Effect of gamma irradiations on pineapple pollen germination and tube growth

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ABSTRACT
Use of irradiated pollen allowed sometimes to induce in situ parthenogenesis, and then to obtain haploid plants. As a first step to develop this technique with pineapple (*Ananas comosus* (L) Merrill), pollen of two varieties, Serrana (Smooth C) and Red spanish, was gamma-ray irradiated with a cobalt-60 source at 16 Gy/min, with rates of doses from 0 to 6 kGy. The in vitro viability (pv) and pollen tube length (ptl) of the both varieties decreased at the highest doses, and 6 kGy destroyed completely their (pv). Serrana smooth cayenne was more radiosensitive than Red spanish. There were correlations between (pv) and (ptl) vs irradiation doses.

Effet de rayons gamma sur la germination du pollen d'ananas et sur la croissance du tube pollinique.

RÉSUMÉ
L'utilisation de pollen irradié a permis, dans certain cas, de provoquer l'induction de parthénogenèse in situ, et donc d'obtenir des plantes haploïdes. En préalable à la mise au point d'une telle technique sur ananas (*Ananas comosus* (L) Merrill), le pollen de deux variétés, Serrana (Cayenne lisse) et Red spanish, a été irradié avec une source de cobalt-60 à 16 Gy/min à raison de doses allant de 0 à 6 kGy. La viabilité in vitro du pollen (vp) et la croissance du tube pollinique (ctp) ont diminué significativement, pour l'un et l'autre cultivar, lors de l'utilisation des plus fortes doses.
En particulier, (vp) est devenue nulle à 6 kGy. Serrana se révèle plus radiosensible que Red spanish. (vp) et (ctp) se sont avérées négativement corrélées avec les doses d'irradiation utilisées.

Efecto de los rayos gama sobre la germinación del polen y crecimiento del tubo polínico en piña.

RESUMEN
La utilización de polen irradiado permitió, en ciertos casos, la inducción de partenogénesis in situ, y asi obtener plantas haploides.
Previo a la puesta a punto de esta técnica en piña (*Ananas comosus* (L) Merrill), el polen de dos variedades, Cayena lisa Serrana y Española roja, se irradiaron con una fuente de cobalto-60 a 16 Gy/min, a dosis de 0 a 6kGy. La viabilidad in vitro del polen (vp) y el crecimiento del tubo polínico (ctp) disminuyeron. Particularmente, (vp) fue nula a 6 kGy. Serrana fuen más radiosensible que Española roja. Existieron correlaciones entre (vp) y (ctp) con las dosis de irradiación utilizadas.

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KEYWORDS
Ananas comosus, pollen, in vitro culture, gamma radiation, radiosensitivity, viability, germinability.

MOTS CLÉS
Ananas comosus, pollen, culture in vitro, rayonnement gamma, radiosensibilité, viabilité, faculté germinative.

PALABRAS CLAVES
Ananas comosus, polen, cultivo in vitro, radiación gamma, radiosensibilidad, viabilidad, poderé germinativo.

introduction

For pineapple, which is a vegetatively propagated species with high level of heterozygosity (CABOT, 1987), the haploid induction could bring about a significative advance in genetic and pineapple improvement. The production of homozygotic plants in pineapple could be used in breeding programs and should allow to obtain new varieties in a short time. Haploid and dihaploid plants would also be useful in providing access to homozygous recessive genes, whenever they remain economically advantageous as in the case of deleting marginal spines (SUBRAMANIAN et al, 1981) and for biotechnological manipulations. However, the usefulness of this approach is as for yet limited in pineapple breeding.

The pollen irradiation technique has been widely used in plant breeding. Recently RAQUIN (1985) developed an in vitro haploidization technique by in vitro cultures of ovaries. It involves the induction of in situ parthenogenesis by pollination with irradiated pollen. One possible explanation of the mechanism of parthenogenesis induction is that the polar nuclei are fertilized by one generative nucleus without oosphere fertilization, giving rise to a normal triploid albumen and a haploid embryo (FALQUE et al, 1992). This technique has permitted the production of haploid plants in petunia (RAQUIN, 1985), *Malus/domestica* (ZHANG et al, 1988), onion (DORÉ and MARIE, 1993), rose/hybrid, cv 'Sonia' (MEYNET et al, 1994), watermelon (SARI et al, 1994) and other species. This strategy offers the possibility for producing haploid in those species in which neither in vitro androgenesis nor gynogenesis have yet been achieved (ZHANG et al, 1990).

