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Creation of Variation through *In-vitro* Mutagenesis using Gamma radiation in Rose (*Rosa hybrida* L.) Variety 'Lửa'

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ABSTRACT

Rosa hybrida L. var. Lửa traditionally an important rose often used for ornamental purposes in Sa Dec city, Dong Thap province. This study aimed to induce variation through in-vitro gamma ray irradiation in the rose variety 'Lửa' for further improvement in rose breeding. In-vitro single node cuttings (25 in-vitro cuttings per treatment with four replications) were irradiated with different doses of γ-rays (0, 5, 10, 15, 20, 25 or 30 Gy) using a ^{60}Co source (India) at Da Lat Nuclear Research Institute. The γ-irradiated explants were then cultured aseptically on Murashige and Skoog's basal medium supplemented with 1.0 mg/L BAP to induce of multiple shoots, shoot proliferation and acclimatization at Division of Vegetables, Flowers and Landscaping of Southern Horticultural Research Institute (SOFRI). The LD_{50} dose was determined at 20 - 25 Gy treatments and these doses affected the multiplication rate, growth in-vitro and ex-vitro survival rate. Three types of mutants with altered or novel flower color in comparison to original flower color were isolated, such as Type 1 with orange-pink (Red 52C), Type 2 with pink (Red 54B) and Type 3 with Red 45AB with Red 54B bicolor.

1. INTRODUCTION

Rose (*Rosa hybrida* L.; Family: Rosaceae) is an important ornamental crop. Its chromosome set is n=7, and the commercial plants could be diploid (2n) or triploid (3n) and comprises more than 150 species. It is valued in the international cut flower market because of its attractive flowers with a long vase life (Datta, 1997). The rose variety 'Lửa' in Viet Nam it is one of two important rose varieties in Sa Dec city, Dong Thap province.

Currently, domestic demand for potted rose flowers is increasing day by day. To meet the market demand, it is necessary to produce new varieties for breeding roses. More than 3,300 mutant plant varieties have been registered in the Food and Agriculture Organization (FAO)/ International Atomic Energy Agency (IAEA) Mutant Variety Database, indicating the widespread use of mutagenesis in modern plant breeding. Gamma radiation in particular has become popular; approximately half of all mutant varieties registered were created using gamma rays (International Atomic Energy Agency, 2021).

Inducing mutation in combination with *in-vitro* culture methods is now considered as an effective method for plant improvement in several vegetative

propagated crops such as flowers and ornamental plants. A large number of studies on *in-vitro* mutagenesis rose by gamma-ray irradiation have published (Smilansky *et al.*, 1986; Datta, 1997; Arnold *et al*., 1998; Mohan Jain, 2006; Chakrabarty and Datta, 2010; Kahrizi *et al.*, 2011; Baig *et al*., 2012; Aamir *et al*., 2016; Bala and Singh, 2016). Many new rose varieties have been developed for commercial production by the use of *in-vitro* mutation-assisted breeding techniques, typically three mutant rose varieties: Rosmarun, Yulikara and Rosanda (Jain, 2006).

In Vietnam, mutation-assisted breeding of rose has been carried out and initial results have created a source of mutant materials, but have so far remined unpublished. The objective of this research is to induce variation through *in-vitro* mutagenesis using gamma radiation in the rose variety 'Lửa' for further improvement of rose breeding in Dong Thap province.

2. MATERIALS AND METHOD

2.1. Plant Materials and Irradiation Treatments

The study was carried out at the Division of Vegetables, Flowers and Landscaping of **Southern Horticultural Research Institute** (SOFRI) in Chau Thanh district, Tien Giang province, Viet Nam, under tropical climatic conditions from January 2017 to April 2018. Shoots from vigorous and healthy *in-vitro* plantlets of *Rosa hybrida* L. var. 'Lửa' were cultured on Murashige and Skoog's (MS) basal medium supplemented with 1.0 mg/L BAP and obtained from the tissue cultural laboratory were used as explants for this study. Cultural conditions were maintained at 26 ± 2^0C under cool-white lamps for a 14 hour photo-period.

