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Induced Mutagenesis in Garlic Cloves (*Allium Sativum* l.) in the cultivar 'Blanco Criollo'

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Abstract

In Cuba, garlic (*Allium sativum* L.) production has low productivity due to biotic and abiotic factors that affect seed quality; Having a protocol for mutagenesis in garlic cloves in vitro constitutes an alternative for obtaining new mutants resistant to the virus complex that contaminate the seed and reduce yield. To determine the effect of gamma radiation on the survival of explants in the in vitro establishment, garlic cloves were treated with five doses (60, 65, 70, 75 and 80 (Gy) and a control based on previous studies. In for in vitro establishment, the culture medium consisting of MS Salts and vitamins was used. Of the applied doses, 70 (Gy) was determined as the optimal dose, which reached 50% of the established meristems. For bulbification, the medium of culture composed of: MS salts and vitamins, 2.0 ml.L-1 of 2ip, 90g of sucrose and 2.5 ml.L-1 of VIUSID Agro® This allowed obtaining bulbs with the appropriate morphological characteristics as seeds in vitro that were established in the acclimatization phase for future plantations in the field, which allows us to conclude that this dose is adequate to favor the production of mutants with desirable agronomic characteristics in the cultivar.

Key words: *Garlic; Allium sativum; Bulbification; Gamma radiation; Mutant; Mutagenesis*

Introduction

Garlic is a herbaceous plant, constituted by an underground bulb, formed by teeth united at its base, around the true stem and covered by white or purple cataphylls, whose tonality varies according to the variety and the height of the planting site (Castillo, 2004). The leaves are elongated, flat and sheathing; the flowers are pink or green. The false stem is soft, smooth and up to 40 cm long from which aerial bulbils are born. The root system consists of numerous simple, thin and poorly branched adventitious roots that grow superficially in the soil. The species of this genus show the same basic pattern of apical growth, both in the root and in the stem, and the frequency of branching of the apical shoot varies with the species, the cultivar and the growing conditions.

According to Eguillor (2010), China is the largest producer of garlic followed by India and the Republic of Korea. It is considered that the Asian continent produces around 80.7% with a yield of 18.1 tons per hectare (ha), while Spain, France and Italy obtain 11.1 % of the harvested area and 6.4% of world production with the lowest yield of 6.2 $t.ha^{-1}$. In Latin America, Argentina has the largest planting area dedicated to cultivation, with 15,600 ha and a yield of 9 $t.ha^{-1}$, followed by Brazil with 10,214 ha and a yield of 9 $t.ha^{-1}$. However, Mexico and the United States reach the highest yields (16.5 t.ha-1) by contributing 2.5% of world production, with only 1.6% of the harvested area (Eguillor, 2010).

Garlic is affected by virus complexes that contaminate the seed and reduce its yield. Conci et al., (2007), affirm that practically all the seed of this crop is infested by a viral complex to a greater or lesser degree. The symptoms of the disease are visible in the form of mosaic or yellow streaks on the leaves, which cause a decrease in the productivity of the bulbs, which depends on the variety and the amount of virus present in the seed. The application of mutagens involves evaluating the biological effects produced in a certain cell or tissue, so factors such as the type of mutagen, the dose of radiation used, and the size and origin of the explants and their radiosensitivity must be considered (Taner and Kunter, 2004).

Due to the fact that the response to a given mutagen is measured mainly based on the percentage of survival and the rate of regeneration and/or multiplication of the irradiated material, it is necessary to determine the dose of radiation that allows the survival of the materials and subsequently select between them. the largest number of mutants with desirable characteristics. Ahloowalia and Maluszynski (2001) carried out radiosensitivity tests in order to establish the range of radiation that can be applied to a given crop.

Within the physical mutagens, gamma radiation is the most used to generate variability in agricultural crops, as well as to reduce post-harvest losses, eliminate early sprouting, minimize contamination, control diseases and pests, extend the useful life of crops, among others (Piri et al., 2011). In this field, gamma rays are considered a type of ionizing radiation usually obtained by radioisotopes, with cobalt 60 (60 Co) and cesium 137 (137 Cs) as the main sources of radiation, being the unit commonly used to measure the amount of exposure to radiation. Radiation the Grays (Gy) or its equivalents the rads (1 rads = 0.01 Grays) and/or the (Gy) (1 (Gy) = 1000 rads) (IAEA, 2005).

In garlic cultivation, research has been carried out from bulbs treated with gamma radiation in order to improve characteristics such as resistance to diseases (Al-Safadi *et al*., 2000; Nabulsi *et al*., 2001), inhibition of sprouting (Croci *et al*., 1987; Taner and Kunter, 2004; Pérez *et al*., 2007), weight gain (Croci and Curzio, 1983), storage durability (AL-Safadi, 2000), morphological changes (Pellegrini *et al*. , 2000; Wi *et al*., 2007) and flavor modifications (Ceci *et al*., 1991), among other characteristics.

