

Radio-sensitivity of *in vivo* and *in vitro* cultures of banana cv. Basrai (AAA)

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Radio-sensitivity of *in vivo* and *in vitro* cultures of banana cv. Basrai (AAA).

Abstract — Introduction. Banana is a worldwide important fruit crop, and improved clones which are resistant/tolerant to different biotic and abiotic stresses are very much required. In this regard, prospects of *in vitro* mutagenesis are high and relevant. **Materials and methods.** The *in vitro* cultures (individual and multiple shoots) and *in vivo* plant materials (suckers and hardened plants) of banana cv. Basrai (AAA) were exposed to ⁶⁰Co gamma ray doses ranging from 0–100 Gy. Radio-sensitivity of suckers and *in vitro* individual shoots was assessed by recording data on survival, multiplication ratio, days to root initiation, root number, root length, shoot length, plant height, number of leaves, leaf area and chlorophyll content. **Results.** Increase in the dose of gamma-rays resulted in corresponding decrease in the growth of the explants. Further, as a trend, the *in vivo* explants were observed to be more vulnerable than the *in vitro* ones. This offers possibilities of obtaining live plant materials even at high doses thereby increasing the probability of higher frequency of mutations. Lower doses of 10 and 20 Gy had an enhancing effect on the multiplication ratio of *in vitro* multiple shoot cultures. **Conclusion.** The *in vivo* and *in vitro* plant materials exhibited differential response to gamma-irradiation. The results obtained from the present studies will be useful in refining strategies for *in vitro* mutation induction aimed at banana improvement.

India / Musa / plant breeding / mutation / gamma radiation / radiosensitivity / *in vitro* plants / ratoons

Radiosensibilité de bananiers « Basrai » (AAA) issus de cultures *in vitro* ou en champ.

Résumé — Introduction. La banane est une importante production fruitière dans le monde et des clones améliorés résistants ou tolérants à différents stress biotiques et abiotiques sont très recherchés. À cet égard, les perspectives offertes par la mutagenèse *in vitro* sont intéressantes et appropriées. **Matériel et méthodes.** Des pousses individuelles ou multiples obtenues *in vitro* et du matériel végétal en champ (rejets et plants acclimatés) de bananiers « Basrai » (AAA) ont été irradiés avec des doses de rayons gamma (⁶⁰Co) variant de 0 à 100 Gy. La radiosensibilité des rejets et des pousses individuelles issues d'*in vitro* a été évaluée par mesure des taux de survie et de multiplication, du temps d'enracinement, du nombre et de la longueur de racines, de la longueur des pousses, de la hauteur des plantes, du nombre et de la surface des feuilles et de la teneur en chlorophylle. **Résultats.** L'augmentation des doses de rayons gamma a entraîné simultanément la diminution de la croissance des plants. De plus, il est apparu que les plants en champ avaient tendance à être plus vulnérables que les plants *in vitro*. Cela laisse envisager la possibilité d'obtenir du matériel végétal vivant, même à fortes doses de radiations, augmentant de ce fait la probabilité d'obtenir une fréquence plus élevée de mutations. Les faibles doses de 10 et 20 Gy ont eu un effet positif sur le taux de multiplication des pousses multiples issues de culture *in vitro*. **Conclusion.** Le matériel végétal étudié a donné une réponse différente à des irradiations gamma selon qu'il était d'origine *in vitro* ou observé en champ. Les résultats obtenus lors de ces travaux pourront être utiles pour redéfinir des stratégies d'amélioration du bananier basées sur l'induction de mutation *in vitro*.

