GAMMA RAYS INDUCED VARIABILITY IN MATURE EMBRYOS OF AVOCADO (*Persea americana* Mill).

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ABSTRACT

Induced mutation and biotechnology techniques are current approaches used in plant breeding. At present work, the induced mutation and embryo zygotic culture techniques were used in order to characterize the radiosensitivity of avocado commercial varieties, Hass and California. The induced diversity in plant material was also evaluated in morphological seedling descriptors as: height seedling, diameter seedling neck, leaves number, length of principal root and secondary root number. The obtained results showed high susceptibility of both varieties to gamma rays. California was the higher sensitivity variety. Percentage of entire shoot induction showed clear dependence of radiation dose in both varieties. Thus dose range for mutagenesis was determined. In general, variation of morphological seedling descriptors not was clearly agreed to increase of radiation dose. In addition, the results suggested that variation in morphological seedling descriptors also could be depending of genotypes. The useful of mature embryo culture of avocado for improvement of breeding approaches in this crop, was discussed.

INTRODUCTION

The avocado is an important fruit tree, which have been incorporated into dietary culture of many countries from our region. In despite of their wide acceptation, pest, disease and abiotic stress as salinity have limited intensive production.

In Cuba, the rot root produced by the fungal *Phytophthora cinnamomi* cause serious damages on the crop yield. Additionally, high tree limits the sanitary control, management and fruit collection. For this reason, is priority objective of avocado breeding programs increase crops yield using varieties with improved resistance to pest and disease and to obtain dwarf genotypes [1].

Genetic breeding in avocado using conventional hybridization methods is quite difficult [2], 1975), therefore, only a few formal genetic studies have been reported [3,4,5,6,7]. The main

problems, which limit it, are the long juvenile period and the large area required for growing trees doing breeding programs very expensive. In or country, breeding efforts have been limited to selection of varieties, its propagation and *ex situ* conservation.

The induced mutation techniques are an alternative breeding methods, which have been widely used for genetic improvement of plant crops. Until today, 2252 new varieties from 175 plant species have been officially released for production. In spice of, wide acceptance of induced mutation techniques for the purpose mentioned above, relatively poor favorable results have been reported in fruit trees [8].

The avocado mutagenesis studies using ionizing radiation are very recent. Salvador Sánchez Colin CICTAMEX Foundation in Mexico initiated studies in 1988 in order to obtain dwarf and architectural improved genotypes. Nowadays, field evaluation of agronomic important traits of the obtained mutants has being conducted yet.

Combined use of biotechnology and induced mutation techniques could be an interesting alternative to breeding in avocado. This approach could optimize selection, shorten breeding schemes and therefore diminish cost of breeding effort [9]. We have reported an *in vitro* propagation method for avocado from mature zygotic embryos, which have proved usefulness into Cuban avocado varieties [1].

Considering this precedent experience, we survey the sensibility of mature zygotic embryos of two avocado varieties (Hass and California) to gamma rays as soon as the induced variability by this treatment on seedling architecture in order to assemble more effective approach for genetic improvement of avocado.

MATERIALS AND METHODS

Plant material

Hass and California varieties fruits were obtained from Cuban avocado germplasm located at Güira de Melena station of the Citrus and Fruits Research Institute.

Mature zygotic embryo culture

Mature zygotic embryos from Hass and California varieties were cultured in half concentration (1/2) of MS [10] medium as described Rodríguez *et al.*, [1], with some modifications. Embryos were rescued in ½ MS medium supplemented with 0.5 mg/l of bencilaminepurine (BAP) and giberelic acid (AG_3). Thirty days olds entire shoots were trespassed to solid ½ MS medium with the same hormone concentrations.

Material Irradiation

Irradiation was conducted in a Russian PX-γ-30M ^{60}Co irradiator at 35ºC. Embryos were irradiated in a dose range from 22-66 Gy. The rate dose value was 46.32 Gy/min, estimated by Fricke dosimeter as described by Prieto and Cañet, [11].

Radiosensitivity curve

Percentage of entire shoot induction was used as criterion in order to know varieties sensibilities to gamma rays. This indicator was calculated as induced entire shoot / total embryo number for each treatment. Forty and thirty embryos were used by each treatment in Hass and California experiments, respectively. Additionally, height seedling (HS), diameter of seedling neck (DSN), leaves number (LN), length of principal root (LPR) and secondary root number (SRN) were also

used in order to evaluate the induced variability by the gamma ray treatments. Morphological descriptor records were achieved using two months old seedling.

