In Vitro Induction of Flower Mutation in *Catharanthus roseus* Using Gamma Irradiation

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ABSTRACT

The purpose of this study was to study the effect of gamma irradiation and *in vitro* culture on mutation induction of flower color in *Catharanthus roseus*. A number of plants were regenerated from irradiated internode explant. Tow cultivars from previous study Rosea and Alba, and four doses of gamma irradiation (0.0, 0.5, 0.75 and 1.0 Gy) were applied. Obtained results indicated that both 0.75 and 1.0 Gy irradiation doses were the most effective in inducing mutation in flower shape and color, while the dose of 0.5 Gy was effective in inducing mutation in leaf form.

Key words: *Catharanthus roseus*, irradiation dose, mutations.

INTRODUCTION

Catharanthus roseus is an important medicinal plant, commonly known as Madagascar periwinkle. It is a perennial, evergreen herb, 30-100 cm height that was originally native to the island of Madagascar. It has been widely cultivated for hundreds of years and can now be found growing wild in the world warmest regions. The leaves are glossy, dark green (1-2 inch long), oblong-elliptic, acute, rounded apex; flowers white to pinkish purple in terminal or axillary cymosely clusters; follicle hairy, many seeded, 2-3 cm long; seeds oblong, minute and black. The plant is commonly grown in gardens for beddings, borders and for mass effect. It blooms throughout the year and is propagated by seeds or cuttings. The blooms of natural wild plants are pale pink with a purple eye in the center. The plant has historically been used to treat a wide assortment of diseases. It was used as folk remedy for diabetes in Europe for centuries (Swanston-Flatt *et al*. 1989). It is also used as prevention of cancer, cancer treatment, reduction of high blood pressure, externally against nose bleeding.

Mutation breeding of ornamental plants has been successfully applied for varietal improvement of many crop species. Some problems remain in inducing mutations of vegetative propagated plants, such as chimera formation (Stewart and Dermen, 1970), relatively low mutation frequency, and a limited mutation spectrum (Broertjies and Van Harten, 1978).

Since Broetjies *et al*. (1976) proposed that *in vitro* regeneration could solve the problem of chimera formation; a mutation induction method using *in vitro* technique has been applied to certain chrysanthemums (De Jong and Custer, 1986; Huttema *et al*., 1991; Preil *et al*., 1991). This *in vitro* technique represents a great potential for mutation breeding. However, the problems with low mutation frequency and the limited mutation spectrum have remained unsolved. Recently, induction of mutations based on ionizing radiations is one of the major breeding approaches for plant improvement. More than 2,300 mutant varieties have been released using irradiation mutagenesis (Jain, 2005) and among them 566 represent ornamental plants. Further, a combination of *in vitro* technique and irradiation induced mutagenesis has been recommended to improve cultivars of vegetatively propagated plants (Maluszynski *et al*., 2000). Some of the selected important agronomical traits of mutant ornamental plants were flower color, flower morphology, flowering time and resistance to abiotic and biotic stress (Das *et al*., 2000; Misra *et al*., 2003). The basic requirement for an effective use of irradiation mutation in plant breeding programs is the analysis of radio-sensitivity of the explants material (Walther and Sauer, 1986). Predieri and Gatti (2001) reported that one of the first steps in mutagenic treatments is the estimation of the most appropriate dose to apply.

Therefore, the purpose of the present investigation is to study the effect of gamma rays doses (0.0, 0.5, 0.75, 1.0 Gy) to induc mutation on two cultivars (Rosea and alba) of *Catharanthus roseus.*

MATERIALS AND METHODS

Source of plant material and *In vitro* mutagenesis

Internodes were collected from the cutting of two cultivars of *Catharanthus roseus* (Rosea and Alba) obtained from previous study (El- Mokadem, 2013). The internode explants were irradiated in a gamma cell with a cobalt 60 source at the National Center of Radiation Research and Technology, Nasr City, Cairo, Egypt. The radiation doses were 0.0 (control), 0.5, 0.75 and 1.0 Gy. Internodes were cut to 5 to 6 mm long. They were first washed with detergent and distilled water, then disinfected with 15% chlorine bleach plus two drops of Tween 20 for 15 min, then placed in ethanol 70% for one minute and rinsed three times with autoclaved distilled water, to ensure that the internodal

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Received Febuary13, 2014, Accepted March 26, 2014

parts (explants) are totally sanitized. Explants were cultured on solidified media containing 4.43 g/l MS with vitamins (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose, 7g/l agar and 4.0 mg/l BA, the pH was adjusted at 5.8 ± 0.1. This medium is reported to be the best for shoot formation (El- Mokadem, 2013), which was poured in tubes (2.5 cm diameter × 15 cm height), Each tube with one explant considered as replication. Ten replications were used for each dose. After culture the explants, tubes were sealed with parafilm and placed under a 16/8h photoperiod at 21 ◦C. After one month of incubation, the explants that formed shoots were sub cultured to the same medium but with addition of 1.0 mg/l IAA for root induction.

