

Gamma irradiation on *Alstroemeria aurea* G. *in vitro* rhizomes: an approach to the appropriate dosage for breeding purposes

Irradiación de rizomas *in vitro* de *Alstroemeria aurea* G. con rayos gamma: una aproximación a la dosis apropiada para utilizar en mejoramiento genético

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Nota científica

ABSTRACT

Gamma irradiation has been widely used as a breeding technique to obtain new cultivars in ornamental species such as *Alstroemeria*, where several cultivars have been obtained through rhizome radiation. The optimum dosage for an appropriate induction of mutation must be considered for breeding purposes and it depends mainly on plant susceptibility. Thus in this study *in vitro* cultured rhizomes of *Alstroemeria aurea* were irradiated with a gamma source using different dosages to evaluate the direct effect produced. Damage and number of rhizome sprouting were observed and recorded during 61 days after irradiation. At the end of this period, rhizomes were weighted and mortality was evaluated. Both mortality and weight increased depending on dosage. All irradiated rhizomes showed early sprouting in comparison with control (0 Gy) and no significant difference in final number of shoots after 61 days among irradiated treatments was observed. Bleaching and necrosis was observed in all irradiated rhizomes and was more evident at higher doses. LD₅₀ was established at about 40 Gy and the optimum dosage to induce mutation was suggested between 2.5 and 5 Gy, when the growth was reduced in 50%, and probably this dosage could be used for breeding purposes.

RESUMEN

La irradiación con rayos gamma ha sido ampliamente utilizada como herramienta en mejoramiento genético para obtener nuevos cultivares de especies ornamentales tales como *Alstroemeria*, en que varios cultivares se han obtenido mediante la irradiación de rizomas. La dosis óptima para una apropiada inducción de mutación debe ser considerada para fines de mejoramiento genético y depende principalmente de la susceptibilidad de la planta. En este estudio, rizomas de *Alstroemeria aurea* cultivados *in vitro* fueron irradiados con una fuente de rayos gamma utilizando diferentes dosis para evaluar el efecto directo producido. El daño y número de rizomas en brotación fueron observados y registrados durante 61 días después de la irradiación. Al final de este periodo, los rizomas fueron pesados y la mortalidad fue evaluada. Tanto la mortalidad como el peso aumentaron dependiendo de la dosis. Todos los rizomas irradiados mostraron una brotación anticipada en comparación con el control (0 Gy) y no se observaron diferencias significativas en el número final de brotes después de los 61 días entre los diferentes tratamientos. Se observó blanqueamiento y necrosis en todos los rizomas irradiados, siendo más evidente a altas dosis.

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Keywords

mutation • radio susceptibility •
sprouting • LD₅₀ • breeding

Palabras clave

mutación • radio susceptibilidad •
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El DL₅₀ fue establecido alrededor de 40 Gy y la dosis óptima para inducir mutación se sugiere entre 2,5 y 5 Gy, cuando el crecimiento se redujo en un 50%, y probablemente esta dosis pueda ser utilizada con fines de mejoramiento genético.

Abbreviations

BA: Benziladenine; **Gy:** Gray
(unit of absorbed radiation dose);
LD₅₀: Median lethal dose

Abreviaciones

BA: Benziladenina; **Gy:** Gray
(unidad de dosis absorbida de radiación);
DL₅₀: dosis letal (50%)

INTRODUCTION

The use of ionizing radiation, such as X-rays, gamma rays and neutrons and chemical mutagens in plant breeding has been widely used in major crops such as wheat, rice, barley, cotton, peanuts and beans (1). However, these techniques have become more popular in floriculture (20). The ornamental crop market is very dynamic and often new cultivars are released by breeding programs because novelty is the main objective followed by this industry. Induction of mutations by ionizing radiations has been widely used in ornamental crops to obtain new cultivars. In early studies, X-rays were used and nowadays gamma (γ) radiation has become an efficient mutagenic agent in ornamental crops such as *Dendranthema* (15), *Petunia* (4), *Gypsophyla* (2) and *Alstroemeria* (6).

