INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 1, p. 256-264, 2014

RESEARCH PAPER OPEN ACCESS

Performance of *in vitro Rosa* mutant lines developed by the application of gamma irradiation and colchicine

Shahid Javed Butt[1*], Atif Sarwar[1], Nadeem Akhtar Abbasi[1], Sunbal Khalil Chaudhari[2]

[1]*Department of Horticulture, PMAS Arid Agriculture University Rawalpindi, Pakistan*

[2]*Department of Botany, PMAS Arid Agriculture University Rawalpindi, Pakistan*

Key words: *Rosa species,* mutant lines, gamma irradiation, colchicine, plant growth and development, flower yield and quality.

http://dx.doi.org/10.12692/ijb/5.1.256-264 Article published on July 11, 2014

Abstract

Present study is aimed to investigate whether mutations caused by gamma radiations and colchicine treatment are any effective in increasing yield and production of rosa varieties. For this purpose, two mutant lines of rose, *Rosa centifolia* and *Rosa gruss-an-teplitz,* which were treated with different levels of gamma rays and colchicine through solution in Plant Tissue Culture Laboratory of Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi were evaluated. These Rose genotype mutants were sown in the field at similar conditions of irrigation, fertilizers and pest/disease management. Data of various parameters like plant height, shoot length, fresh leaf weight, dry leaf weight, flower diameter, rose water, number of shoots, number of flowers/plant/week, weight of 10 flowers and number of petals was recorded. Statistical analysis of obtained data showed significant variation. When compared to control, gamma radiations showed greater improvement in *Rosa centifolia* but colchicine impact was more pronounced on *Rosa gruss-an-teplitz.*

* **Corresponding Author:** Shahid Javed Butt ✉ sbutt61@yahoo.com

Introduction

Rose is one of the most important commercial flower crops belonging to family Rosaceae which is mostly used in perfumery, cosmetic industry and for medicinal purposes. Origin of Rose is from China and Asia and the name "Rose" was taken from Latin (Rosa) which means pink (Gault and Synge, 1971). One hundered and fifty wild rose species and eighteen hundred cultivars recorded in the literature until now (Weiss, 1997; Gudin, 2000). Rose varieties are grown extensively in the mild part of Northern Hemisphere spreading among Europe, America, Middle East and Asia particularly in Western China (Phillips and Rix, 1988). Major centers of rose are Turkey, Bulgaria, Iran and France dealing in the production of rose-oil and rose-water that are frequently used for multifarious health advantages. For example, rose-oil is highly effective for hard and rough skin consequently improves the complexion, particularly for sensitive and ageing skin (Kataz, 2001). Major species of roses grown in Pakistan for oil production include *R. centifolia, R. damascena*, *R. bourboniana* and *R. gruss-an-teplitz*. Among all *Rosa* species, about 20% species are considered as scented, 50% are low scented and the rest are non-scented (Staikov and Kalaidzhiev, 1972; Huxley, 1992).

Mutation induction methods largely increased gene mutation frequency in vegetatively propagated plants which made them a thousand times more evolved then cultivars produced through natural or conventional methods (Liu and Chen, 1998). The spectrum of useful variations was broadened in plants by application of gama irradiations and colchicine which was necessary for obtaining target mutations speeding up the selection process (Liu, 1998). The turff mutants (GF, GF2) from gamma irradiation compared with germplasm collections (Elm4, Ray, and WS) and standard (Raleigh) indicated the significant difference of growth for disease incidence, winter survival, leaf length, internode length and stolon length by using digital imaging and lines intersect method (Reynolds *et al* 2009).

The frequent genotypic variation in Rosa species (tetraploid , 2n=4x=28), including *R. centifolia* and *R. gruss-an-teplitz* shows high level of genotypic variations which offers better opportunities for genetic crosses. Other than the aspect of beautification, the petals of these two Rosa species have commercial significance and used in perfume industry, food stuff and medicines (Lavid *et al*., 2002). Rose breeders may have the preference for required traits of roses in a breeding program by the application of mutants within shortest possible time. In the present study, two mutant lines *R. centifolia* and *R. gruss-an-teplitz* were used. These lines which were developed using Gamma irradiation and colchicine treatment established in vitro. The focus of the study was to access the field performance of these two species in terms of morphological and yield attributes that will further assist for reliable plant multiplication practices that can be utilized in horticulture for commercial entrepreneurs.