Statistical analysis

Means values of HS, DSN, LN, LPR and SRN and their standard deviation were calculated using Kolmogorov-Smirnov test. Variance homogeneity was estimated by the F maximum test. Mean values of seedling morphological descriptors for each radiation dose were compared with those obtained on not irradiated material using student test.

RESULTS AND DISCUSSION

Dose effect studies developed with Hass and California varieties showed that gamma dose greater than 27 Gy inhibited entire shoot induction. Thus, only results of treatments 23 and 27 Gy as well as no irradiated embryos could be recorded. Unfortunately, dose smaller than 15 Gy (transient dose) could be studied neither.

Previous study [12], has evaluated lethality of gamma ray on mexican avocado germplasm using irradiated scions as experimental model. This study showed that doses higher than 60 Gy produce zero percentage of grafted scions. The results here obtained suggested that mature embryos are highly sensible to gamma rays, maybe by more elevated moisture content than those present in scions. Effect of moisture content on radiosensitivity of avocado varieties has been clearly demonstrated [13].

Based on recovered data the percentage of entire shoot obtained from both varieties was calculated. Then it was used in order to know varieties sensibility to gamma rays (Figure 1). ID_{50}, were defined as dose which diminish in 50 % the induction of entire shoot. It was in range from 20-30 Gy for both varieties. This result agrees to those obtained by de la Cruz *et al.*, [14] using irradiated avocado scions. These authors founded that DL_{50} was 29.54 Gy for Hass variety.

Sensibility to gamma ray was higher in California variety. This variety showed a dramatic diminishes of entire shoot number throughout dose of 23 Gy. Although at present study was not identified optimal dose for mutagenesis, is clear that useful dose ranges could not exceed the 30 Gy. Previous studies [14] showed that useful dose range for gamma-mutagenesis in trees of Hass variety was between 20-25 Gy. The results here founded using mature embryos as experimental model agree with those obtained by these authors.

Induced variability on mature zygotic embryos was evaluated in five morphological seedling descriptors (Table 1). Since the only one entire shoot from California variety was obtained for treatment of 27 Gy, dates are not enable for this dose.

Radiosensitivity curve using Hass variety suggested dose dependent variation in HS, DSN and SRN descriptors. Thus, HS significantly decreased throughout dose of 22 Gy and DSN and SRN to do at 27 Gy. Contrarily, the variation of LN not agreed to increase of radiation dose. Increase in LPR was apparently dependent of radiation dose, however it not was significant. By the other way, the conducted experiment using California variety was not sufficient to evidence variation dependent of radiation dose. The unique dose, which could be recorded shown significant variation respect to non-irradiated material only in HS, LPR and SRN descriptors.

In general, variation of morphological seedling descriptors not was clearly agreed to increase of radiation dose. A limited dose number evaluated at present study could be cause of this picture. In addition, the results suggested that variation in morphological seedling descriptors also could be depending of genotypes.

At present study, dose range for mutagenesis in Hass and California varieties were defined. However, the results here showed are preliminary and mutagenic dose for each genotype could be determined. Additionally, the usefulness of gamma rays to induce variability in seedling architecture was demonstrated. At this regard, morphological seedling descriptors could be useful for radiosensibility studies as well as *in vitro* selection markers in avocado breeding approach. Futures studies will be necessary in order to demonstrate the utility of mature culture embryos methodologies for genetic improve of avocado.

