Original article

Mitotic instability in banana varieties. V – Chromosome loss in plants derived from gamma-irradiated cultures of a triploid genotype

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Mitotic instability in banana varieties.
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Abstract — **Introduction**. The objective of this study was to report the impact of gamma-irradiation on chromosome loss and deficiencies in triploid Silk bananas. **Materials and methods**. Three sets of AAB cultivar Maçà (2n = 3x = 33) plants of different origin were analysed. While one set was an untreated control, two had been subjected to irradiation with Co^{60} at a dose of 2.5 kR applied on in vitro proliferating shoot tips and later exposed in culture to toxin filtrate of *Fusarium oxysporum* sp. *cubense*, to select resistant genotypes. A cytological analysis was performed on cells from root tips of the plants. **Results**. In contrast to control plants, those subjected to gamma-irradiation were found to show an extremely high level of chromosome loss. In the two distinct lots, half or more of the cells examined had less than 33 chromosomes with the most extreme cell having only 23 chromosomes. **Discussion**. As high mitotic instability can occur after mutagenic treatments, somatic karyotype analysis of products should form an integral component part of any project for the induction of mutations in *Musa* with gamma-irradiation. © Éditions scientifiques et médicales Elsevier SAS

Brazil / gamma irradiation / chromosome aberrations / chromosome numbers

Instabilité mitotique des variétés de bananier.
V – Perte de chromosomes chez des plants issus de cultures de triploïdes irradiés par rayons gamma.

Résumé — **Introduction**. L'objectif de l'étude a été d'étudier la perte de chromosomes et les déficiences obtenues sur bananiers triploïdes de type Figue-Pomme, après irradiation par rayons gamma. **Matériel et méthodes**. Trois ensembles de plants du cultivar Maçà (AAB, 2n = 3x = 33) de différente origine ont été analysés. Alors qu'un lot témoin restait non traité, deux autres étaient irradiés au Co^{60} à la dose de 2,5 kR appliquée à des cultures proliférantes in vitro d'explants de tiges, soumises ensuite à un filtrat de toxines obtenues à partir de *Fusarium oxysporum* sp. *cubense*, pour sélectionner des génotypes résistants. Après traitement, un dénombrement de chromosomes a été effectué sur des pointes racinaires de ces plants. **Résultats**. À l'inverse des plantes témoins non traitées, les plantes irradiées ont présenté de fortes pertes de chromosomes dans les pointes racinaires. Pour les deux lots de plantes traités, plus de la moitié des cellules étudiées avaient moins de 33 chromosomes dont un cas extrême de cellule n'ayant que 23 chromosomes. **Discussion**. Comme les traitements mutagènes sont propices à une certaine instabilité mitotique, l'analyse des caryotypes somatiques des plants obtenus devrait constituer une composante essentielle de tout projet d'induction de mutations par irradiation chez le bananier. © Éditions scientifiques et médicales Elsevier SAS

Brésil / irradiation gamma / aberration chromosomique / nombre chromosomique

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1. introduction

Bananas are crops of primary importance for many tropical and subtropical countries, either as local foodstuff or for exportation. Historically, diseases have been the most important constraint, and most of the improvement programs throughout the world are still oriented today towards the creation of new varieties resistant to diseases (Black Leaf Streak, Panama disease) [1]. Because of the difficulties, i.e. crossing bananas and selecting outstanding new clones, alternative methodologies have been developed by several authors to induce mutations with new sources of resistance in the cultivated varieties.

In Austria, the International Atomic Energy Agency (IAEA) selected, through induced mutations, a new clone of Cavendish (Grande Naine – 60A) showing greater precocity with bigger and more cylindrical bunches [2]. In Australia, the Queensland Department of Primary Industry (QDPI), starting from a natural extra dwarf Cavendish (Dwarf Parfitt) with no commercial value but resistant to Panama disease race 4, selected a taller mutant, called Giant Parfitt having acceptable agronomic and commercial features. In Brazil, at Embrapa's *Centro Nacional de Recursos Genéticos e Biotecnologia* (Cenargen) in Brasilia, mutations were induced in proliferating shoot tip cultures to select new clones of Silk bananas resistant to Panama disease.

In earlier papers in this series, the first author and associates have identified anomalous chromosome numbers and breakage deficiencies in plants obtained from meristem cultures, as well as in their conventionally propagated counterparts [3, 4]. Mitotic instability in bananas has also been reported by other authors [5] without mutagenic treatment application in Cavendish subgroup vitroplants. Now that the improvement of bananas by induced mutations is being developed increasingly in several countries, an account of the chromosome situation of plants obtained from irradiated meristem cultures is seen to have enhanced value. The objective of this study was to report the impact of gamma-irradiation on chromosome loss and deficiencies in triploid Silk bananas.

2. materials and methods

The present study was performed with the AAB cultivar Maçà (Silk or Rastali). Three sets of plants of different origins were analyzed. While one set was an untreated control, two had been subjected to irradiation with a dose of 2.5 kR Co^{60} applied on in vitro proliferating shoot tips. They were later exposed in culture to toxin filtrate of *Fusarium oxysporum* sp. *cubense* to select resistant genotypes. The stocks subjected to analysis was as follows: K 86, irradiated plants and exposed to toxin; K 216, irradiated plants and exposed to a different batch of toxin; K 87, not irradiated nor exposed to toxin.