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1. Introduction

Banana is the world's largest fruit crop with a production of 58.3 Mt [1] and is also the most important fruit crop of India with a great socio-economic significance. One of the major objectives of banana breeding has been to develop genetically diverse cultivars for resistance against endemic diseases [2]. However, banana is intractable in terms of genetic improvement because of its long production cycle, polyploidy, seed sterility, predominant vegetative mode of propagation, etc. [2–4]. Owing to the complex nature of the *Musa* genetic system, inclusion of non-conventional methods in support of conventional breeding programs is of utmost importance. The prospects of using biotechnology for potential benefits are very high [3, 5–7]. Mutation breeding using micropropagation techniques has been proposed as an important tool to achieve the goals [4, 8, 9]. Understanding the radio-sensitivity of the explants to be mutagenized would then be the first step in this direction. Herein, the effects of ^{60}Co gamma irradiation on various *in vivo* and *in vitro* explants of banana are described.

2. Materials and methods

2.1. Plant material used and culture establishment

Basrai (AAA), a popular Cavendish type cultivar in India, was selected for the study. The various plant materials used for experimentation were (a) suckers of field grown plants, (b) individual as well as multiple shoots grown *in vitro* and (c) hardened plants. The multiple shoots were obtained by culturing *in vitro* the shoot apices from the field grown suckers [10]. The suckers were initially surface cleaned with liquid detergent and, then, with distilled water. After the removal of several ensheathing leaves, the explants were treated with 5% chlorine water, 70% ethanol and 0.1% HgCl_2 for 15, 8 and 7 min respectively, under aseptic conditions, with a minimum of four rinses with sterile double distilled water

after each treatment. Finally, the innermost shoot tips were cultured on MS medium [11] supplemented with benzylaminopurine (BAP, $5 \text{ mg} \times \text{L}^{-1}$) and adenine sulfate (AS, $30 \text{ mg} \times \text{L}^{-1}$) and gelled with 0.2% Gelrite. The cultures were incubated in a culture room with a light intensity of 1,000 lux, $25 \pm 2 \text{ }^\circ\text{C}$, relative humidity of 55–60% and 10 h / 14 h day / night cycle. At the end of three weeks of culture, the shoot-tip explants produced numerous multiple shoots. These were excised under aseptic conditions and cultured on the medium of same composition for shoot multiplication. The multiple shoots were dissected to obtain individual shoots which were placed on MS medium supplemented with naphthaleneacetic acid (NAA, $1 \text{ mg} \times \text{L}^{-1}$) for induction of rooting. The plantlets with good root systems were hardened in the greenhouse, in polybags filled with a mixture of Soilrite and soil (1:1).

2.2. Irradiation experiments

The *in vivo* as well as *in vitro* plant materials were exposed to ^{60}Co gamma-rays ($18 \text{ Gy} \times \text{min}^{-1}$) ranging from 0–100 Gy in an irradiator (Gammacell-220, Atomic Energy of Canada Ltd.). The suckers were trimmed to uniform size and weight, while the hardened plants were decapitated so as to facilitate the irradiation procedure. After irradiation, the suckers were transferred to earthen pots while the irradiated hardened plants were maintained in a greenhouse for assessment of survival.

The *in vitro* material was kept intact in test tubes with culture medium and then submitted to irradiation. Immediately after irradiation, the multiple shoots were cultured on the MS medium supplemented with BAP ($5 \text{ mg} \times \text{L}^{-1}$) and AS ($30 \text{ mg} \times \text{L}^{-1}$), whereas the *in vitro* individual shoots were cultured on MS medium supplemented with NAA ($1 \text{ mg} \times \text{L}^{-1}$) to test the post-irradiation rooting response.

Radio-sensitivity of suckers and *in vitro* individual shoots was assessed by recording survival % after 30 d. The multiplication ratio (from one sub-culture to the next one) of *in vitro* multiple shoots was recorded after 30 d for each sub-culture cycle. The

post-irradiation rooting response of *in vitro* individual shoots was determined by recording days to root initiation, root number, root length (cm), shoot length (cm) and rooting score, during and after 40 d of treatment. In the case of hardened plants, the plant height (cm), number of leaves, leaf area (cm²) and chlorophyll content (mg × cm⁻²) were scored monthly.