Before γ-irradiation treatment, *in-vitro* single node cuttings (size of cutting around 1 cm) were cultured on Petri dishes containing MS medium supplemented with 2.0 mg/L BAP. After 14 days in shoot proliferation medium (Figure 1a), *in vitro* single node cuttings (25 *in-vitro* cuttings per treatment with four replications) were irradiated with different doses of γ-rays (0, 5, 10, 15, 20, 25 or 30 Gy) using a ^{60}Co source (India) at Da Lat Nuclear Research Institute.

2.2. Shoot Proliferation, Shoot Elongation and Acclimatization

The γ-irradiated explants were then cultured aseptically on MS basal medium supplemented with 1.0 mg/L BAP to induce shoot multiplication, then sub-cultured twice for 30 days per time on the same medium for shoot proliferation. Shoot clusters were continuously transferred onto an elongation medium consisting MS medium supplemented with 0,7% agar and 30 g/L sucrose. These clusters were transferred into a MS without any PGRs medium in the growth room. The shoot clusters were sub-cultured twice, 30 days for each.

For rooting stage, elongated shoots (with 3-4 leaves) were transferred individually to plastic bags containing 1/3 MS basal medium supplemented with 0,7% agar and 30 g/L sucrose. After 30 days of culture, *in-vitro* rooted plantlets were carefully, take out of plastic bags and washed to removed agar. The roots were then dipped in 0.1% copper oxychloride for 30 second and plantlets were planted on 112 holes of tray with a 1:1 (v/v) cocopeat and white peat potting substrate for acclimatization. Plantlets were maintained in net-house for 3 days with 60-70% moisture, 50% shading and 30 ± 2^0C condition.

2.3. Screening

Plantlets were planted in plastic pot (5 x 6 cm) with a 1:1 (v/v) cocopeat and white peat potting substrate for morphological characters of screening at the similar condition.

2.4. Data and Statistical Analysis

The percentages of survived explants at 60 days after treatment, number and diameter of *in-vitro* clusters of explants at 110 days after treatment, in each treatment were recorded as the data of gamma irradiation study. Lethal dose 50% (LD_{50}) were determined as the dose at which 50 percentage of the explants survived.

The data were statistically processed using the SPSS 22.0 program, and the mean was compared by Duncan's multiple range test at $p\leq0.05$. Means followed by the same letters are not significantly different according to Duncan's multiple range test ($p\leq0.05$).

The plantlets derived from gamma irradiation treatments were compared against the untreated parental control plantlets grown under the same condition. After one-month planting, the plantlets height (cm), number of leaves, flower diameter (cm), number of petals were measured in M1V1 plantlets. The flower colors were measured with the Royal Horticultural Society (RHS) color chart, London, United Kingdom. Ratio of flower color

change was calculated as the percentage of the plants according to their screened plant number.

3. RESULTS AND DISCUSSION

3.1. Effect of gamma irradiation on survival rate of in-vitro explants and plantlets after hardening

The results in Figure 1 showed that in the shoot proliferation stage, all gamma rays had an effect on the survival rate of *in-vitro* explants. At 60 days after treatment, the survival rate was different between the treatments, ranging from 40 - 92%. The dose of 15 Gy showed the highest survival rates (92%) as compared with 20 Gy, 25 Gy and 30 Gy doses but was not different when compared with 0 Gy (88%), 5 Gy (76%) and 10 Gy (86%).

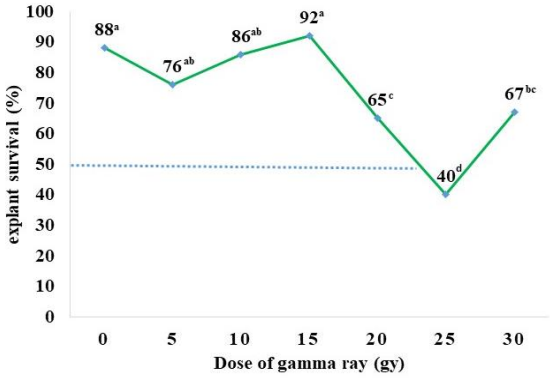

Figure 1. Effect of gamma irradiation on the survival explants in rose (*Rosa hybrida* L.) var. 'Lửa'

According to Bala and Singh (2015), high dose irradiation on the rose variety Raktima made the explants grow poorly, stunted growth and they did not survive in the first subculture. In addition, high dose irradiation also caused morphological abnormalities in explants and leaves.

