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 has been one of the most important roses for a long time and is most often used for ornamental purposes in Sadec city, Dong Thap province. The 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 60Co 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 multiple shoots, shoot proliferation, and acclimatization at Division of Vegetables, Flowers and Landscaping of Southern Horticultural Research Institute (SOFRI). The LD50 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 the 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.

Keywords
Gamma ray irradiation, in vitro, mutagenesis, Rosa hybrida, variation

1. INTRODUCTION

Rose (Rosa hybrida L.; Family: Rosaceae) is an important ornamental crop, chromosome set is n=7, and 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 long vase life (Datta, 1997). The rose variety ‘Lửa’ in Vietnam is one of two important rose varieties in Sadec city, Dong Thap province.

Currently, domestic demand for potted flowers in rose 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 been 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 being considered as an effective method for plant improvement in several vegetative propagated crops such as flowers and ornamental plants. Many pieces of research on in-vitro mutagenesis rose by gamma-ray irradiation had been 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 were created in the world and developed for commercial production by the use of in-vitro mutation-assisted breeding techniques, typically three mutant rose varieties: Rosmarin, Yulikara, and Rosanda (Jain, 2006).

In Vietnam, mutation-assisted breeding on roses has been carried out and initial results created a source of mutant materials but have not been published yet. The objective of this research is to induce variation through in-vitro mutagenesis using gamma radiation in the rose variety ‘Lùa’ for further improvement on 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 Longdinh commune, Chau Thanh district, Tien Giang province, Vietnam, under tropical climatic conditions from January 2017 to April 2018. Shoots from vigorous and healthy in-vitro plantlets of Rosa hybrida L. var. ‘Lùa’ cultured on Murashige and Skoog’s (MS) basal medium supplemented with 1.0 mg/L BAP and obtained from the tissue culture laboratory were used as explants for this study. Cultural conditions were maintained at 26±2°C under cool-white lamps for 14-hour photo-period.

Before γ-irradiation treatment, in-vitro single node cuttings (size of cutting around 1 cm) were cultured on Petri dishes contained with 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 60Co source (India) at Dalat 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 subcultured twice for 30 days per time on the same medium for shoot proliferation. Shoot clusters were continuously transferred onto an elongation medium consisting of MS medium supplemented with 0.7% agar and 30 g/L sucrose. These clusters were transferred into an MS without any PGRs medium in the growth room. The shoot clusters were subcultured twice, 30 days for each.

For the 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 taken out of plastic bags and washed to removed agar. The roots were then dipped in 0.1% Copper oxychloride for 30 seconds and plantlets were planted on 112 holes of a 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°C condition.

2.3. Screening

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

2.4. Data and Statistical Analysis

The percentages of survived explants at the 60 days after treatment, the number and diameter of in-vitro clusters of explants at 110 days after treatment, in each treatment were recorded as the data of the gamma irradiation study. A lethal dose of 50% (LD50) was determined at the dose of the 50% of survived explants.

The data were statistically processed using the SPSS 22.0 program, and the mean was compared by Duncan’s multiple range test at p≤0.05. Means followed by the same letters are not significantly different according to Duncan’s multiple range test (p≤0.05).

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 plantlet height (cm), number of leaves, flower diameter...
and number of petals were measured in M1V1 plantlets. The flower colors were measured with the Royal Horticultural Society (RHS) color chart, London, United Kingdom. The ratio of flower color change were calculated the percentage of the plants according to their screened plant number.

3. RESULTS AND DISCUSSION

3.1. Effect of gamma irradiation on the 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 affected the survival rate of in-vitro explants. At 60 days after treatment, the survival rate was a difference between the treatments, ranging from 40 - 92%. The dose of 15 Gy showed the highest survival rates (92%) were different as compared with 20 Gy, 25 Gy and 30 Gy doses but were not different as compared with 0 Gy (88%), 5 Gy (76%) and 10 Gy (86%).

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

Thus, in-vitro ‘Lira’ rose cultures were significantly affected by gamma-ray irradiation with different irradiation doses. A lethal dose of 50% (LD₅₀) 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 had less effect.

According to Kahrizi et al. (2011), LD₅₀ in two rose varieties 'Mourossia' and 'Apollo' were 66 and 67 Gy. According to Bala and Singh (2015), LD₅₀ was determined to be 40 Gy in the rose variety Raktima.

There were many studies 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 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 significance between treatments, ranging from 10.7 – 18.8 shoots (Figure 2a). The 0 Gy dose showed the highest (18.8 shoots) and significant difference with 25 Gy dose (10.7 shoots) but non-significant difference with 5 Gy, 10 Gy, 15 Gy, 20 Gy, and 30 Gy doses.

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Gy dose (2.42 cm) were different compared with other treatments, and 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) but not different between each other.

Thus, gamma-ray irradiation at doses of 5, 10, 15, 20, 25, and 30 Gy showed affecting on shoot development of the in-vitro ‘Lưu’ rose; however, the higher doses showed stronger affecting than lower doses. The affecting on the growth of explants included reducing the diameter of shoot clusters and the ability to multiply shoots.

This result is also consistent with studies on mutagenesis in roses by gamma-ray irradiation that the higher 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, number, and diameter of in-vitro clusters of explants at 30 Gy dose were also higher than at 25 Gy. It was shown that at the 30 Gy dose, it was tended to increase the vigor and growth potential of in-vitro explants on rose var. ‘Lưu’.

### 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)).

| 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 result of Thanh (2020), the percentage of survival plantlets after 15 days of hardening on ‘Lưu’ roses ranged from 59.5 to 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 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 non-irradiation doses. The higher dose from 20 Gy onwards strongly affected the survival rate of plantlets at the hardening stage.

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 had 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 large leaves than at the 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 with M1V1 plantlets exposed by all treatment were different from the control. The number of leaves of
all treatments were 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. ‘Liña’

| 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 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 affected flower diameter, number of petals and flower color on ‘Liña’ rose variety. The flower diameter in the gamma rays from 5.4 - 5.6 cm showed a larger mean value than the control certificate of 5.1 cm, but the flower diameter in the gamma rays was much higher and more variable with certificate.

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 doses 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 certificate (16.5 petals).

Table 3. Effect of gamma irradiation on flower characters in rose (*Rosa hybrida* L.) var. ‘Liña’

| 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            | Red 43AB (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                              |
|                          |                      |                       | 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                           |
| 15                      | 5.4 ± 0.5            | 16.6 ± 3.4            | Red 45B (17)                               | 100                            |
| 20                      | 5.6 ± 0.5            | 17.7 ± 3.9            | Red 45B (4)                                | 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 brackets is the number of plants 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 bicolor (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%) 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 from 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).

Figure 4. Flower of rose (*Rosa hybrida* L.) cv. ‘Liña’ (control) and three types of flower color mutants: Type 1 (b), Type 2 (c), and Type 3 (d).
Thus, gamma irradiation-induced changes in flower color, number of petals, and flower diameter in rose (Rosa hybrida L.) cv. ‘Lúra’. 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, to determine whether these types were stable and genetically distinct, further evaluation at M1V4 and genetic analysis studies are necessary.

4. CONCLUSIONS

The results showed that these treated doses affected the multiplication rate, growth in-vitro, and ex-vitro

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