2.3. Statistical analysis

The experiments were conducted with five replicates. The replication means were obtained by averaging the data of three samples. The data were statistically analyzed as per the standard procedures [12]. A regression analysis was carried out for per cent survival of the suckers and *in vitro* individual shoots, and rooting parameters of *in vitro* individual shoots to quantify the degree of dependence of the variables on irradiation dose. Data obtained from the irradiation of the multiple shoots and hardened plants were analyzed using a completely randomized design.

3. Results

3.1. Effect of gamma irradiation on suckers from field grown plants

A general decrease in per cent survival was observed when the suckers were irradiated with gamma rays. At 10 Gy, the per cent survival decreased to 83.3% whereas, at 20 Gy, the survival decreased to 66.7%, as compared to the control with 100% survival. Doses exceeding 40 Gy were found to be lethal. A regression equation $y = 86.48 - 1.63x$ was derived to quantify the degree of dependence of per cent survival on irradiation dose (figure 1).

3.2. Effect of gamma irradiation on the survival of *in vitro* individual shoots

Single shoots isolated from multiple shoot cultures exposed to gamma irradiation

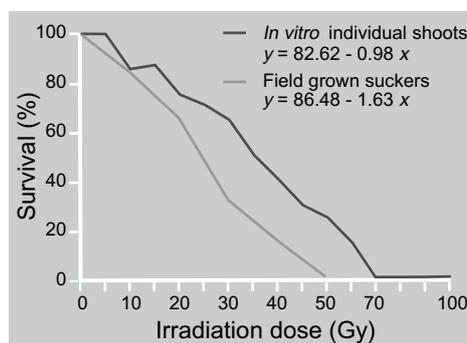


Figure 1. Effect of different ⁶⁰Co gamma irradiation doses on survival of field grown suckers and *in vitro* individual shoots of banana cv. Basrai.

exhibited a decline in the per cent survival of individual shoots with increase in the irradiation dose, (figure 1) indicating an inverse association between the two parameters as was evident from the negative magnitude of the regression coefficient (- 0.98). It was observed that shoots irradiated with doses higher than 60 Gy necrosed completely.

3.3. Effect of gamma irradiation on *in vitro* multiple shoots

Multiple shoot cultures were exposed to gamma irradiation to assess the multiplication ratio. No significant differences were observed in multiplication ratio among four sub-culture generations. However, within a sub-culture generation, enhancing effects of low dose irradiation at 10–15 Gy ($M_1V_0-M_1V_1$ and $M_1V_1-M_1V_2$)¹ were observed to be significant (table I). In $M_1V_2-M_1V_3$ and $M_1V_3-M_1V_4$ generations, significantly stimulating effects persisted even at 20 Gy. At doses exceeding 60 Gy, the multiplication ratio steadily declined reaching 'unity', indicating complete inhibition of shoot multiplication.

3.4. Effect of gamma irradiation on *in vitro* rooting of individual shoots

In an earlier study, it was observed that rooting seldom occurred for doses exceeding 20–30 Gy when individual *in vitro* shoots were immediately cultured, after irradiation, on auxin rich medium (data not presented). Hence in the present study, the experiment was repeated with a finer

¹ M_x : mutants of the generation x; V_x : vegetative plants at the generation x.

Table I.Effect of gamma irradiation on the multiplication ratio of *in vitro* multiple shoot cultures of banana cv. Basrai.

Dose (Gy)	$M_1V_0-M_1V_1$	$M_1V_1-M_1V_2$	$M_1V_2-M_1V_3$	$M_1V_3-M_1V_4$
0	2.6	2.8	3.0	1.8
5	2.4	3.0	3.0	2.0
10	4.4	4.2	4.4	4.0
15	4.2	5.0	4.8	4.0
20	3.4	3.2	5.2	4.6
25	2.4	2.6	3.8	2.8
30	2.2	1.6	2.2	2.0
35	1.4	1.8	2.0	1.2
40	1.6	1.2	1.2	1.0
45	1.4	1.0	1.0	0.0
50	1.0	1.0	0.0	0.0
60	1.0	1.0	0.0	0.0
70	1.0	0.0	0.0	0.0
80	1.0	0.0	0.0	0.0
90	1.0	0.0	0.0	0.0
100	1.0	0.0	0.0	0.0
Standard error	0.35	0.38	0.42	0.39
Critical difference (5%)	0.97	1.05	1.20	1.13
Critical difference (1%)	1.27	1.38	1.44	1.35