REFERENCES

1. Rodríguez, N.N.; V. Fuentes; O.L. Rodríguez, y M. Alvarez. 1997. Cultivo *in vitro* de embriones maduros e inmaduros de aguacatero (*Persea americana* Mill). Agricultura Técnica (Chile) 57 (2):154-158.
2. Bergh B.O., 1975. Avocado. In: Advances in fruit breeding. Janick J and Moore J.N. (ed.), Purdue Univ. Press, West Lafayette, Ind, pp:541-567.
3. Degani C. and Gazit S., 1984. Selfed and crossed proportions of avocado progenies produced by caged pair of complementary cultivars. HortScience 19:258-260.
4. Lahav E., Zamet D., Gazit S. And Lavi U., 1986, Girdling as a means of shortening the juvenile period of avocado sedlings HortScience 21:1038-1039.
5. Lavi U., Lahav E., Genizi A., Degani C., Gazit S. And Hillel J., 1991. Quantitative genetic analysis of traits in avocado cultivars Plant Breeding 106:149-160.
6. Lavi U. , Degani C. and Gazit S., 1993a. Genetics of skin color, flowering group and anise scent in avocado. The Journal of Heredity. 84: 82-84.
7. Lavi U., Lahav E., Degani C., Gazit S. and Hillel J., 1993b. Genetic variance components and heritatabilities of several avocado traits. J. Amer. Soc. Hort. Sci. 118: 400-404.
8. Maluszynski M., Nichterlien K., Van Zanten L. y Ahloowalia S., 2000. Officially released mutant varieties-The FAO/IAEA database. Mutation Breeding Review. No 12:1-84.
9. Maluszynski M., Ahloowalia B.S. and Sigurbjornsson B., 1995. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. Euphytica 85:303-315.
10. Murashige T. y Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiology 15:473-493.
11. Prieto E. y Cañet F., 1990. Aspectos a considerar en el dosímetro Fricke. Tecnología Química . Año XI. No 2 p 19.
12. Sánchez S., Rubi M. and Sosa R., 1990. Variability induction by irradiation of avocado (*Persea americana* Mill) scions. Proceeding of Salvador Sánchez Colín foundation meeting , CICTAMEX, S.C., Coatepec Harinas, Mexico, pp:41-48.
13. Rubi M., Saavedra C., de la Cruz E. and Hernández M., 1993. Effect of moisture content on radiosensitivity of avocado (*Persea americana* Mill). Proceeding of Salvador Sánchez Colín foundation meeting , CICTAMEX, S.C., Coatepec Harinas, Mexico, pp:111-119.
14. De la Cruz E., Hernández M., Rubi M. and Savedra C., 1993. Radiosensibility study on Hass avocado for breeding purposes. Proceeding of Salvador Sánchez Colín foundation meeting, CICTAMEX, S.C., Coatepec Harinas, Mexico, pp:121-128.

Table 1. Induced variability on morphological seedling descriptors. Mean and their standard deviation are presented.

Doses (Gy)	HS (mm)	DSN (mm)	LN	LPR (mm)	SRN
Hass variety					
0	38.16 ± 20.3	3.32 ± 0.48	8.00 ± 2.19	123.16 ± 74.09	0.63 ± 0.95
22	26.08 ± 16.97*	3.15 ± 0.61$^{n.s.}$	5.38 ± 2.04***	121.92 ± 75.22$^{n.s.}$	0.15 ± 0.46$^{n.s.}$
27	19.09 ± 10.44**	2.73 ± 0.65 **	6.09 ± 2.84*	168.18 ± 51.15$^{n.s.}$	0.09 ± 0.3*
California variety					
0	39.26 ± 17.46	3.12 ± 0.73	10.91 ± 2.44	211.62 ± 101.10	1.12 ± 1.68
22	17.50 ±11.34**	2.62 ± 0.52$^{n.s.}$	9.25 ± 2.43$^{n.s.}$	205.00 ± 100.71**	0.12 ± 0.35**
27	-	-	-	-	-

Student test signification were 95 (*), 99 (**) and 99,9 % (***).

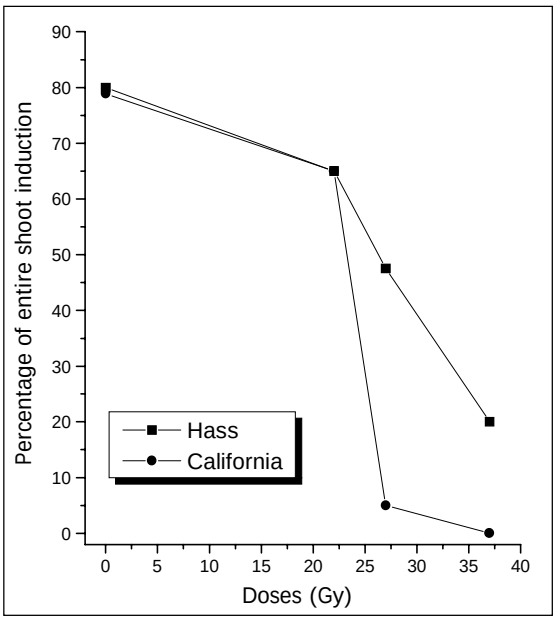

Figure 1. Radiosensitivity curve based on percentage of entire shoot induction for Hass and California varieties.