Thus, *in-vitro* 'Lửa' rose cultures were significantly affected by gamma-ray irradiation with different irradiation doses. Lethal dose 50% (LD_{50}) was observed at 25 Gy (40%) at 60 days after irradiation. Two doses of 20 Gy and 30 Gy were also shown to have a strong effect on the survival rate of the explants, while doses of 5, 10 and 15 Gy showed less effect.

According to Kahrizi *et al*. (2011), LD_{50} in two rose varieties 'Mourossia' and 'Apollo' were 66 and 67 Gy. According to Bala and Singh (2015), LD_{50} was determined to be 40 Gy in the rose variety Raktima. Many studies have reported a significant effect of gamma-ray irradiation on rose such as Smilansky *et al*. (1986); Datta (1997); Arnold *et al.* (1998); Jain (2006); Chakrabarty and Datta (2010); Kahrizi *et al.* (2011); Bala and Singh (2015, 2016). However, in the different varieties of roses, the reported lethal dose was different.

3.2. Effect of gamma irradiation on number and diameter of in-vitro clusters of explants

The number of shoots per cluster at 110 days showed significant differences between treatments, ranging from 10.7 – 18.8 shoots (Figure 2a). The 0 Gy dose showed highest (18.8 shoots) and significant different with 25 Gy dose (10.7 shoots) but no-significant difference between 5 Gy, 10 Gy, 15 Gy, 20 Gy and 30 Gy doses was detected.

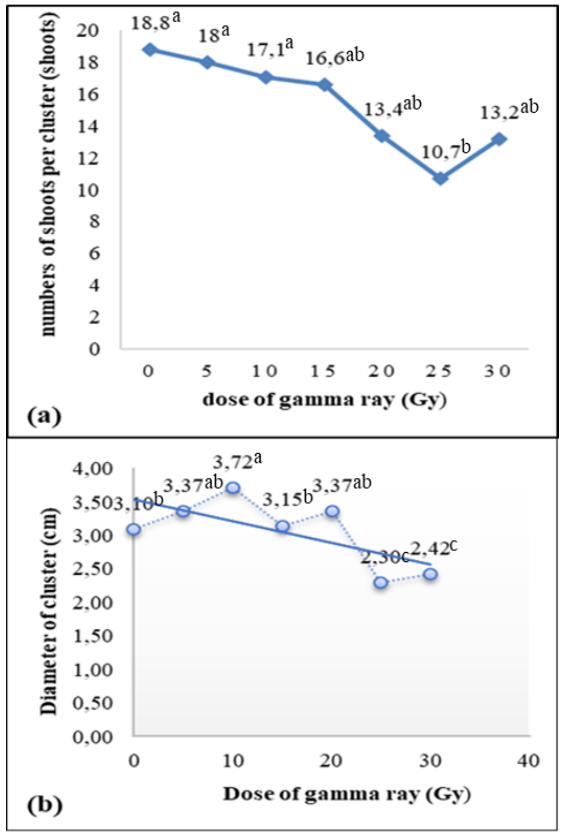

Figure 2. Effect of gamma irradiation on the number (a) and diameter of clusters (b) at 110 days after *γ*-irradiation treatment of *in-vitro* explants in rose (*Rosa hybrida* L.) var. 'Lửa'

Figure 2b show that at 110 days after treatment, the smallest cluster diameter was recorded for the dose of 25 Gy (2.3 cm) followed by the 30 Gy dose (2.42

cm), which were different compared with other treatments. Moreover, the largest cluster diameter was recorded at 10 Gy dose (3.72 cm), followed by 0 Gy, 5 Gy, 15 Gy and 20 Gy doses (3.37 cm).

Thus, gamma-ray irradiation at doses of 5, 10, 15, 20, 25 and 30 Gy showed an effect on *in-vitro* shoot development of the rose variety 'Lửa'. However, the higher doses showed stronger effects than the lower doses. The effect on the growth of explants included reducing shoot diameter and the ability to multiply shoots.

This result is also consistent with studies on mutagenesis in roses by gamma-ray irradiation, which hightlight that higher of gamma-ray irradiation dose tended to reduce the growth potential of *in-vitro* explants on rose (Smilansky *et al*. (1986); Arnold *et al*. (1998); Kahrizi *et al*. (2011); Bala and Singh (2015)).