Materials and methods

Present research work was carried out at the research area of PMAS-Arid Agriculture University, Rawalpindi, during the year 2012-2013. Design used for this purpose was randomized complete block design (RCBD) with 3 replications. The experimental material consist of plant of two varieties (*Rosa centifolia* and *Rosa gruss-an-teplitz*) treated *in vitro* with mutagens (Table 1 and Table 2). Plants were treated with different level of gamma radiations (Table 1) and colchicine through solution (Table 2). These treated plants were proliferated and rooted in Plant Tissue Culture Laboratory before acclimitization in green house. Now the mutant lines were taken from the Plant Tissue Culture Laboratory of Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi.

Field was prepared by ploughing and hoeing the field followed by planking. Different plots were prepared for transplanting the rose genotypes mutants of two varieties (*Rosa centifolia* and *Rosa gruss-an-teplitz*). Rose genotypes mutants of two varieties (*Rosa centifolia* and *Rosa gruss-an-teplitz*) were sown in the field on October, 2011. The total length of the

research area was 130 ft and width 33 ft. The plant to plant distance was 3 ft and row to row distance was also 3 ft. All the cultural practices such as irrigation, weeding, hoeing, insects and pest control measures were given uniformly to all the treatments. The parameters such as plant height, shoot length, fresh leaf weight, dry leaf weight, flower diameter were taken and rose water was collected. The data obtained was statistically analyzed through the analysis of variance techniques and the tables of variance were constructed.

Results and discussion

Effect of gamma radiations on Rosa mutant lines

Plant height

Plant height of two rose lines depicted significant difference under varying levels of gamma radiations (Fig. 1). Results revealed the maximum plant height (32.66 inches) for *Rosa centifolia* at T_2. T_1 showed height of 29 inches and it was recorded as 16 inch in control conditions. For *Rosa gruss-an-teplitz,* control showed minimum plant height (15.66 Inch). Pal *et al.*, 1984 also observed that plant height increase by increasing the irradiation doses and the best result of irradiation was shown on sapota and blue blood plant height (Hase *et al.*, 2002).

Table 1. Gamma radiations.

Rose species	Gamma radiation (Gy)						
	T_0	T1	T_2	T_3	T_4	T_5	T_6
Rosa centifolia	00	10	20	30	40	50	60
Rosa gruss-an-teplitz	00	10	20	30	40	50	60

Table 2. Colchicine solutions.

Rose species	Colchicine ($mg^{-1}l$)				
	T_0	T_1	T_2	T_3	T_4
Rosa centifolia	00	100	300	500	700
Rosa gruss-an-teplitz	00	100	300	500	700

Shoot length

Shoot length of both the lines depicted a great difference at different levels of gamma radiations. Maximum shoot length was shown by *Rosa centifolia* (11.33 inches) at T_2 and *Rosa gruss-an-teplitz* showed 7.33 inches at T_1, minimum plant length was shown by control (3.00 inches) in *Rosa centifolia*. The difference between Rosa *centifolia* and *Rosa gruss-an-teplitz* was about (4.00 inches). Calucci *et al.*, (2003) had also studied effect of gamma radiations on shoot length of some herbs and got significant results.

Fresh leaf weight

There was a significant difference seen in fresh leaf weight of both the lines at different levels of gamma radiations. *Rosa gruss-an-teplitz* depicted the maximum fresh leaf weight (74.56 mg) at T_1 and *Rosa centifolia* showed (60.36 mg) at T_2, minimum fresh leaf weight was shown by control (39.20 mg) in *Rosa centifolia*. Fresh leaf weight in case of *Rosa gruss-an-teplitz* and *Rosa centifolia* were showing a significant difference of about (14 mg) and the difference between *Rosa centifolia* and control were observed about (21.36 mg). The decline in fresh weights coincided with the onset of flower wilting and desiccation (Reid *et al.*, 1989). According to our findings gamma rays were most important for inducing fresh leaf weight increase.

Dry leaf weight

Maximum dry leaf weight was observed in *Rosa gruss-an-teplitz* (27.16 mg) at T_1, *Rosa centifolia* shows the dry leaf weight (19.32 mg) at T_2 and the minimum dry leaf weight were recorded in control *Rosa centifolia* (9.26 mg). At comparison *Rosa gruss-*

an-teplitz and *Rosa centifolia* were showing a significant difference of about (7.84 mg) and the difference between *Rosa centifolia* and control were observed about (10.06 mg).Similar results were found by Hong *et al.* (1990) who observed that dry weight of leaves of rose plant was significantly increased as result of gamma rays compared with control in different seasons. (Fig 2).