Knowledge about radiosensitivity is important for developing such techniques and the study of pollen tube growth is a good method for its evaluation (CUNY and ROUDOT, 1991). Therefore, this paper presents initial studies on the effect of gamma-rays on in vitro pollen viability and pollen tube growth in two pineapple cultivars, Serrana smooth cayenne and Red spanish.

materials and methods

plant material

The pineapple plants, belonging to the experimental station, came from the University of Ciego de Avila.

pollen irradiation

Freshly opened flowers of Serrana smooth cayenne and Red spanish cultivars were picked up for 4 consecutive days. Twelve stamens, each from different flowers, were placed inside petri dishes on a microscope slide and sealed with Parafilm. They were irradiated at the laboratory of irradiation techniques in CENSA, La Habana, with gamma-rays of cobalt-60 (radiation flow rate: 16 Gy/min) at doses of 0, 1, 2, 3, 4, 5 and 6 kGy. For the 0 kGy dose, the flowers were transported to and from the irradiation source.

in vitro culture of irradiated pollen

The irradiated pollen was spread on solid medium (WEE and RAO, 1979) in petri dishes (44 mm), which were close into a glass containing distilled water. Cultures were incubated at 27 °C. Samples were fixed using FAA (formaldehyde, ethanol 95°, acetic acid) 6 h later. The dishes were then stored in a refrigerator at 5 °C until the in vitro viability and pollen tube length rates were recorded with a microscope eyeglass micrometric. The in vitro pollen grain viability was achieved when pollen tube length had overcame the diameter of them. Moreover, pollen tube length was recorded just on in vitro germinated pollen grain. Two replicates of 200 pollen grains were observed for each treatment.

results and discussion

Results in figure 1 show that the in vitro pollen viability was affected by gamma-ray irradiation. There were significant differences between different doses and studied culti-

vars. Nevertheless, there was no difference on pollen viability between 0 and 1 kGy in Serrana smooth cayenne (43.9% and 41.18% respectively) and Red spanish (50.16% and 48.70% respectively). However, there were differences in the analysis of this parameter between cultivars on each irradiation doses. Red spanish (r = –0.8215, y = 57.2685 – 10.0270 * doses) was in general more radio-resistant than Serrana smooth cayenne (r = –0.9213, y = 49.7400 – 9.0080 * doses). It was confirmed by the high LD_{50} values achieved in Red spanish (3.65 kGy), whereas Serrana smooth cayenne shows it at 2.65 kGy. Meanwhile, the lethal dose was reached with the same dose (6 kGy) in both cultivars.

CUNY and ROUDOT (1991) reported that high irradiation doses (4 kGy) were necessary in melon to obtain complete inhibition of the pollen viability, whereas ZHANG and LESPINASSE (1991) stated that the apple pollen viability was not significatively influenced by gamma irradiations up to 1000 Gy.

The effect of gamma irradiations on pollen tube growth after 6 h of evaluation is illustrated in figure 2. There were no statistical differences between 0, 1 and 2 kGy doses in Red spanish pollen. The pollen tube length values oscillated from 555.67 µm at 0 kGy dose to 549.70 µm and 556.96 µm with 1 and 2 kGy doses respectively. A slight increase was obtained until 2 kGy dose. The maximum length of pollen tubes was affected only by doses greater than 2 kGy. Moreover, doses greater than 1 kGy were sufficient to reduce pollen tube growth rates in Serrana smooth cayenne. This effect was clearer with doses greater than 2 kGy.

In general, pollen tube length reductions were correlated by increasing the irradiation doses as Red spanish (r = –0.88, y = 655.0876 – 100.03 38 * doses) as Serrana smooth cayenne (r = –0.91, y = 561.8257 – 88. 1924 * doses). However, the highest doses (6 kGy) promoted an absolutely decreasing of the pollen tube length in the two cultivars.