M_x : mutants of the generation x ; V_x : vegetative plants at the generation x .

resolution of doses from 0 to 40 Gy (step of 4 Gy) to examine irradiation effects on days to root initiation, number of roots, root length and shoot length (*table II*). It was observed that increase in irradiation dose significantly delayed root initiation. A minimum number of days (2.4) was required in the control cultures for the first root emergence whereas other treatments required 4.0–15.4 d. The number of roots and root length decreased significantly in magnitude with increase in irradiation as compared to control cultures. However, a decline in shoot length was not perceptible with increase in the dose. The regression analysis conducted to estimate the degree of dependence of the above variables on increase in dose of gamma rays showed that the parameters, except days to root initiation, were negatively dependant on the increase in dose levels (*table II*).

3.5. Effect of gamma irradiation on hardened plants

Exposure to gamma rays was found to distinctly affect the plant morphological characteristics of the surviving population. Plant height, leaf area and chlorophyll content decreased in magnitude while the number of leaves increased with increase in gamma irradiation dose. The hardened plants irradiated at 30 Gy were shorter (7.63 cm) as compared to the control (11.72 cm) and 10 and 20 Gy irradiated plants (10.62 cm and 9.74 cm, respectively) at the end of one month. However, this effect persisted for about 2 months after irradiation and thereafter the growth resumed normally like control plants (*table III*).

The number of functional green leaves was found to increase with irradiation dose. The plants at 30 Gy had significantly more

Table II.Effect of gamma irradiation on *in vitro* rooting of individual shoot cultures of banana cv. Basrai.

Dose (Gy)	Days to first root initiation	Number of roots	Root length (cm)	Shoot length (cm)	Rooting score
0	2.40	26.40	6.42	11.64	+++
4	4.20	25.40	5.72	9.40	+++
8	5.80	23.80	4.94	8.38	++
12	7.20	22.20	4.60	8.00	++
16	7.40	19.60	4.46	7.46	++
20	8.40	16.80	4.76	6.30	+
24	11.40	13.80	4.50	6.18	+
28	11.00	11.00	4.28	4.32	+
32	15.40	8.40	3.80	3.78	+
36	–	–	–	–	–
40	–	–	–	–	–
Standard error	0.41	0.65	0.32	2.47	
Critical difference (5%)	1.17	1.86	0.91	7.08	
Critical difference (1%)	1.41	2.24	1.09	8.51	
Regression equation	$y = 6.13 + 0.024 x$	$y = 27.97 - 0.59 x$	$y = 6.63 - 0.13 x$	$y = 11.33 - 0.27 x$	
R ²	0.0052	– 0.98	– 0.73	– 0.94	

+, ++, +++ sparse, moderate, profuse rooting, respectively.

leaves (8.60) as compared to the control and 10 Gy irradiated plants (5.00 and 5.20, respectively). The differences were observed to persist even at the end of 2 and 3 months of growth (*table III*).

The plants exhibited a decrease in mean individual leaf area with an increase in dose as compared to the control plants. The plant at 30 Gy had significantly less leaf area at all the stages of growth as compared to the

Table III.

Effect of gamma irradiation on hardened plants of banana cv. Basrai.