Table 3. Effect of gamma radiations on both *Rosa* varieties.

Sr.#	Parameters	Control	*Rosa centifolia*	*Rosa gruss-an-teplitz*
1.	Plant height (inches)	16.00 ± 2.73 15.66 ± 1.45	32.66 ± 1.86	29.00 ± 1.53
2.	Shoot length (inches)	3.00 ± 0.88 4.66 ± 0.33	11.33 ± 0.88	7.33 ± 0.33
3.	Fresh leaf weight (mg)	39.20 ± 4.20 56.40 ± 1.48	60.36 ± 4.93	74.56 ± 1.45
4.	Dry leaf weight (mg)	9.26 ± 1.22 15.06 ± 0.69	19.32 ± 1.01	27.16 ± 0.45
5.	Flower diameter (cm)	2.33 ± 0.60 4.80 ± 0.15	5.76 ± 0.21	6.26 ± 0.12
6.	Rose water (%)	2.13 ± 0.20 0.50 ± 0.15	3.70 ± 0.35	1.36 ± 0.12
7.	Number of shoots	1.66 ± 1.20 1.33 ± 0.58	6.00 ± 0.58	4.33 ± 0.58
8.	Number of flowers/plant/week	4.33 ± 0.58 5.33 ± 0.33	11.00 ± 1.00	7.66 ± 0.33
9.	Weight of 10 flowers (gm)	19.09 ± 1.92 12.30 ± 0.56	31.99 ± 2.36	17.33 ± 0.07
10.	Number of petals	21.66 ± 0.88 21.00 ± 1.20	36.00 ± 3.52	34.33 ± 0.58

Floral diameter

Flower diameter of *Rosa gruss-an-teplitz* and *Rosa centifolia* showed a significant at various treatments of gamma radiations. Maximum flower diameter was observed in case of *Rosa gruss-an-teplitz* (6.26 cm) at T_1 and the *Rosa centifolia* revealed the flower diameter of (5.76 cm) at T_2, minimum flower diameter was seen in control (2.33 cm) followed by *Rosa centifolia*. *Rosa gruss-an-teplitz* and *Rosa centifolia* were showing a significant difference of about (0.5 cm) Difference between *Rosa centifolia* and control were observed about (3.43 cm). Our results are not relevant to Bendini *et al.* (2002) who had given the statement based upon his own research that flower diameter has no significant results with the application of gamma radiations.

Rose water percentage

Percentage of rose water in *Rosa centifolia* was recorded as (3.70 %) at T_3 with control (2.13 %) and *Rosa gruss-an-teplitz* (1.36 %) at T_3 with minimum water content in control (0.53 %). The difference between *Rosa centifolia* and *Rosa gruss-an-teplitz* was about (2.34 %) and between *Rosa centifolia* and control was (1.57 %). The difference between *Rosa gruss-an-teplitz* and control was about (0.83 %). Results agreed with Hanson *et al.* (1995) who also observed that gamma radiation show significant increase in rose water percentage in case of *Rosa centifolia*.

Number of shoots

Maximum number of shoots (6) was recorded in *R. centifolia* as compared to lesser number of shoots (1.66) in T_0 (control) treatment. On the other hand, in case of *Rosa gruss-an-teplitz* number of shoots was high in T_1 treatment (4.33) and seen low in T_0 (1.33). Difference between *Rosa centifolia* and control were observed about (4.34). The results supported the observations of Muthuswamy and Pappiah (1976), who conducted experiment on *Jasminum auriculatum* under varying climatic conditions; They found that gamma rays produced beneficial effect on quantity of new shoots. They concluded from their

experimentation that the application of gamma radiation at different ratios increased the number of branches compared to untreated plants in *J. sambac* and *J. auriculatum*.

Table 4. Effect of colchicine solution on both *Rosa* varieties.