On the other hand, the combination of techniques such as tissue culture and mutagenesis has facilitated, in a great diversity of agricultural crops, the regeneration of a high number of desired variants, obtaining materials free of pests and diseases and the possibility of irradiating a high number of explants (Zhen, 2001; Zheng *et al*., 2007). Due to the aforementioned, the objective was to study the effect of gamma radiation on the survival and development of in vitro explants, which allow obtaining seeds with phytosanitary quality to scale the results and establish plantations in the field.

Materials and Methods

The research was carried out at the Instituto de Investigaciones de Viandas Tropicales (INIVIT), where garlic meristems of the cultivar 'Blanco Criollo' were used as plant material. For the development of the experiments, the inorganic salts and vitamins proposed by Murashige and Skoog (1962) (MS) were used as basal culture medium. The pH of the culture media was adjusted to 5.7 with 0.5 $mol.L^{-1}$ NaOH and 0.5 $mol.L^{-1}$ HCl before autoclaving.

The culture media and culture systems used in the experiments were steam sterilized in a vertical autoclave (BK-75) at 121°C and 1.20 $kg.cm^{-2}$. The teeth were packed in nylon bags to facilitate the irradiation process. Five doses of radiation were applied: (60, 65, 70, 75 and 80). The teeth, once irradiated, were established in the culture medium made up of the MS Salts and vitamins, until 21 days after establishment they were placed in a semi-automated culture system for bulbification with the culture medium made up of: Salts and vitamins MS; 2.0 $ml.L^{-1}$ of 2ip; 90 g of sucrose and 2.5 $ml.L^{-1}$ of VIUSID Agro®.

To determine the radiosensitivity of the materials, the optimal dose was established as that which allowed the survival of 50% of the meristems. The variables were evaluated: "Survival" (%); "Plant height" (cm); "Equatorial perimeter of the base" (cm); "Equatorial perimeter of the bulb" (cm); "Number of bulbs". The effect of VIUSID Agro® for bulbification in explants treated with gamma radiation was studied.

Effect of gamma radiation on the survival of explants in the in vitro establishment

In order to obtain the effect of gamma radiation for the determination of radio sensitivity in garlic cloves established in vitro, five doses (60, 65, 70, 75 and 80 Gy) and a control were evaluated. The teeth were disinfected, according to the best variant obtained in previous experiments. 50 meristems were established in vitro for each treatment and five variants plus a control were studied. The number of explants that presented the appropriate morpho-physiological characteristics to place them in the bulbification medium was evaluated at 21 days. This experiment had two replications per treatment.

Effect of gamma radiation on the development of explants in the in vitro establishment

In order to analyze the effect of gamma radiation on the development of explants in the in vitro establishment, the "Height of the plant" (cm) and the "Equatorial perimeter of the base" (cm) were evaluated. The number of explants that presented the appropriate morpho-physiological characteristics to place them in the bulbification medium was evaluated at 21 days. This experiment had two replications per treatment.

Effect of VIUSID Agro® on explants treated with gamma radiation on in vitro bulbification in a semi-automated culture system

With the objective of achieving the formation of bulbs, the combination of 2ip, plus sucrose and VIUSID Agro® in the semi-automated culture medium was evaluated; for the bulb phase, two treatments were studied.

Treatments

Control. MS salts and vitamins + 2.0 $mg.L^{-1}$ of 2ip + 90 g sucrose.

Treatment 1. MS salts and vitamins + 2.0 $mg.L^{-1}$ of 2ip + 90 g sucrose + 2.0 $ml.L^{-1}$ VIUSID Agro®.

Treatment 2. MS salts and vitamins + 2.0 $mg.L^{-1}$ of 2ip + 90 g sucrose + 2.5 $ml.L^{-1}$ VIUSID Agro®.

In each culture system, 20 explants and 1 500 mL of bulb culture medium were placed. For the Temporary Immersion System (SIT) an immersion time of 15 minutes and immersion frequency every three hours was used. This experiment had two repetitions for each culture system. The bulbing coefficient was evaluated after 30 days of cultivation. 0.5 mL VIUSID Agro® was added to each bottle every seven days up to 21 days from the day of sowing. The explants used came from treatment 3 and the control. Twenty explants were used per treatment and bulb formation was evaluated after 30 days of culture for each treatment. This experiment had two replications per treatment.

Results and Discussion

Effect of gamma radiation on the survival of explants in the in vitro establishment

The effect of gamma radiation for the determination of radiosensitivity in garlic cloves established in vitro, both the control and the irradiated ones, 60 and 65 (Gy) presented survival percentages greater than 50%, this indicates that it favored the development and/o growth of the in vitro meristems of garlic, while the one treated with 70 (Gy) showed a lower average (50%) and those treated with 75 and 80 (Gy) had no survival (Table 1). According to Al-Safadi *et al*., (2000); Nabulsi *et al*., (2001); Zheng *et al*., (2007) and Wi *et al*., (2007) found, in bulbils or 'teeth' of (Allium sativum L.), that high doses of radiation can affect cells, tissues and even organelles such as chloroplasts. It is also widely known that gamma radiation frequently produces both gene and chromosomal mutations (Donini, 1997), which not only inhibit cell division processes, but also multiplication and consequently the growth or development of tissues.