Even though the biochemistry base of mutation is still not completely clear, it is thought that mutagenic treatments can generate chromosome rearrangements or produce changes in some genes to other allelic forms, explaining the phenotypic variation (12). The mutation induced using these techniques is specific to each case because it is determined by inherent chemistry and genetics of the species or genotype (5). Furthermore, different levels of tolerance can be found in the same genotype depending on developmental stage. Moreover, tissues with high rate of growth are more susceptible to damage due to irradiation exposure (21). Susceptibility to radiation must be evaluated on the plant before attempting mutation induction in order to establish the optimum dosage, which has been described as the point at which 50% of growth reduction occurs compared to the control (17).

Another parameter used in plant breeding to calculate dosage is the determination of the median lethal dose (LD₅₀) (7, 13), where mortality of the irradiated explants reaches 50%. Mutation rates are usually very low (< 1%) and large populations must be treated to achieve successful results (6). *In vitro* culture techniques can be considered as an appropriate tool for inducing mutations because it can supply a large amount of homogeneous and virus free individuals to be irradiated (1). In addition, *in vitro* culture also provides an efficient propagation system for irradiated material allowing assessment of mutant stability and easy later selection (1).

At the moment *Alstroemeria* is one of the major products in the cut flower industry (11) and the breeding of new cultivars of this species have been based on the induction of mutagenesis through irradiation of rhizomes with x-rays (6) and gamma rays (18, 19). *Alstroemeria* mutagenesis has been focused in changing few traits on outstanding cultivars (1, 6). Even though most of mutations are recessive and negative (12), variation in interesting traits are still relatively easy to obtain by mutation induction in *Alstroemeria*. In this species the most common induced variations are color and size of the flower, shape and stripes on the tepals, plant vigour, productivity and blooming time (6, 20).

In this study we evaluated the direct effect of gamma radiation on rhizomes of the wild species *Alstroemeria aurea* in order to establish the optimum dosage to be used under *in vitro* conditions.

MATERIALS AND METHODS

Plant material

Alstroemeria aurea rhizomes were sterilized in 2.5% NaOCl for 10 min, rinsed with sterile water and then cultured *in vitro* on a 25% MS medium (14) under dark conditions at 24°C. The developed plants were placed in liquid MS medium (multiplication medium), supplemented with 3 mg·L⁻¹ of benziladenine (BA) and 30 g·L⁻¹ of sucrose and the pH was adjusted to 5.7. Rhizomes were cultured in 250-ml flasks with 15 ml of multiplication medium in a growing chamber at 24°C with a 16/8 h light/dark photoperiod. Rhizomes were subcultured every eight weeks until enough material was obtained to be irradiated.

In vitro rhizome irradiation

Before the irradiation, each rhizome was weighted and sized. Rhizomes were trimmed to 0.5-1.8 cm and placed in aseptic and sealed Petri dishes to be irradiated with 0, 2.5, 5, 10, 20 and 40 Gy of gamma rays from a Co⁶⁰ source at the facilities of the Chilean Commission of Nuclear Energy located in Santiago, Chile. Irradiated rhizomes were transferred to the same multiplication medium and maintained at the same temperature and photoperiod described before.

Evaluation

Rhizome damage and sprouting were observed and recorded at 4, 7, 14, 21, 28, 38, 42, 49 and 61 days after irradiation. At the end of this period, rhizomes were weighted and sized again and mortality was evaluated.

Data analysis

A completely randomized design was used using six irradiation doses. The experimental unit was a 0.5-1.8 cm rhizome and each treatment comprised 15 replicates.

Analysis of covariance (ANCOVA) for sprouting and weight increase, analysis of variance (ANOVA) for rhizome growth, and Student t-statistic analysis for mortality were performed using SPSS 12.0 for Windows.

RESULTS AND DISCUSSION

Rhizome growth and sprouting

All irradiated rhizomes showed an early sprouting compared with the control, suggesting an initial stimulation of shoot elongation by the gamma rays. Nevertheless, from day 14 this effect decreased coinciding with the beginning of sprouting of non- irradiated rhizomes (figure 1, page 195).