Dose (Gy)	Plant height (cm)			Number of leaves			Leaf area (cm ²)			Chlorophyll content (mg·cm ⁻²)		
	1M	2M	3M	1M	2M	3M	1M	2M	3M	1M	2M	3M
0	11.72	14.38	18.20	5.00	5.20	5.80	96.68	103.75	118.00	63.49	35.33	37.49
10	10.62	12.40	14.32	5.20	5.40	5.80	57.42	67.23	70.60	51.73	33.45	30.89
20	9.74	11.20	12.12	8.80	6.20	5.20	31.60	42.93	45.82	41.00	28.26	24.66
30	7.63	10.44	10.12	8.60	7.20	9.20	18.70	24.95	38.99	28.65	22.82	15.44
Standard error	0.69	0.59	1.64	0.62	0.42	1.36	2.73	2.79	3.67	0.68	0.74	0.58
Critical difference (5%)	2.07	1.77	4.92	1.86	1.26	4.67	8.17	8.39	11.00	2.05	2.22	1.75
Critical difference (1%)	2.85	2.44	6.77	2.56	1.73	5.62	11.26	11.52	15.16	2.83	3.07	2.41

1M, 2M, 3M: plant of 1-month, 2-months and 3-months age, respectively.

rest of the treatments (*table III*). The irradiated plants showed variegated chlorophyll patches on the leaves. The non-irradiated control plants had maximum chlorophyll content as compared to the irradiated plants throughout the period of growth (*table III*).

4. Discussion

In the present studies, radio-sensitivity of different plant materials was found to be directly proportional to the irradiation dose. The per cent survival of the irradiated material decreased with increase in irradiation dose. The hardened plants were most sensitive followed by the suckers, while individual *in vitro* shoots were least sensitive to increase in dose of gamma irradiation. There have been reports where 20–30 Gy was the most preferred dosage level for *in vivo* plant material, and a decrease in regeneration capacity with linear increase in irradiation dose of X-rays or gamma rays [13]. Doses exceeding 70 Gy have been reported to be completely lethal to the *in vitro* multiple shoots with LD-50 of around 35 Gy [4]. Similar results have also been reported by other investigators [9, 14, 15].

Irradiation of the *in vitro* multiple shoots adversely affected multiplication, except for doses 10–20 Gy, which were observed to significantly enhance multiplication. The enhancing effects were prominent within a sub-culture generation but among sub-culture generations the multiplication ratios were similar. Such stimulatory effects of low dose irradiation on multiplication of *in vitro* shoots were also observed in earlier studies [4, 16]. However, Yang et al. [9] did not observe any such enhancing effects, which may have been due to the genotypic differences. The radio-sensitivity is known to be a direct function of mitotic activity of cells [17]. Shoot-tip meristems being mitotically active regions exhibit considerable radio-sensitivity. However, in the present studies, elevated multiplication ratios were observed for the *in vitro* multiple shoots irradiated at 10–20 Gy indicating a stimulatory effect on proliferation of the tissue.

Gamma-irradiation of individual *in vitro* shoots was found to adversely affect the

in vitro rooting response. Such harmful effects have often been considered to be due to interference in the normal physiological functioning. This implies that the observed phenomenon cannot be related to any specific genetic aberration, although both, DNA damage and modification of gene expression are involved [18].

In the case of hardened plants, the magnitudes of plant height, leaf area and chlorophyll content were observed to decrease, while the number of leaves increased with increase in gamma irradiation. Chlorophyll deficiencies, narrowing of leaves and more leaves in the field growing plants coming from irradiated suckers have also been reported previously [19]. The chlorophyll variegatedness in the irradiated plants [20] was due to changes in the biosynthetic pathways of chlorophyll pigments and the time of chlorophyll synthesis in palisade and spongy mesophyll cells. In the present studies, the unexpected increase in 'number of leaves' was related to the decrease in 'leaf area', and possibly was the result of splitting of the meristematic regions due to irradiation. The studies on neutron irradiation of *Lycopersicon* spp. [21] clearly indicated that, as a result of displacement of the centres of apical meristematic activity towards the flanks of the generative region, two new shoot apical meristems could develop. Similar findings have also been reported earlier [19].