Transfer of *in vitro* grown plantlets to greenhouse

After shoots and roots development, regenerated plantlets were washed with tap water to remove any medium traces from the roots and were transplanted in pots filled with peat moss, perlite and sand (1:1:1 v/v/v). The pots were incubated under moist conditions in the greenhouse for acclimatization. Plants in the greenhouse were screened for variation in morphological characters of leaves and flowers.

Statistical design and analysis

Data were statistically analyzed as a factorial experiment with two factors; radiation dose and cultivar in a randomized complete design (RCD) with 10 replicates (4 gamma ray doses x 2 cultivars x 10 replicates = 80 internodes). All obtained data were subjected to analysis of variance (ANOVA) and data with percentage was subjected to arcsine transformation prior to statistical analysis according to Steel and Torrie (1980). Comparisons among means were made using the least significant differences test (LSD) at 0.05 level of probability. The data were analyzed using SAS program version 6 (1985).

RESULTS AND DISCUSSION

Effect of gamma irradiation on morphological characters

Plantlets transferred to the greenhouse (after rooting and acclimatization) were screened for variation in the morphological characters.

Leaf variation

The obtained data of both cultivars showed a wide range of leaf shape abnormalities compared to the leaves of control plantlets which was oblong-elliptic (Fig.1). Plantlets treated with 0.5 Gy gave divided leaf form while, the 0.75 and 1.0 Gy treated plantlets resulted in leaves with wavy margins.

More than one factor may be responsible for such variation in leaf patterns. A possible explanation is that the alteration in the ontogeny of leaf tissues as a result of irradiation is through the selective destruction of one or more cell layer in the shoot meristem (El-Shennawy *et al*.2011 on *Encelia farinosae)*. Other leaf changes, especially those with distorted patterns of development may be resulted as induced polyploidy as reported by Love (1966) on *Euphorbia pulcherrima*.

Flower variation

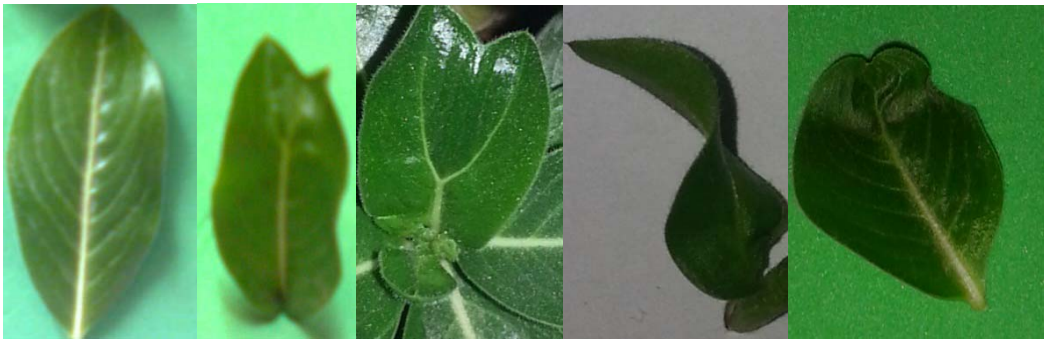
Among the plants that continued their growth in the greenhouse until flowering, two new colors appeared with the 0.75 Gy dose. One plant from Rosea produced purple colored flowers with white margins compared to the dark purple center and light purple margins of the control flowers (Fig. 2A). As for the Alba cultivar, one plant showed flowers with large pink center compared to the small pink centre and white petals in the control (Fig. 2A).

In the meantime, flower shape variation was observed in both cultivars. The 0.75Gy dose caused plants with four petals compared with five ones in the control in both Rosea and Alba (Fig. 3A and B). Also, in Alba cultivar, flowers with four normal sized petals and small one and abnormal petal were obtained with 1.0 Gy irradiation (Fig. 3B).

The obtained results go in line with those reported by Bouman and De Klerk (1996). They examined the extent of variability of leaf shape and flower color in ornamental plants and observed a large increase in variability after regeneration from non-organized callus. In addition, Nagatomi *et al*. (2000) stated that, the most variability in flower color of the regenerators was induced with gamma irradiation and not by a culturing process. Frequent studies reported the ionizing radiation effects on the shape and form of the floral organs: Smilansky *et al*. (1986) working on *Rosa spp*., Soedjone (1989) on *Begonia semperflorens*, Datta (1990) on *Rosa spp*., Banerji *et al*. (1996) on rooted cutting of *Chrysanthemum morifolium*, Khalaf (2008) on *Amaranthus caudatus* and El-Shennawy *et al*. (2011) on *Encelia farinosa.* They concluded that these changes in flower color might be attributed to the effect of radiation treatments along with the temperature and light influence on the development of pigments. They added that, the phenotypic expression of the genes concerned should be dependent upon the temperature, since temperature is one of the factors controlling reaction velocity.

