Mutation induction in zygotic embryos of avocado (*Persea americana Mill*)

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INVESTIGACIÓN

ABSTRACT

Mutation induction and biotechnological techniques are current approaches used in plant breeding. Here, both methodologies were combined to obtain a mutation-breeding model in avocado. Based on the inhibition of the entire sprout fraction, radiosensitivity curves for the Duke and Hass varieties were developed. The inhibition of the entire sprout fraction was described by a second order polynomial equation. Fit of the experimental data and the theoretical model was of 0.95 and 0.96 for Duke and Hass radiosensitivity curves, respectively. Mean lethal doses (LD_{50}) were determined in 28 and 27 Gy for Duke and Hass varieties, respectively. *In vitro* germination and rooting were very similar for non-irradiated and irradiated embryos at doses lower than DL_{50} values. Radiation induced variability was evaluated at plantlet state by plantlet morphological descriptors. Doses values lower than DL_{50} induced significant variation in both avocado varieties. The usefulness of the combined approach to improve avocado varieties is discussed. This methodology appears to be an alternative to traditional breeding methods, particularly for improving agronomic characteristics as root-rot resistance and salt tolerance in avocado, where *in vitro* selection methods could be determinant.

Key words: Zygotic embryo culture, mutation breeding, γ-rays, avocado.

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RESUMEN

Inducción de mutaciones en embriones cigóticos de aguacatero (Persea americana Mill). Las técnicas de inducción de mutaciones y biotecnológicas son metodologías actuales usadas en el mejoramiento de plantas. En el presente trabajo, ambas metodologías se combinaron con el objetivo de obtener un modelo para el mejoramiento de aguacateros. Se determinaron las curvas de radiosensibilidad para las variedades Duke y Hass sobre la base de la inhibición de la fracción de brotes enteros. Esta inhibición fue descrita por un polinomio de segundo orden. El ajuste de los datos experimentales y el modelo teórico fue de 0.95 y 0.96 para las variedades Duke y Hass, respectivamente. Los valores de dosis letal media (LD_{50}) fueron 28 y 27 Gy para las variedades Duke y Hass, respectivamente. La germinación y el enrraizamiento *in vitro* fueron muy similares para los embriones no irradiados y aquellos irradiados a dosis inferiores a los valores de LD_{50}. La variabilidad inducida por radiación se evaluó en plántulas usando descriptores morfológicos. Dosis inferiores al DL_{50} inducen significativa variación en ambas variedades. Fue discutida la utilidad de la metodología usada en el mejoramiento de variedades de aguacatero. Esta metodología puede resultar una alternativa que complemente a los métodos de mejoramiento convencionales de aguacatero, particularmente para mejorar características como resistencia a la pudrición de la raíz y la tolerancia a la salinidad, donde los métodos de selección *in vitro* deben ser muy importantes.

Palabras claves: Cultivo de embriones cigóticos, mejoramiento por mutaciones, rayos γ, aguacatero

*I*ntroduction

Avocado breeding using conventional hybridization methods has been limited because trees require long juvenile periods and large areas, making breeding programs very expensive. In Cuba, breeding efforts have been limited to variety selection, vegetative propagation and *ex situ* conservation [1].

Mutation induction techniques are alternative breeding methods, which have been widely used for the improvement of major crops, ornamental plants and eventually perennial fruit trees [2]. However, studies on avocado improvement using this technique are very scarce. Collaborative efforts were made by Salvador Sánchez Colin CICTAMEX Foundation and the Institute for Nuclear Research (ININ) of Mexico to obtain dwarf and architecturally improved genotypes. These studies demonstrated the usefulness of mutation induction to modify plant architecture, vegetative growth, flowering, fruit setting and certain changes on fruiting behavior in avocados [3-5].

In addition to mutation induction, biotechnological techniques appear to be important approaches to improve avocados because of the versatile micropropagation and regeneration systems now available [6-14]. It has been also indicated [15], that the combined use of mutation induction and biotechnological techniques is a more effective approach for plant breeding because it may allow for selection optimization, shorter breeding schemes and there-

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fore, diminishing costs of breeding efforts. Now, a FAO/IAEA Coordinated Research Project aimed at improving fruit crops by mutation induction and biotechnology has been established [16].

We have recently indicated the potential of zygotic embryo culture as a breeding method in avocados [1]. The hypothesis of the present study is that *in vitro* culture of zygotic embryos could be an effective model for mutation breeding in avocados. According to this, our objectives were: (1) to develop radiosensitive curves of zygotic embryos of the Duke and Hass varieties and to establish their γ-rays mutagenic doses; (2) to compare *in vitro* response of irradiated and non-irradiated zygotic embryos of both avocado varieties; and (3) to evaluate the induced variability at the plantlet state by this type of treatment by means of plantlet morphological descriptors.

Materials and methods

Plant material

Fruits were obtained from open-pollinated trees of the Duke and Hass varieties located at the Güira de Melena station of the Tropical Fruit Research Institute (IIFT). Genotypes were selected on the basis of their relevance for breeding purposes in Cuba.

