Radiosensitivity to gamma rays (^{60}Co) in shoot tips of henequen

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ABSTRACT
The current research was carried out with the objective of evaluating the radiosensitivity of shoot tips from henequen (*Agave fourcroydes*) to gamma radiations ^{60}Co and to determine the optimum dose of radiation for breeding program in the cultivar Sac Ki. Shoot tips of 20 days of *in vitro* culture were selected and irradiated with five doses between 10, 20, 30, 40 and 50 Gy using a ^{60}Co source. Evaluations were aimed to determine increasing of fresh weight of explants in relation to the control (no irradiated material), number of dead explants and average number of axillary shoots per explants. Percentage of mortality was bigger when irradiation dose was increased. Irradiation 20 dose of Gy was GR_{50}, increasing fresh weight of explants in a 49.6% in relation to control. LD_{50} was 30 Gy and a 50% of irradiated explants died. When increasing irradiation dose the number of explants decreased, though starting of sprouting took place after 90 for both, irradiated and non irradiated explants, suggesting that this parameter was not radiostimulated nor radioinhibited. Results enable to suggest the application of 20 Gy in studies aimed to create genetic variability in breeding programs by mutation induction.

Key words: *Agave fourcroydes,* plant breeding, tissue culture

RESUMEN
El presente estudio se desarrolló con el objetivo de evaluar la radiosensibilidad de ápices de henequén (*Agave fourcroydes*) a los rayos gamma ^{60}Co. Para su ejecución se seleccionaron e irradiaron ápices de la variedad Sac Ki con 20 días de sembrados *in vitro*. Las dosis de radiación fueron 10, 20, 30, 40 y 50 Gy. Las evaluaciones consistieron en determinar el incremento en masa fresca de los explantes en relación con el control (material no irradiado), el número de explantes muertos y el número promedio de brotes axilares por explante. Se comprobó que el porcentaje de mortalidad se elevó con el incremento de las dosis de radiación. La dosis de 20 Gy fue la GR_{50}, con esta los explantes incrementaron su masa fresca en relación con el control en un 49.6%. La LD_{50} fue 30 Gy donde el 50% explantes irradiados murió. Con el incremento de las dosis de radiación se redujo el número de brotes por explante, sin embargo el inicio de la brotación fue posterior a los 90 días tanto para los explantes irradiados como los no irradiados, lo que sugiere que este parámetro no fue radioestimulado ni radioinhibido. Los resultados del estudio permiten sugerir la aplicación de la dosis de 20 Gy en los trabajos dirigidos a la creación de variabilidad genética en programas de mejora por inducción de mutaciones en esta especie.

Palabras clave: *Agave fourcroydes,* mejoramiento genético, cultivo de tejidos

INTRODUCTION

Henequen (*Agave fourcroydes*) is cultivated for its hard fiber which is used to make agricultural twin, saks, carpets and others products (Gonzalez *et al.*, 2004). Furthermore it is used as a source of carbohydrates (inuline), spirits and steroidal sapogenin (hecogenin) (Infante *et al.*, 2003). Due to its high levels of ploidy (5n) it is sterile and produces few seeds with very low viability at the end of its long life cycle (Piven *et al.*, 2002). Moreover, it has undesirable characterists as: marginal thorne leaves. This problem is a handicap during its harvest (Eastmond *et al*., 2000). The problems mentioned above make very difficult to carry out a traditional cross breeding program, that is why henequen has never been genetically improved. Therefore, other methods to induce variability with gamma rays and *in vitro* culture are needed to be used (Nichterlein, 2000). Use of combination of both techniques may favor the program of genetic improvement (Pérez, 1998).

An assay aimed to apply different dose of gamma radiation on henequen shoot tips previously cultured *in vitro* to determine the optimum range to be used in the improvement program was carried out.

MATERIALS AND METHODS

Plant material

Bulbils were prepared and sterilized according to González *et al.* (1997) and Peña *et al.* (1997). Once isolated shoot tip was placed on 10 ml of MS culture medium (Murashige and Skoog, 1962)

(slightly modified by Robert *et al.*,1992), supplemented with 1.6 mM naphtalenacetic acid (NAA) and 4.4 mM 6- benzylaminopurine (6-BAP) under 16 h illumination and 8h darkness at 22 ºC for 3 weeks.

Explants were irradiated with five doses (10, 20, 30, 40, 50 Gy) using a ^{60}Co source in the irradiator Gammacell 500 with a dose potency of 13.7 Gy min^{-1}. They were planted *in vitro* 24 h after being irradiated using the methodology developed by González *et al.* (2004). Explants without irradiation were used as control.

Explants were evaluated 45 days after the irradiation to measure the radiosensitivity index, the increase of fresh weight percentage in relation to control plant and mortality percentage (%). The rate production of new axillary shoots per explant was evaluated 45, 90 and 135 days after irradiation too.

Statistical analysis

A total of five replications with four explants were used for each treatment and the experimental trial was repeated twice. Significance of the treatment effects was determinated by non parametric analysis of variance Kruskal-Wallis test (Statgraphics ver. 5.0 Statistical Graphics Corp.), using a completely random design. Percentage data were subjected to arcsine transformation before analysis. Variations among treatment means were analyzed by Student-Newman-Keuls´s procedure.