The pollen tube length of *Cucumis melo* (CUNY and ROUDOT, 1991) was reduced and there was a delayed effect in the increase of

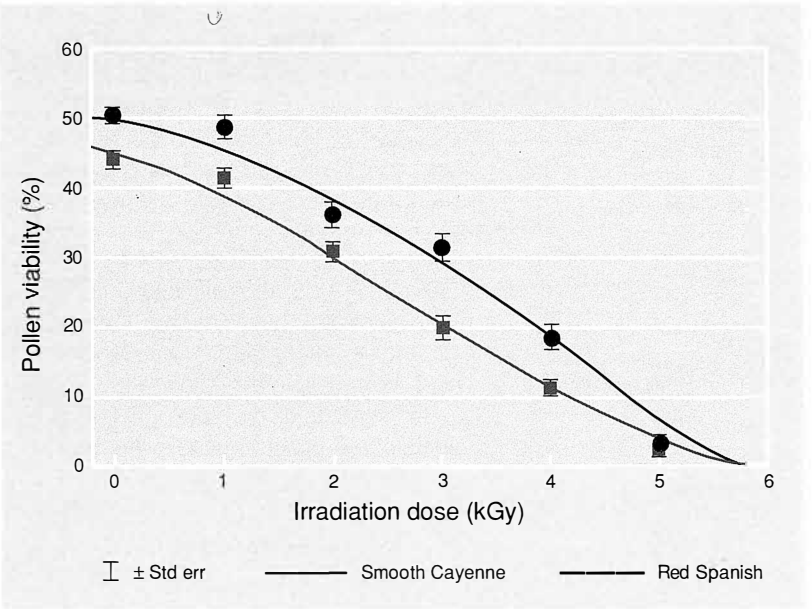

Figure 1
Effect of gamma-rays on the in vitro pollen viability from two pineapple cultivars, observed after 6-h irradiated pollen incubation.

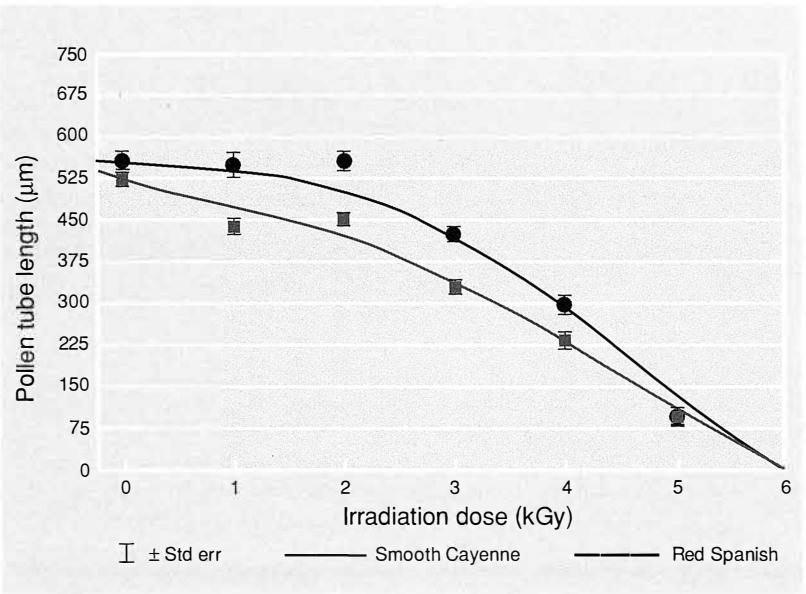

Figure 2
Effect of gamma-rays on the in vitro pollen tube growth from two pineapple cultivars, observed after 6-h irradiated pollen incubation.

velocity of them with the irradiation doses. Meantime, the pollen germination and pollen tube growth were reduced at higher doses on barley and rye varieties (ROHILLA and KHANNA, 1993). In pineapple, SUBRAMANIAN et al (1981) demonstrated that pollen irradiation could overcome self-incompatibility. Besides, they observed that irradiated pollen at doses higher than 25 kr inhibited the seed set over all pollinated fruits. These results show the high radioresistance of the two studied pineapple cultivars. Moreover, it could be possible to know the effect of gamma irradiations on pineapple pollen germination and pollen tube growth. Thus, this research will allow to begin the works on in situ parthenogenesis induced by pollination with pineapple irradiated pollen.

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