The chromosome analyses reported were performed during the period of 1987–1988, using the cytological methods described by Shepherd and Dos Santos [3] with vitroplants established in a glass house. The comment of Shepherd and Da Silva [4] on the response of this triploid genotype to 8-oxyquinoline is again relevant that well-spread chromosomes at mitosis were not readily achieved. For this reason, the count numbers per individual plant, of acceptable level of certainty, were lower than would have been hoped.

3. results

The control plants of K 87 were relatively stable, with only three aberrant mitotic plates among 44: two of these were deficient in chromosome number (31 and 32 chromosomes) and one had 34 instead of 33 chromosomes (*table I*).

For the K 86 and K 216 plant strains, statistical data show that the two irradiated strains did not significantly differ in their behavior, but they were both different from the control plants. The irradiated strains manifested a very marked tendency to chro-

Table I.
Chromosome counts obtained from two irradiated strains (k 86 and k 216) and one non-irradiated control group (k 87) of AAB cultivar Maçà at the CNPMF (Brazil), 1987–1988.

Strain number	Number of plants	Total cells	Numbers of cells with a given chromosome number												% with 33 chromosomes
			23	24	25	26	27	28	29	30	31	32	33	34	
K 86 (a)	16	73	–	–	–	–	1	–	1	1	4	28	35	3	48
K 216 (a)	8	69	1	–	–	1	1	1	2	3	7	28	23	2	33
K 87 (b)	6	44	–	–	–	–	–	–	–	–	1	1	41	1	93

χ^2 analysis: distributions of the different strains having the same letter do not differ significatively at the level of 1 %.

mosome loss, affecting half or more of the cells assessed. The majority of these had 32 chromosomes, but a significant number also had lower numbers (*table I*).

Only one mini-chromosome was identified and this was in the most deficient cell, that with only 23 chromosomes. A cell not in the table was "hexaploid" with 64 chromosomes.

Moreover, chromosome deficiencies in irradiated plants showed considerable changes at the level of individual plantlets and, in some instance, within the plates derived from a single root tip (data not shown in the table). These last results suggest that irradiated plants were considerably affected in the chromosome stability control during mitosis in roots whereas control plants were more stable.

Morphological abnormalities were often visible in the plants taken from irradiated cultures, whereas plants derived from control plants were relatively normal. Irradiated plants showed leaf narrowing and chlorotic interveinal streaking in greenhouse.

4. discussion

The results reported the occurrence of high mitotic instability with a Brazilian Silk variety of banana. Most cells showed losses usually ranging from one to seven chromosomes, with one case of cell with ten chromosomes. In some instance, chromosome losses have been associated with delayed plant growth and leaf abnormalities.

Chromosome damages are rather common in plants after mutagenic treatments [6]. In *Lactuca sativa*, irradiation-induced mutants led to the identification of chromosomal rearrangements associated with deletions [7]. In *Arabidopsis*, translocations were induced by fission neutrons [8]. Nevertheless, there are very few experiments with induced-γ ray mutagenesis reporting the loss of complete chromosomes. In our observations on irradiated shoot tips, it cannot be certainly claimed, on this limited evidence, that disturbances of the karyotype of such magnitude would invariably be consequences of irradiation in triploid bananas, no matter what dosages or other variations in method are employed such as the in vitro post-treatment selection with the toxin filtrate of *Fusarium oxysporum*. What the authors wish to do is to issue a warning that such events are possible and that somatic karyotype analysis of products should form an integral component part of any project for the induction of mutations. Somatic cytology by chromosomes counts on root tips or flow cytometry on every type of tissue must be used as a guide to the best protocols to be followed.

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Inestabilidad mitótica de las variedades de banano. V – Pérdida de cromosomas en las plantas procedentes de cultivos de triploides irradiadas con rayos gamma.

Resumen — Introducción. El objetivo del estudio era estudiar la pérdida de cromosomas y las deficiencias obtenidas en bananos triploides de tipo 'Figue-Pomme' tras irradiación con rayos gamma. **Material y métodos**. Se analizaron tres grupos de plantas del cultivar Maça (AAB, 2n = 3x = 33) de diferente origen. Se dejaba un grupo testigo sin tratamiento y los dos restantes se irradiaban con Co^{60} con una dosis de 2,5 kR aplicada a cultivos proliferantes in vitro de explantes de tallos que seguidamente se sometían a un filtrado de toxinas obtenidas a partir de *Fusarium oxysporum* sp. *cubense*, para seleccionar genotipos resistentes. Tras el tratamiento, se contaron los cromosomas en las puntas radiculares de dichas plantas. **Resultados**. Al contrario de las plantas testigo no tratadas, las plantas irradiadas presentaron importantes pérdidas de cromosomas en las puntas radiculares. En los dos grupos, más de la mitad de las células estudiadas tenía menos de 33 cromosomas y una de ellas sólo tenía 23. **Discusión**. Al ser los tratamientos mutagénicos propicios a cierta inestabilidad mitótica, el análisis de los cariotipos somáticos de las plantas obtenidas debería ser un componente esencial de cualquier proyecto de inducción mediante mutaciones en banano.

Brasil / *Musa* / irradiación gamma / número de cromosomas