Zygotic embryo culture

Seeds with different developmental stages as those of fruits of between 20-43 weeks old from fruit-set were used. The embryo was considered mature when it was extracted from ripe fruits. Seeds were dipped into 90% (v/v) ethanol and flamed for surface sterilization. Aseptic seeds were divided in two into separated cotyledons, excising the plumule-radicle axes together with 1 cm thick sections of the cotyledon, and transferring them into tubes containing a nutrient medium.

For all experiments, zygotic embryos were put on filter paper bridges into glass tubes containing 5 ml of Murashige and Skoog [17] salt medium diluted to half strength (½ MS) supplemented with 30 000 mg/L of sucrose, 100 mg/L of *i*-inositol, pH 5.7±0.1. Four week-old entire plantlets were transferred to glass pots containing 10 ml of the fresh medium and grown for eight more weeks. Cultures were grown in a climatized room with a relative humidity of 60%, temperature of 25±2 ºC and light intensity of 2 500 lx provided by Chiyoda lux fluorescent lamps and measured using a Yu116 Luxometer (Russia). A 16 hour light photoperiod was used.

Radiosensitivity curves

Glass tubes containing mature zygotic embryos of the Duke and Hass varieties were irradiated in a dose range of between 15 and 50 Gy. Then, the embryos were immediately transferred to new tubes with a fresh medium. Irradiation was conducted in a Russian PX-γ-30M ^{60}Co irradiator at 35 ºC. The rate dose values ranged between 38-46 Gy/min, estimated by a Fricke dosimeter.

The percentage of induction of the entire sprout was used as the criterion to determine variety sensitivity to gamma rays. This indicator was calculated for each treatment (radiation dose) as induced entire sprout/total embryo number. At least three experiments were carried out for each treatment and a minimum of 20 embryos were used in each experiment. Embryo survival data were computed for polynomial fit analysis according Origin-PC package (Microcal Software, Inc.).

Induced variability at plantlets state

Plantlet height (PH), diameter of plantlet neck (DPN), number of leaves (LN) and length of the principal root (LPR) descriptors were used to evaluate the induced variability by γ-rays. Mean values of PH, DPN, LN and LPR and their standard error were calculated using a Kolmogorov-Smirnov test. Variance homogeneity was estimated by the F maximum test. Mean values of plantlet morphological descriptors for each radiation dose was compared with those obtained from a non-irradiated sample using a Dunnett test.

Results and discussion

Radiosensitivity curves for the Duke and Hass varieties are shown in Figure 1. The inhibition of the entire sprout fraction depended on the radiation dose according to the equation $Y(x)=a+b_1x+b_2x^2$, where $Y(x)$ is the logarithm of the entire sprout fraction, x is the radiation dose; and a, b_1 and b_2 are the equation parameters (Table 1). The fit between the experimental data and the theoretical model was of 0.95 and 0.96 for the Duke and Hass radiosensitivity curves, respectively. In contrast to that observed in radiosensitivity curves based on the entire sprout fraction, the variation of plantlet morphological descriptors was not correlated with an increase in radiation dose (data not shown), which demonstrates their inadequacy as indicators of the toxicity produced by radiation.

Based on the equation parameters, mean lethal dose (LD_{50}) values, here defined as the dose at which 50% of the entire sprout fraction is obtained, were calculated in 28 and 27 Gy for the Duke and Hass varieties,

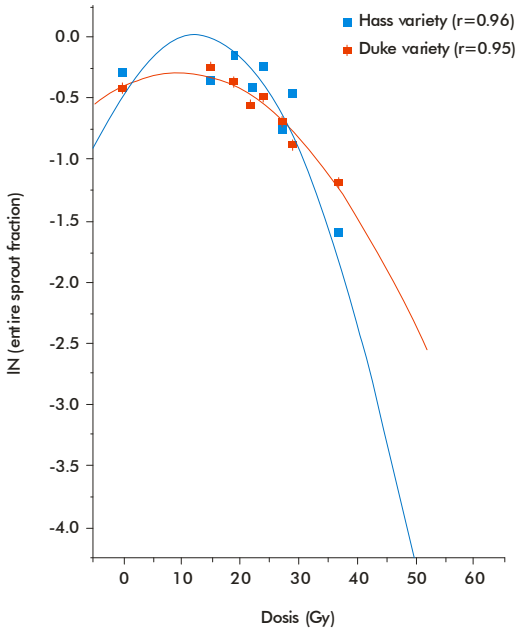

Figure 1. Effect of γ-rays on the survival of avocado embryos of the Duke and Hass varieties.