5. Conclusions

Global banana production is being seriously threatened by several biotic/abiotic causes and resistant/tolerant cultivars are urgently required. In view of the difficulties in conventional breeding, mutagenesis *in vitro* can offer as a feasible tool to achieve these goals. Evaluation of the effects of the mutagen (gamma-rays in present investigations) on different *in vivo* and *in vitro* plant material is imperative and essential.

The investigations indicated that increasing the dose of gamma-rays resulted in a corresponding decrease in the growth of the explants. As a trend, the *in vivo* explants

were observed to be more vulnerable to irradiation than the *in vitro* ones. It is thus possible to obtain live plant materials even at high doses thereby increasing the probability of higher frequency of mutations. Lower doses of gamma-irradiation had an enhancing effect on the *in vitro* multiple shoot cultures. It can be speculated that the observed aberrations in plant morphology were due to the irradiation induced disturbances in the normal physiological functioning. The contribution of DNA damages resulting in altered gene expression is difficult to judge at this stage. In view of the present experiments and published literature, it appeared that the radio-sensitivity is a complex function of several factors such as physiological status of the plant tissue, method of post-irradiation handling (*in vitro* or *in vivo*), degree of differentiation of tissue, stage and rate of growth (dividing or non-dividing), the irradiation dose and dose rate, and also, the genetic architecture of the species. Nevertheless, the use of *in vitro* regeneration techniques for mutation induction in banana is advantageous over the *in vivo* system because it offers (i) a high propagation rate facilitating proper chimera separation, (ii) generation of large plant populations for screening, (iii) the possibility of exposing the *in vitro* cultures to higher irradiation doses (than *in vivo* explants) and thereby expecting a high frequency of mutations, (iv) reduction in time and space requirement, (v) the optional facility of *in vitro* selection and (vi) better chances of selecting dominant or desired mutations. The results of the present studies will serve as useful hints for designing *in vitro* mutagenesis experiments in banana improvement.

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Radiosensibilidad de bananos ‘Basrai’ (AAA) procedentes de cultivos *in vitro* o en campo.

Resumen — Introducción. El banano es una de las producciones frutales más importantes del mundo y, por ello, se buscan continuamente clones mejorados resistentes o tolerantes a diferentes estrés bióticos y abióticos. En este sentido, la mutagénesis *in vitro* ofrece perspectivas interesantes y adecuadas. **Material y métodos.** Se irradiaron brotes individuales o múltiples obtenidos *in vitro* y material vegetal en campo (hijos y plantas aclimatadas) de bananos ‘Basrai’ (AAA) con dosis de rayos gamma (^{60}Co) que iban de 0 a 100 Gy. Se evaluó la radiosensibilidad de hijos y brotes individuales de procedencia *in vitro* midiendo las tasas de supervivencia y multiplicación, tiempo de enraizamiento, número y longitud de raíces, longitud de los brotes, altura de las plantas, número y superficie de las hojas y contenido de clorofila. **Resultados.** El aumento de las dosis de rayos gamma produjo simultáneamente la disminución del crecimiento de las plantas. Además, se observó que las plantas en campo tenían una tendencia a ser más vulnerables que las *in vitro*. Esto lleva a pensar en la posibilidad de obtener material vegetal vivo, incluso con altas dosis de radiación, incrementando así la probabilidad de obtener una frecuencia más alta de mutaciones. Las bajas dosis de 10 y 20 Gy tuvieron un efecto positivo sobre la tasa de multiplicación de brotes múltiples procedentes del cultivo *in vitro*. **Conclusión.** El material vegetal estudiado proporcionó respuestas diferentes en función de su origen *in vitro* u observado en campo. Los resultados obtenidos durante estos trabajos podrán ser útiles para redefinir las estrategias de mejoramiento del banano basadas en la inducción de mutación *in vitro*.

India / *Musa* / fitomejoramiento / mutación / radiación gamma / radiosensibilidad / vitroplantas / renuevo