Similar results were found by Bassam and Philipp (3) on carrot germination after gamma radiation of seeds. Non-irradiated rhizomes showed higher sprouting and larger shoots compared to irradiated rhizomes, especially during the period between day 7 and 21.

Similar results were obtained by Duron and Decourtye (9) who concluded that both cell elongation and shoot regeneration are affected by gamma radiation as the dosage of exposure increased. The negative effect of gamma irradiation over rhizome sprouting observed in this study (figure 1) is also coincident with previous results obtained in banana cultured *in vitro* (16).

At the end of the evaluation (after 61 days), there was no statistical difference in shoot production among irradiated rhizomes (figure 1, page 195) and all of them showed a lower shoot production (2.42 - 3.85 shoots per rhizome) compared to the control (12.96 shoots per rhizome).

These results suggest that shoot production is highly influenced by the irradiation but not by the dosage used. Susceptibility of *Alstroemeria* rhizomes to radioactivity has been studied before by Broertjes and Verboom (6) who reported a growth decrease of 60% with a gamma radiation of 5 Gy. Predieri and Gatti (17) established the optimum dosage to induce mutation for breeding purposes in plum as the dosage at which the growth is reduced in 50%.

In this study the optimum dosage of gamma radiation on *A. aurea* rhizomes was determined to be between 2.5 and 5 Gy. Similar results were found by Przybyla (19), who obtained three *Alstroemeria* cultivars using 3 Gy of gamma radiation ('Catalina', 'Carlota' and 'Juanita'), one cultivar with 4 Gy ('Paula'), two cultivars with 5 Gy ('Erendira' and 'Isabel'), two cultivars with 6 Gy ('Ines' and 'Matilde') and one cultivar with 10 Gy ('Azucena').

Rhizome weight

Rhizome weight reduction was observed specially on treatments with doses over 5 Gy which were significantly different from the 2.5 Gy treatment. The control showed a significant weight increase compared to irradiated rhizomes, gaining an overall of 2.04 g in 61 days (figure 2, page 195). This negative effect of radiation on weight increase has been also described in plum (19) and carrot (3).

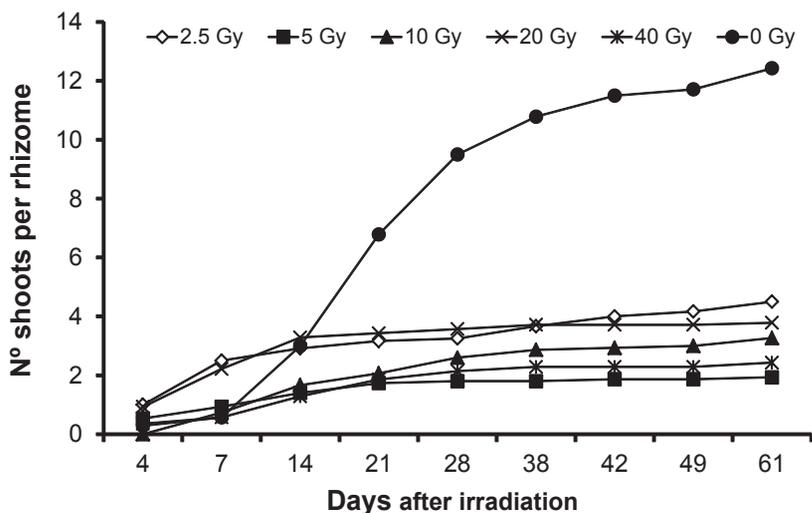


Figure 1. Sprouting evolution of *A. aurea* rhizomes grown *in vitro* during 61 days, after different gamma irradiation treatments.

Figura 1. Evolución de la brotación de rizomas de *A. aurea* cultivados *in vitro* durante 61 días, después de diferentes tratamientos con radiación gamma.