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Table 1. Fit and equation parameters obtained from polynomial analysis

Varieties	R-Square	Equation parameters		
		a	b_1	b_2
Duke	0.95*	-0.3943	0.0166	0.0009
Hass	0.96*	-0.4711	0.0775	0.0004

(*) Significant for p<0.0001

Table 2. *In vitro* response of irradiated† and non-irradiated embryos of the Duke and Hass varieties cultivated in ½MS medium

	Non-irradiated embryos		Irradiated embryos	
	Duke	Hass	Duke	Hass
Number of cultivated mature embryos	203	99	698	317
Percentage of germinated entire embryos	71	71	52	76
Percentage of non-germinated embryos	2	5	7	3
Percentage of germinated incomplete embryos	26	12	35	9
Percentage of contaminated cultures	1	12	6	12

(†) Considering only irradiated embryos at doses lower than LD_{50} values

respectively. Doses lower than the LD_{50} were not toxic for either variety and did not significantly change variety performance in culture (Table 2). *In vitro* germination, rooting and contamination levels were very similar for non-irradiated and irradiated avocado zygotic embryos. This result suggested a similar sensitivity to γ-rays for both varieties, however; doses higher than LD_{50} evidenced that the Hass variety was more sensitive to the lethal effect of radiation.

Sánchez-Colín *et al* [3], have previously established LD_{50} values of between 20 and 40 Gy for avocado ecotypes based on the loss of grafting capacity of irradiated avocado scions. Using the same criterion, an LD_{50} of 30 Gy for the Hass variety has been also reported [18]. The results here obtained with Hass variety suggested that zygotic embryos are slightly more sensitive to gamma rays than scions, perhaps due to differences in the radiosensitivity of both tissue types or a higher moisture content in embryos than in scions. The effect of moisture content on the radiosensitivity of avocado varieties has been previously demonstrated [4].

Radiation induced variability was evaluated at plantlet stage by means of plantlet morphological descriptors (Table 3). Dose values below DL_{50} induce significant variation in both avocado varieties. Thus, the treatment of the Hass variety with 15 Gy produced variation in plantlet height and number of leaves, while a dose of 22 Gy varied the diameter of the neck and the number of leaves. In addition, the irradiation of zygotic embryos of the Duke variety using doses of 15 and 21 Gy produced variation in the length of the principal root and the number of leaves, respectively; while a dose of 26 Gy varied the plantlet height and the number of leaves. Interestingly, other qualitative indicators such as leaf and root anomalies, atrophied and chlorophyll-deficient sprout and albinism were observed at doses higher than the LD_{50} values. However, it was not possible to quantify these indicators because the percentage of complete sprouts dramatically decreased for doses above DL_{50}.

Visser [19] indicated that doses ranging between LD_{60}-LD_{70} were useful for mutagenesis in fruit trees. According to this, we have calculated mutagenic doses of between 19 and 26 Gy for the Hass and Duke varieties, which agree with those obtained by De la Cruz *et al* [18] irradiating scions of the Hass variety. However, we demonstrated that a dose of 15 Gy could induce variability in our experimental model, suggesting that doses that are useful for mutagenesis cannot be restricted to this range. According to this, the election of mutagenesis doses based on toxicity (LD_{50} value) could be more effective for breeding purposes in avocados.

The present study demonstrated the usefulness of gamma rays to induce variability in plantlet architecture in accordance to previous reports [4, 5]. However, further field studies would be necessary to evaluate the true usefulness of the induced variability.

It has been indicated [15], that the use of *in vitro* techniques such as anther/microspore culture, shoot organogenesis and somatic embryogenesis can overcome some of the limitations in the application of mutation techniques; these are the lack of effective mutant screening techniques and the unrealistically large but necessary size of the mutated population, calculated on the basis of an expected mutation frequency for a desired trait.

The use of embryos culture techniques combined with mutation induction seems particularly suitable for the improvement of avocado varieties. This combined methodology could be an alternative to traditional breeding methods to improve important characteristics such as root-rot resistance and salt tolerance in avocados, where *in vitro* selection methods could be determinant in obtaining favorable genotypes.

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Table 3. Gamma rays induced variability in avocado plantlets as measured by morphological plantlet descriptors+

Doses (Gy)	PH (mm)	DPS (mm)	LN	LPR (mm)
Duke Variety				
0	35±2	1.26±0.09 n.s	14±0	116±11
15	34±3 n.s	1.31±0.16 n.s	18±2 n.s	224±42*
19	43±5 n.s	1.19±0.09 n.s	20±2 n.s	57±10 n.s
21	36±1 n.s	1.26±0.06 n.s	19±1*	108±12 n.s
24	35±6 n.s	1.17±0.17 n.s	16±4 n.s	108±37 n.s
26	44±2*	1.20±0.05 n.s	19±1*	121±14 n.s
Hass variety				
0	34±4	2.54±0.21	11±1	131±15
15	55±6*	2.17±0.22 n.s	19±2*	193±39 n.s
19	38±4 n.s	2.25±0.15 n.s	15±2 n.s	122±13 n.s
22	26±3 n.s	3.15±0.12*	5±0*	122±15 n.s
24	37±5 n.s	2.20±0.13 n.s	15±2 n.s	144±39 n.s
27	19±3 n.s	2.73±0.19 n.s	6±1 n.s	168±15 n.s

(+) Mean and standard error are presented (*) Significant for p<0.05 in a Dunnett test

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