In addition, Abdel-Maksoud (1992) stated that changes in flower form may be a result of chromosomal deletion or change of the factor governing the normal form or structure, as well as of the effect of gamma-rays on the ontogeny of flower organ tissue through the

selective destruction of one or more cell layer in the apical floral meristem.

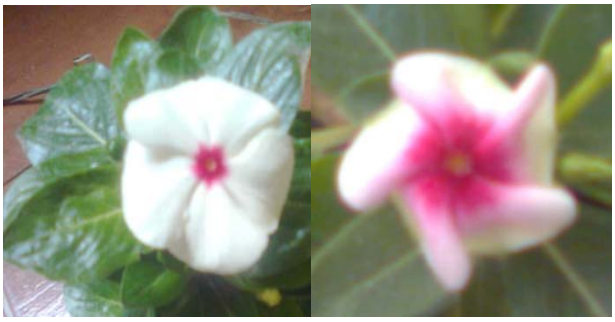


Control **Rosea divided form at 0.5 Gy** **Alba** **Rosea Wavy margins at 0.75 and 1.0 Gy** **Alba**

Fig. 1. The effect of radiation dose on leaf shape variation in the two *Catharanthus rosues* cultivars

Control **0.75Gy**

A. Change in color of Rosea.



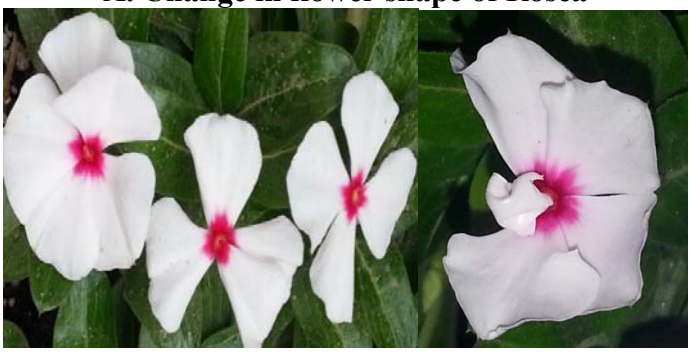
Control **0.75Gy**

B. **Change in color of Alba**

Fig. 2.The effect of gamma radiation on flower color variation in *Catharanthus rosues* cultivars.

Control **0.75Gy**

A. Change in flower shape of Rosea



0.75Gy **1.0 Gy**

B. Change in flower shape of Alba

Fig. 3. The effect of gamma radiation on flower shape variation in *Catharanthus rosues* cultivars

CONCLUSION

From the obtained results of the present study, it may be necessary to try other doses of irradiation, as well as some chemical mutagens to obtain better phenotypes characteristics of both Rosea and Alba. These new plants will be confirmed by using RAPD to differentiate the regenerated plants from their parents before releasing as commercial cultivars.

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الملخص العربي

إستحداث طفرات لنباتات الونكا معمليا بإستخدام أشعة جاما

هدى السيد المقدم

تم زراعة نباتات الونكا معمليا بإستخدام صنفين من أصناف الونكا وهما Rosea, Alba وإستخدام المنفصل النباتى Internode (السلاميات) حيث أعطى أفضل نتائج فى إنتاج المجاميع الخضرية وتم أيضا تحديد أفضل بيئة غذائية وكانت MS+ 4.0 mg/l BA بينما كانت البيئة الغذائية MS+ 1.0 mg/l IAA + 4.0 mg/l BA هى أفضل بيئة لتكوين أعلى معدل جذور.(El Mokadem,2013).

ثم تم إجراء هذه التجربة حيث تم تعريض السلاميات لأربع جرعات من أشعة جاما وهى (0.0, 0.5, 0.75 , 1.0 Gy) ثم زراعة المنفصل النباتى (السلاميات) internode على بيئة إنتاج المجاميع الخضرية والتى تم التوصل إليها فى التجربة الأولى لمحاولة إحداث طفرة فى النبات من خلال أشعة جاما. وقد تم الحصول على مجاميع خضرية مختلفة عن النبات الأم فى شكل الأوراق وكانت الجرعتان (0.75 , 1.0 Gy) الأكثر تأثيرا فى إحداث تغيرات فى شكل ولون الأزهار بينما كانت الجرعتان (0.5 , 1.0 Gy) الأكثر تأثيرا فى إحداث تغيرات فى شكل الورقة وذلك فى كلا الصنفين.