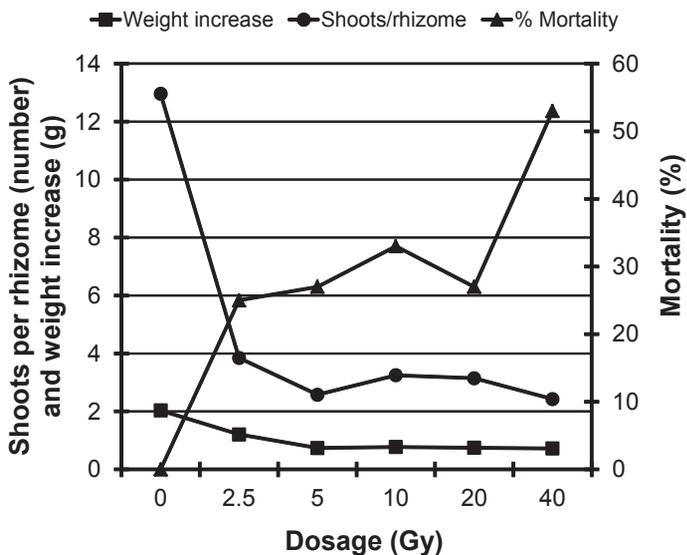


Figure 2. Weight increase, sprouting and mortality evaluated at 61 days after different gamma irradiation treatments on *A. aurea* rhizomes grown *in vitro*.

Figura 2. Incremento de peso, brotación y mortalidad de rizomas de *A. aurea* cultivados *in vitro*, evaluados a 61 días después de haber sido tratados con irradiación gamma.

Rhizome damage

Bleaching and necrosis was observed on irradiated rhizomes, appearing earlier and being more severe at higher doses. Thus the 40 Gy treatment showed these symptoms in some of the shoots right after radiation. Seven days after irradiation, treatments over 10 Gy showed necrosis and after 21 days all treatments showed this symptom. In this sense Ahloowalia and Maluszynski (1) suggested that *in vitro* cultures are more susceptible to necrosis compared to other explants irradiated such as seeds or stem cuttings. Bleaching was observed in rhizomes after 14 days in treatments over 20 Gy and after 28 days in all treatments. Douglas (8) also observed bleaching on *in vitro* cultured stems of irradiated poplar.

Rhizome mortality

All treatments produced higher rhizome mortality than the control plants, which was even more significant with doses higher than 20 Gy (figure 2). Similar responses to this dosage have been observed on *Alpinia purpurata* (10) and plum (19). Forty Gy gamma radiation produced 53% of mortality; meanwhile control (0 Gy) did not show any mortality. In this sense Fereol *et al.* (12) suggested that cell nuclei injury by irradiation would be the responsible for the tissue lethal effect. Furthermore *A. aurea* rhizomes showed lower susceptibility to gamma radiation than other *Alstroemeria* genotypes reported in previous studies, like the one performed by Przybyla (19) who irradiated *in vivo* rhizomes and established the LD₅₀ at about 10 Gy, meanwhile in this study LD₅₀ was established at about 40 Gy. This result could be explained due to inherent differences among genotypes and to size, weight and developmental stage of the rhizomes irradiated.

On the other hand, Ahloowalia and Maluszynski (1) indicated that *in vivo* cultures would be less susceptible to radiation than *in vitro* cultures because susceptibility would be correlated to the size of irradiated material. Usually, *in vitro* explants are smaller than *in vivo* material, but in this case explants used (rhizomes) were larger than those normally used in other species, and they were probably similar to the *ex vitro* explants used by Przybyla (19).

Summarizing, weight increase, mortality and injury of irradiated rhizomes of *Alstroemeria aurea* are related to the dose of gamma radiation, meanwhile sprouting depends only on the gamma treatment regardless the dosage used. Furthermore, gamma radiation can become lethal or induce bleaching and necrotic symptoms. Therefore it is necessary to perform previous irradiation trials to avoid these problems. Finally the data presented here concerning the determination of optimum dosage of gamma irradiation when growth decreases 50%, and LD₅₀ when mortality reaches 50%, could be useful to consider in *Alstroemeria* breeding.

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