعطت النتائج بين تركيزات إيثيل ميثان سلفونيت تقارب الكنترول مع تركيز 0.05% وتباعد الكنترول عن تركيز0.2%.

تبين انه كلما أزدادت جرعات التطفير الفيزيائيه أو الكيميائية كلما أزدادت درجة التباعد الوراثي عن الأصل.