Sr.#	Parameters	Control	*Rosa centifolia*	*Rosa gruss-an-teplitz*
1.	Plant height (inches)	15.66 ± 1.45 22.33 ± 0.88	25.00 ± 0.58	25.33 ± 0.88
2.	Shoot length (inches)	4.66 ± 0.33 4.33 ± 0.33	8.00 ± 0.58	7.33 ± 0.33
3.	Fresh leaf weight (mg)	32.18 ± 1.48 31.56 ± 0.81	47.52 ± 0.46	56.06 ± 1.57
4.	Dry leaf weight (mg)	15.06 ± 0.69 10.86 ± 0.65	13.66 ± 0.35	20.10 ± 0.87
5.	Flower diameter (cm)	4.80 ± 0.15 3.70 ± 0.06	5.76 ± 0.15	5.56 ± 0.09
6.	Rose water (%)	1.90 ± 0.06 0.76 ± 0.13	2.83 ± 0.03	1.46 ± 0.15
7.	Number of shoots	1.33 ± 0.56 2.33 ± 0.33	6.33 ± 0.24	4.33 ± 0.18
8.	Number of flowers/plant/ week	6.33 ± 0.55 3.66 ± 0.33	11.33 ± 0.33	8.66 ± 0.33
9.	Weight of 10 flowers (gm)	18.90 ± 0.58 11.90 ± 0.21	28.63 ± 0.56	18.90 ± 0.35
10.	Number of petals	28.00 ± 0.58 19.33 ± 0.33	37.00 ± 0.58	25.66 ± 0.88

Number of flowers / plant / week

In *Rosa centifolia,* maximum number of flowers / plant / week (11.00) was found in T_2 treatment with minimum number (4.33) in T_0 (control) treatment. But in *Rosa gruss-an-teplitz*, it was found that maximum number of flowers / plant / week (7.66) was in T_1 treatment with minimum number (5.00) in T_3 treatment. The difference between *Rosa centifolia* and control were observed about (6.67). Similar findings are reported by Khattak *et al.,* (2011) he recorded 20.6 as maximum number of flowers.

Floral weight

Weights of 10 flowers in *Rosa centifolia* was found maximum in T_2 treatment (31.99) as compared to lowest weights of 10 flowers which were observed in T_0 (control) treatment (19.09). *Rosa gruss-an-teplitz*, the highest weights of 10 flowers were observed in T_1 treatment and in T_0 (control) treatment (12.30). The difference between *Rosa centifolia* and control were observed about (12.00), while *Rosa gruss-an-teplitz* and control showed a difference of (5.03).The results are in consonance with the findings of Nikabakht (2008) who observed that *Rosa gruss-an-teplitz* and *Rosa indica* show the lowest values (1.358g and 1.388g, respectively) for flower weight.

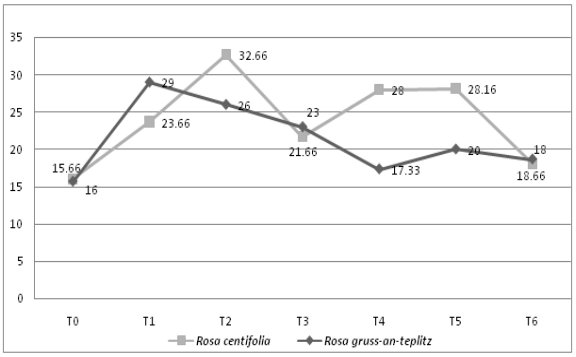

Fig. 1. Stacked line graph showing the effect of gamma radiations on Plant height (inches) of *Rosa*

centifolia and *Rosa gruss-an-teplitz.*

Number of Petals

maximum number of petals (36.00) were noted in T_2 treatment of *Rosa centifolia*, in comparison with lowest number of petals (21.66) in T_0 (control) treatment. While in *Rosa gruss-an-teplitz*, maximum numbers of petals (34.33) were noted in T_1 treatment with lowest number of petals (21.00) in T_0 (control) treatment. *Rosa centifolia* and control difference was about (14.34) and *Rosa gruss-an-teplitz* and control had shown a difference of (13.33). The present results are so much agreed with the findings of Kaur *et al.* (2007) who found that number of petals were 38, 32 and 47, respectively. Similar findings were observed by Tabaei- Aghdaei *et al.* (2007) who pointed out positive correlation between number of petals and number of stamens.