Treatment (Gy)	Survival (%)
Control	100 a*
1	80 a
2	75 ab
3	45 bc
4	0c
5	0c

Table 1: *Average survival of garlic meristems (Allium sativum L.), treated with gamma radiation.*

Mean ranges with uncommon letters in the same column differ, according to the Kruskal-Wallis/Mann-Whitney nonparametric test for $p<0.05$ (n=150).

Legend: Control. Without treating. Treatment 1. 60 (Gy), Treatment 2. 65 (Gy), Treatment 3. 70 (Gy), Treatment 4. 75 (Gy), Treatment 5. 80 (Gy).

Figure 1: *Developed from the meristems of garlic (Allium sativum L.) treated with gamma radiation.*

Effect of gamma radiation on the development of explants in the in vitro establishment

When analyzing the effect of gamma radiation on the development of explants in the in vitro establishment (Table 2), it was observed that there are statistical differences between the treatments studied. The growth of the meristems was determined during the establishment phase and in terms of height, both the control and treatment 1 and 2 were greater with respect to treatment 3, but treatment 3 differed in the equatorial perimeter. These results indicate that the Application of 70 (Gy) of gamma radiation stimulated mitotic activity in the apical buds located at the base of the stem or stem disc, directing the bulbification process rather than elongation. This allowed them to be placed in temporary immersion systems for bulb formation, with significant differences compared to the rest of the treatments.

Treatment	Plant Height (cm)	Equatorial perimeter of the base (cm)
Control	15,3 a	0, 7 b
1	14,8 a	0, 7 b
2	14,6 a	0,8 b
3	11,4 b	1,4 a

Table 2: *Vegetative development of garlic (Allium sativum L.) meristems in vitro treated with gamma radiation.*

Mean ranges with uncommon letters in the same column differ, according to the Kruskal-Wallis/Mann-Whitney nonparametric test for $p<0.05$ (n=150).

Legend: Control. Untreated, Treatment 1. 60 (Gy), Treatment 2. 65 (Gy), Treatment 3. 70 (Gy).

Effect of VIUSID Agro® in a semi-automated culture system (SIT) of explants treated with gamma radiation for in vitro bulbification of garlic (*Allium sativum* L.)

The culture conditions that are created in the SIT allow the best growth of the garlic explants. According to Escalona (2006) and Cabrera et al., (2012), semi-automated culture systems allow a more efficient supply of nutritional elements and a periodic renewal of the internal atmosphere of the culture flask.

Taking into account the above, it was determined to use an immersion frequency of 15 minutes every 3 hours in 5 L flasks, a density of 20 shoots, a volume of 100 mL of culture medium per shoot, with the addition of 2.5 ml $.L^{-1}$ VIUSID Agro® with a complete formula and a cultivation time of 30 days. Table 3 shows the result of the new bulbification method of the garlic explants treated with gamma radiation, using the stimulant VIUSID Agro® with a dose of 0.5 $ml.L^{-1}$ every seven days until the best development of the bulbs. 21 days, from the day of sowing. In the case of bulbification, a possible explanation may be the effect of amino acids in the plant, such as arginine, which has the function of stimulating root growth, being one of the main amino acids of the rhizosphere, in addition to acid aspartic acid, which intervenes in almost all the metabolic processes of the plant (Galvez D. et al., 2021).

Treatments	Equatorial Perimeter of the Bulb (cm)	Number of Bulbs
Control	5.50 ± 0.24 b	1.00 ± 0.02 b
Treatment 1	14.10 ± 0.65 a	12.00 ± 0.50 a
Treatment 2	13.60 ± 0.69 a	13.80 ± 0.43 a

Means with uncommon letters differ, according to Tukey's test for $p<0.05$

Table 3: *Effect of VIUSID Agro® on the in vitro bulbification of (Allium sativum L.) in a semi-automated culture system (SIT) treated with gamma radiation.*

It was decided to study the control treatments and treatment 3 in the SITs, because the latter complied with the protocol standards for in vitro mutagenesis in the "target cultivar", which also offers an additional advantage by facilitating the selection and maintenance via vegetative growth of solid or stable mutants, with desirable characteristics in short periods of time. Treatment 1 there was no difference with the control being excluded from the study.

With these culture parameters, the best response of the plant material and the highest bulbing coefficient (13.80 CM) were obtained in treatment 3, for which it was taken into account to establish a protocol for in vitro mutagenesis in the cultivar Blanco Criollo de Garlic. In this way, the irradiation of garlic cloves for in vitro establishment can be part of genetic improvement programs in the production of basic garlic seed.

Figure 2: *Bulbification of in vitro garlic plants (Allium sativum L.) in temporary immersion system treated with gamma radiation after 30 days of cultivation and establishment in the acclimatization phase.*

Conclusions

- A protocol for in vitro mutagenesis was established in the cultivar Blanco Criollo (Allium sativum L.) with 70 (Gy), in the establishment of tooth meristems.
- With the use of gamma radiation, garlic microbulbs as pre-basic seed can be taken directly to the acclimatization phase.
- The procedure offers an additional advantage since it facilitates the selection and maintenance of solid or stable mutants by vegetative means, with desirable characteristics in short periods of time.

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