However, similar to the survival rate, the number and diameter of *in-vitro* clusters of explants at 30 Gy dose were also higher than at 25 Gy. The 30 Gy dose tended to increase the vigor and growth potential of *in-vitro* explants on rose var. 'Lửa'.

3.3. Effect of gamma irradiation on the number and ratio of survived plantlets

At the hardening stage (Table 1), the total number of plantlets obtained to the stage of the nursery was 1,096 (154 plants derived from 5 Gy treatment, 272 plants (10 Gy), 344 plants (15 Gy), 83 plants (20 Gy), 12 plants (25 Gy), 38 plants (30 Gy) and 193 plants (the control)).

Table 1. Effect of gamma irradiation doses on the number and ratio of survived plantlets in rose (*Rosa hybrida* L.) var. 'Lửa'

Dose of gamma rays (Gy)	No. of survived plantlets	Ratio of survived plantlets (%)
0 (Control)	193	51,0
5	154	21,7
10	272	21,9
15	344	30,0
20	83	15,6
25	12	17,0
30	38	11,4
Total	1,096	

The survival rate after 30 days of hardening was 51,0% for the non-irradiated control dose. This was similar to the research results of Le Nguyen Lan Thanh *et al*. (2020), who reported that the percentage of survival plantlets after 15 days of hardening on 'Lửa' roses ranged between 59.5 - 69.0%. At irradiated treatments, the survival rate of plantlets after hardening was lower than in the control dose, the highest survival rate was only 30% at the dose of 15 Gy and the lowest survival rate was 11.4% at the dose of 30 Gy. This shows that gamma-ray irradiation doses affected on the survival rate of plantlets at the nursery stage.

Thus, at the stages of rooting and hardening, the plantlets at irradiated doses were still affected by the effects of gamma-ray irradiation compared with the non-irradiation doses. The higher dose from 20 Gy onwards strongly affected the survival rate of plantlets at the hardening stage.

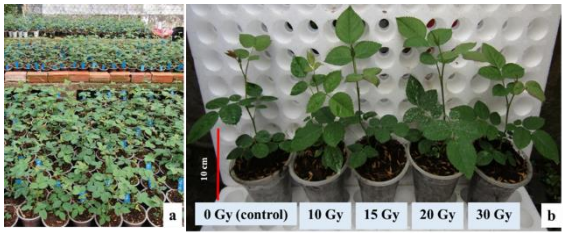

Figure 3. All untreated (control) and treated plantlets in net-house at SOFRI (a), some plantlets at control and γ-irradiation treatments in one month after acclimatization (b)

This result is in accordance with the results of Bala and Singh (2015), gamma-ray irradiation still affected the survival rate of rose 'Raktima' at *ex-vitro* stage. However, in this study, at 30 Gy dose, some plantlets showed better vigor than at the dose of 25 Gy. This was recorded obviously at the stage of planting, some plantlets at 15, 20 and 30 Gy doses were higher and stronger with larger leaves than at control dose (Figure 3b).

3.4. Effect of gamma irradiation on morphological characters

3.4.1. Effect of gamma irradiation on plant characters

Of the 1,096 plantlets (Figure 3a), there were some screened flowered plants (Table 2). Gamma irradiation would induce noticeable morphological changes in plants. The observation about morphological characters made in 199 M1V1 plants are summarized Table 2.

The plant height derived from explants treated of M1V1 plantlets exposed by all treatment were different than control. The number leaves of all

treatments also changed when compared with the control, but not clearly.

Table 2. Effect of gamma irradiation on plant characters in rose (*Rosa hybrida* L.) var. 'Lửa'

Dose of gamma rays (Gy)	No. of screened plants in field (199)	Plant height (cm)	No. of leaves
0 (Control)	17	18.0 ± 1.1	6.3 ± 1.0
5	32	19.1 ± 1.7	5.9 ± 0.9
10	55	17.9 ± 2.2	6.1 ± 0.9
15	59	18.9 ± 1.6	6.7 ± 0.8
20	17	19.4 ± 2.4	6.5 ± 1.1
25	4	17.3 ± 2.2	5.8 ± 1.0
30	15	19.2 ± 2.6	7.0 ± 1.0

Gamma irradiation and/or variable temperatures, low humidity, or high light intensity, might have contributed to the reduction in survival percentages when plantlets were transferred to *ex-vitro* environments. An inhibitory effect of γ-radiation on rose plant height was noticed, as has been reported previously (Ferol 1996, Banerjee and Datta 2002 and Senapati and Rout 2008).