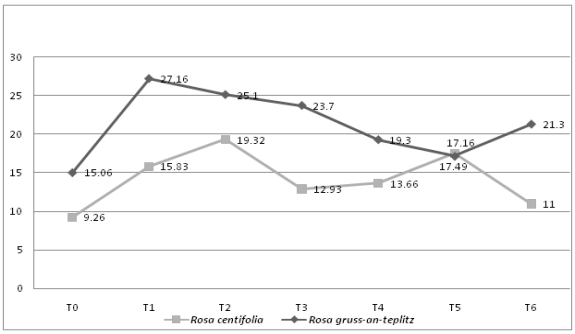

Fig. 2. Stacked line graph showing the effect of gamma radiations on Dry leaf weight (mg) of *Rosa centifolia* and *Rosa gruss-an-teplitz*

Effect of colchicine solutions on Rose mutant lines

Plant Height

Plant height of two rose lines depicted significant difference under varying colchicine treatments. Results revealed the maximum plant height (25.33 inches) in line *Rosa gruss-an-teplitz* for T_1 and (25.00 inches) for T_2 in case of *Rosa centifolia* but the control showed the minimum plant height (17.00 inches) at T_0 in *Rosa centifolia*. These findings were similar to the conclusions made by Mensah *et al.* (2006) earlier. They reported colchicine application increases plant height at moderate application but on higher levels of colchicine, decrease in height occurs.

Shoot length

Maximum shoot length was recorded in *Rosa centifolia* (8.00 inches) at T_2 and *Rosa gruss-an-teplitz* showed (7.33 inches) at T_1, minimum plant length was shown by control (4.33 inches) in both *Rosa* varieties. Amiri *et al.* (2010) had discovered earlier that colchicine treatment enhances shoot growth. Our results confirmed their findings.

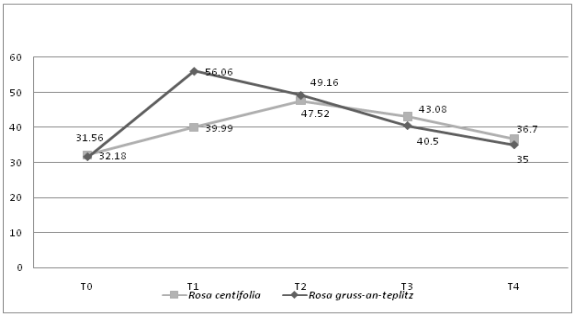

Fig. 3. Stacked line graph showing the effect of cholchicine solutions on Fresh leaf weight (mg) of *Rosa centifolia* and *Rosa gruss-an-teplitz.*

Fresh leaf weight

There was a significant difference seen in fresh leaf weight of both the lines (Fig. 3) at different levels of colchicine treatments. Minimum weight (32.18 mg) was obtained at control T_0 treatment in *Rosa centifolia*. *Rosa gruss-an-teplitz* showed (56.06 mg) at T_2. Fresh leaf weight in case of control and *Rosa gruss-an-teplitz* were showing a significant difference of about (23.88 mg) and the difference between control and *Rosa centifolia* was observed about (8.88 mg). Difference between *Rosa gruss-an-teplitz* and *Rosa centifolia* were observed about (15.34 mg). Data shows the increase in fresh leaf weight (mg) over the application of colchicine confirming results obtained by Amiri *et al* (2010).

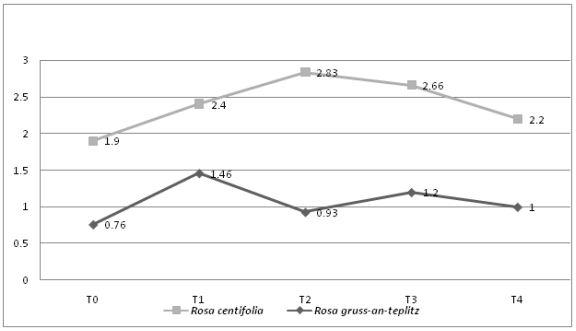

Fig. 4. Stacked line graph showing the effect of cholchicine solutions on Rose water (%) of *Rosa centifolia* and *Rosa gruss-an-teplitz*

Dry leaf weight

At comparison *Rosa gruss-an-teplitz* and control were showing a significant difference of about (14.84 mg) and the difference between *Rosa gruss-an-teplitz* and *Rosa centifolia* was observed about (5.04 mg). *Rosa centifolia* and control difference were observed about (8.35 mg).It confirmed earlier findings that shoot dry weight increases by the application of colchicine (Mensah *et al*., 2006).