RESULTS AND DISCUSSION

In the irradiated explants, different responses were observed in relation to the dose of radiation applied. The non irradiated explants (control explants) presented an increase of fresh weight of 6.31 g and 100% of survival, however, irradiated explants showed a decrease from increase of fresh weight and a greater mortality percentage as the dose of radiation increased.

In *Musa* spp., cultivar Grande Naine, the mortality was superior and the growth decreased as the dose increased (García *et al.*., 2000).

Figure 1 shows the decrease of fresh weight in relation to the control explant, and an increase of mortality percentage in irradiated explants. A reduction between 40-60% of their fresh weight in relation to control explants was noted in irradiated explants with 10, 20, 30 Gy. The dose that brought about a reduction between 40-60% of fresh weight in irradiated explants has been recommended for the improvement by *in vitro* mutation (Pérez, 1998), because these doses produce a proper frequency of mutation without multiple mutations (Afza *et al.*, 1994).

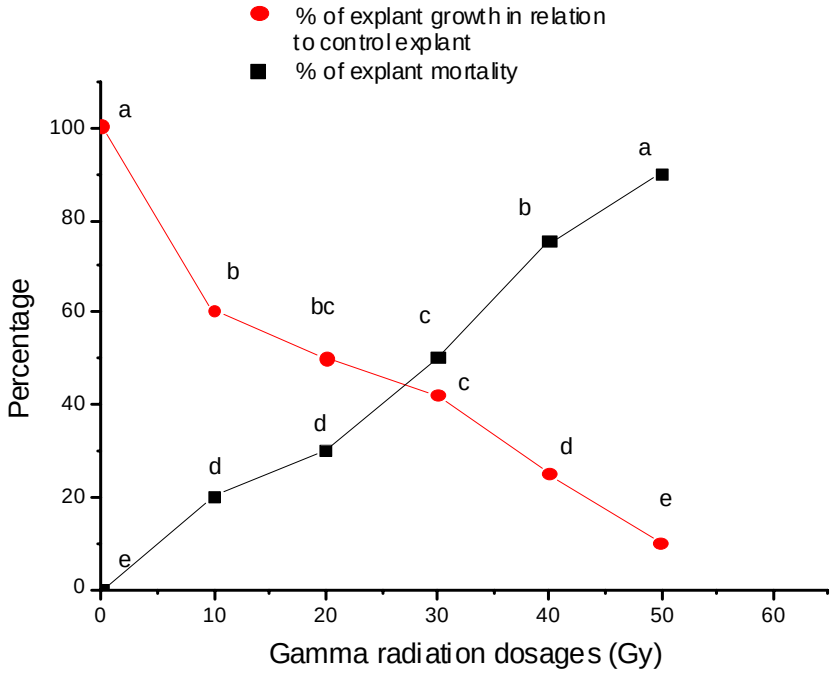

Figure 1. Effect of different doses of gamma rays (^{60}Co) over growth and mortality of the irradiated explants of henequen cultured *in vitro.* Means followed by different letters are significantly different at 95 % confidence level. H: Statistical of Kruskal-Wallis test.(41.23 ***)

Table 1. Effect of different doses of gamma rays over production rate of new axillary shoots per explant.

Doses Gy	Production rate of new axillary shoots/ explant		
	45 days	90 days	135 days
0	0	0	3.5 a
10	0	0	2.7 b
20	0	0	2.4 b
30	0	0	1.3c
40	0	0	0.8 d
50	0	0	0.1 e
H	-	-	42.74 ***

Means followed by different letters are significantly different at 95% confidence level. H: Statistical of Kruskal-Wallis test.

A reduction of 49.6 % was reached in the increase of fresh weight with the dose of 20 Gy and for this reason it was considered as GR_{50}. However, the 50% of mortality (LD_{50}) was produced in the dose of 30 Gy.

The dose of 50 Gy produced a superior mortality percentage (over 90%) and 10% in the increase of fresh weight in relation to control explants. That is why this dose is considered as radioinhibiting.

Production of new axillary shoots per explant was influenced with the dose used, as it is shown in table 1, however, the onset of production of axillary shoots in all treatments was postponed to 90 days. This result evidenced that the beginning of the production of shoots is neither radiostimulated nor radioinhibited by the different doses used.

The number of axillary shoots regenerated from control explants was higher and statistically different than the rest of the treatments (Table 1). Decrease under 3.0 of production rate of new axillary shoots, in all dose used, evidenced the ruptuture of hormonal balance that allows a good proliferation of shoots. An either endogenous or exogenous hormonal balance, which allowed reaching a production rate of new axillary shoots superior to 3.0, was established in explants of henequen (Miguel *et al.*, 2006).

The higher dose of gamma rays (50 Gy) was not acceptable due to the serious necrosis it causes to the explants furthermore only an axillary shoot was obtained. Phytotoxic action at this dose (50 Gy) was extremely harmful.

The parameters used in this work; increase of fresh weight percentage in relation to control explants, mortality percentage and production rate of new shoots were good indicators of the radiosensitivity. The dose of 20 Gy was selected to continue studies aimed to create genetic variability in breeding programs by mutation induction because it was considered the GR_{50} and it reached a production rate of new axillary shoots per explant near to the control explants.

All plants from the different doses grow properly in nursery condition. This will be studied in furthers experiments.

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