3.4.2. Effect of gamma irradiation on flower characters

The results in Table 3 show that gamma ray irradiation has an effect on flower diameter, number of petals and flower color on 'Lửa' rose variety. The flower diameter in the gamma rays ranged from 5.4 -5.6 cm and showed a larger mean value than the control certificate of 5.1 cm, yet the flower diameter in the gamma rays was much higher and more variable than the control.

The corresponding results were recorded in the petal index and in the 25 Gy dose with the least number of petals (8.7 petals). In addition, irradiation dose for the average number of petals were 16.8-17.7 petals high, such as 10, 15, 20 and 30 Gy doses, but varied more than the control (16.5 petals).

Table 3. Effect of gamma irradiation on flower characters in rose (*Rosa hybrida* L.) var. 'Lửa'

Dose of gamma rays (Gy)	Diameter flower (cm)	No. of petals (petal)	Color	Ratio flower color change (%)[x]
0 (Control)	5.1 ± 0.1	16.5 ± 3.1	Red43AB (17)	0
5	5.5 ± 0.4	16.4 ± 3.0	Red 52C (1), Red 43AB (41)	0.03
10	5.5 ± 0.5	16.8 ± 2.9	Red 43AB (55)	0
15	5.4 ± 0.5	16.6 ± 3.4	Red 43 AB (53), Red 42A (1), Red 44A (1), Red 45AB (1), Red 46A (1), Red 54B (1) and Red 45AB with Red 54B bicolor (1)	0.11
20	5.6 ± 0.5	17.7 ± 3.9	Red 45B (17)	100
25	5.4 ± 0.3	8.7 ± 4.7	Red 45B (4)	100
30	5.5 ± 0.7	16.6 ± 2.6	Red 45AB (15)	100

Note: Number in barcket is number of plant measured by RHS, [x] : percentage of the plants according to their screened plant number.

As for flower color, the results in Table 3 show that gamma ray irradiation has a large change in flower color in this rose at high irradiation doses of 15, 20, 25 and 30 Gy. However, the flower color diversity was recorded at 15 Gy dose (0.11%) with many different color levels according to the RHS color chart such as Red 42A (1), Red 44A (1), Red 45AB (1), Red 46A (1), Red 54B (1) and bicolor Red 45AB with Red 54B (1) compared with a control color level Red 43 AB.

Meanwhile, although high exposure doses of 20-30 Gy give a high rate of flower color change (100%) but only 1-2 higher red levels than Red 45AB. In addition, at a low irradiation dose of 5 Gy, a flower color change rate of 0.03% was obtained with a color level of Red 52C.

The flower colors of plants were different with control plants such as Type 1 with orange-pink (Red 52C) derived from 5 Gy treatment, Type 2 with pink (Red 54B) and Type 3 with Red 45AB with Red 54B bicolor derived from 15 Gy treatment (Figure 4).

Thus, gamma irradiation induced changes in flower color, number petals and flower diameter in rose (*Rosa hybrid* L.) cv. 'Lửa'. The induction of color mutations after gamma-ray irradiation agrees with previous results in ornamental crops such as chrysanthemum, carnation, gladiolus and roses (Singh *et al*. 2000, Nonomura et al. 2001, Dao et al. 2006, Kim *et al*. 2006, Yamaguchi *et al*. 2008, Bala and Singh, 2016). However, in order to determine whether these types are stable and genetically distinct, further evaluation at M1V4 and genetic analysis studies are necessary.

Figure 4. Flower of rose (*Rosa hybrida* L.) cv. 'Lửa' (control) and three types of flower color mutants: Type 1 (b), Type 2 (c) and Type 3 (d).

4. CONCLUSION

The results show that the treated doses affected the multiplication rate, *in-vitro* growth and *ex-vitro* survival rate of the rose variety 'Lửa'. The LD_{50} was determined at 20 - 25 Gy treatments. Three types of flower color mutants with altered or novel flower color in comparison to the original could be isolated, including Type 1 with orange-pink (Red 52C), Type 2 with pink (Red 54B) and Type 3 with Red 45AB with Red 54B bicolor.

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