Floral diameter

Maximum flower diameter was observed in *Rosa centifolia* (5.76 cm) at T_2 and the *Rosa gruss-an-teplitz* reveal the flower diameter of (5.56 cm) at T_1, minimum flower diameter was seen in control treatment of (3.70 cm). *Rosa centifolia* and *Rosa gruss-an-teplitz* were showing a significant difference of about (0.20) and the difference between *Rosa centifolia* and control was observed about (2.06 cm). Hessayon (1988) observed that varying flower diameters in different rose cultivars showed similar results as found in this research study.

Rose water percentage

Rosa centifolia had shown rose water % age of (2.83 %) at T_2 and it was seen in *Rosa gruss-an-teplitz as* (1.46 %). The difference between *Rosa centifolia* and *Rosa gruss-an-teplitz* was about (1.37 %), *Rosa gruss-an-teplitz* and control difference was (2.07%) and the difference between *Rosa centifolia* and control was observed to be (0.70 %).

Number of Shoots

Number of shoots was significantly different from each other. *Rosa centifolia has shown* maximum shoots (6.33) which were noted at T_1 treatment in comparison with lowest number of shoots (1.33) in T_0 (control) treatment. While *Rosa gruss-an-teplitz* showed number of shoots which was high in T_1 treatment (4.33) and seen low in T_0 (control) treatment (2.33). Senapati and Rout (2010) observed that *Rosa gruss-an-teplitz* and *Rosa centifolia* showed significant results having 2.809 and 2.158 number of shoots, respectively. Shoot multiplication rate varied in different species and was specific to culture medium.

Maximum number of flowers / plant / week

Maximum number of flowers / plant / week showed significant results at various colchicine treatments. Minimum number of flowers / plant / week (6.33) was noticed in T_0 (control) treatment. *Rosa gruss-an-teplitz* showed maximum number of flowers / plant / week (8.66) which was observed in T_1 treatment, while minimum number (3.66) flowers / plant / week was noticed in T_0 (control) treatment. The difference between *Rosa centifolia* and *Rosa gruss-an-teplitz* was about (2.67). The useful mutant lines isolated and treated with colchicine to establish any changes in locus for the increase of number of flower/plant/week.

Floral weight

Weights of 10 flowers showed significantly different results from each other. *Rosa centifolia* showed maximum weights of 10 flowers in T_2 treatment (28.63) as compared to lowest weights of 10 flowers which were observed in T_0 (control) treatment (18.90). *Rosa gruss-an-teplitz* showed highest weights of 10 flowers which was observed in T_1 treatment (18.90). Previously it was noted that, Colchicine solution has no significant effect on increase in flower weight reported by (Barnabas *et al.* 2009). In another study it is revealed that *R. centifolia* showing great variation in its weight as compared to *R. grusa-an-teplitz* (Ojomo *et al*., 2007). However in this research, significant increase in floral weight was recorded.

Number of petals

Number of petals created a significant difference in between both lines. *Rosa centifolia* have shown maximum number of petals (37.00) in T_2 treatment in comparison with lowest number of petals (28.00) in T_0 (control) treatment. The results were followed by other treatments T_3 (35.00), T_1 (33.66) and T_4 (30.33) respectively. *Rosa gruss-an-teplitz* found to have maximum numbers of petals (31.00) in T_1 treatment in comparison with lowest number of petals (19.33) in T_0 (control) treatment.

Conclusion

Gamma radiations in *Rosa centifolia* enhanced growth by increasing plant height (32.66 inches), shoot length (11.33 inches), rose water percentage (3.70 %), number of shoots (6.00), number of flowers/plant/week (11.00), weight of 10 flowers (31.99) and number of petals (36.00). Treatment with gamma rays in *Rosa-gruss-teplitz* increased fresh leaf weight, dry leaf weight and flower diameter significantly. Colchicine treatment in *Rosa gruss-an-teplitz* increased plant height, fresh leaf weight and dry leaf weight up to (25.33 inches), (56.06 mg) and (20.10 mg) respectively. Colchicine in *Rosa centifolia* increased shoot length, flower diameter, rose water percentage (8.00 inches), (5.76 cm), (2.83 %) respectively. This data was statistically analyzed and showed greater variation in its results by the application of gamma radiations and colchicine treatment. Gamma radiations enhanced growth in *Rosa centifolia* but colchicine impact was more pronounced on *Rosa gruss-an-teplitz* . Control results were less significant as compared to treatment applications.

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