Micropropagated banana plants induced by gamma irradiation and resistant to the root-knot nematode reproduction

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Abstract: Micropropagation of banana plants has been used successfully in commerce. Banana plantation has a great interest in Egypt due to the suitable climate. However, some problems might thwart the plantation progress like the root-knot nematode; *Meloidogyne incognita* infection which can affect the acclimatization and plantation of *in vitro* plants. Thus, our investigation was aimed to produce nematode resistant *in vitro* plants using gamma radiation (γ). Banana *in vitro* plantlets were exposed to gamma radiation at several doses then replanted *in vitro*. Sensitivity of banana plants to irradiation was determined. Acclimatized plants were infected with *M. incognita* in greenhouse to see their capability for tolerance. Results indicated that irradiated plants could manage the infection as they tolerated *M. incognita* infection when exposed to 10 Gy. This response was clear as plant length, leaf number, and leaf width, fresh and dry weight when compared to control. Irradiated plants affected nematode parameters as number of juveniles in soil, developmental stages, galls, and egg masses in banana roots. These were reduced gradually by increasing γ -doses up to 10 Gy. The highest reduction percentages of total nematode populations and build-up of root-knot nematode were also achieved by 10 Gy dose. Gamma irradiation and/or infection by nematode caused variations in leaf content as free amino acids, free proline, glycinebetaine, choline, phenolic compounds, and flavonoids. **Keywords:** *Musa sp.*, micropropagation, gamma radiation, *Meloidogyne incognita*, free amino acids

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1 Introduction

Banana (*Musa* sp.) is one of the most economic tropical fruit crops in the world, reached an estimated world production of 113.28 million tones, in 2016. India, China, Philippines, Ecuador and Brazil accounted for 61% of global banana production. In Egypt, its harvested area, in 2016, reached about 27632 ha, with the production of 1341478 tones (FAO, 2018).

Nowadays, banana plants are successfully propagated

by tissue culture technique in commercial trend. However, banana tissue culture plants are highly susceptible to parasitic nematodes such as root knot nematode (*Meloidogyne incognita*), lesion nematode (*Pratylenchus coffeae*), burrowing nematode (*Radopholus similes*), and spiral nematode (*Helicotylenchus multicinctus*). These enter the plant roots through contaminated water or through the pot mixture used for hardening of tissue culture plants (Singh et al., 2011).

The root-knot nematode received a great attention in banana orchards and some crops in Egypt (Eissa et al., 2005; El-Nagdi and Abdel-Fattah, 2011; El-Nagdi et al., 2017 and Hafez et al., 2017). *Meloidogyne spp*. was the most prevalent nematode in banana samples; about 76% frequency of occurrence (Mokbel et al., 2006). The

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management of the previous pest using chemicals is not recommended due to their risks to human and environment.

However, many beneficial uses of radiation were presented and offered few risks when properly employed. Many reports were interested in the effect of gamma radiation (γ) on plants, focusing on metabolic alterations, modifications of growth and development, and changes in biochemical pathways especially physiological behavior (Jan et al., 2012). It is well-known that ionizing radiation induces various alterations in the physiological and biochemical processes in plants (Rayis and Abdallah, 2014). Gamma radiation has widely been used to induce genetic variability in crop plants (Kamel et al., 2011). High doses of gamma radiation produce deleterious effects, such as poor growth and genetic damage. Relatively low doses usually alter the growth characteristics, while lower doses have shown to stimulate plant growth (Watanabe et al., 2000; Kamel et al., 2011).

The main objective of this study is to find out the relative effect of gamma radiation on *in vitro* banana plants and their resistance to root-knot nematode (*Meloidogyne incognita*) infection and some correlated morphological and biochemical parameters.

2 Materials and methods

This investigation was carried out at Tissue Culture Technique Lab, Central Labs Net, greenhouse of Pomology Dep., and Nematology Lab of Plant Pathology Dep., Agricultural and Biological Division, National Research Centre, Dokki, Egypt during the seasons of 2016 to 2017. Exposure of banana *in vitro* shoots to gamma irradiation and biochemical analyses after infection were measured at Middle Eastern Regional Radioisotope for Arab Countries Center, Dokki, Egypt.

2.1 Banana in vitro culture

2.1.1 Explants preparation

Well-developed suckers (four months old) cv. Grandnain were carefully separated from mother plants, brought to the Lab. The shoot tip (4.0-5.0 cm in length and 2.0 cm in width at the base) was taken out carefully from suckers, washed thoroughly under running tap water for 30 min, immersed in Rhizolix 50 WP (1.0 g L⁻¹) for 20 min then in 30% Clorox (Sodium Hypochlorite: 5.25%) for 10 min. After that, explants were immersed in Tochopherol 40% as an antioxidant (Mostafa et al., 2013). Explants were transferred to the culture room and sterilized with 50% Clorox for 30 min then washed with sterilized distilled water for three times under aseptic conditions. The outer white leaflets were removed from shoot tips until a suitable volume (1.0-2.0 cm in length and 1.0 cm in width at the base). Shoot tips were carefully cultured on starting medium and incubated in controlled conditions.

2.1.2 Media preparation

MS medium (Murashige and Skoog, 1962) was used for all stages of banana tissue culture:

1-Starting stage: full MS salts + 3 mg L^{-1} BAP + 30 g L^{-1} Sucrose + 100 mg L^{-1} Inositol + 2 g L^{-1} Gelrite.

2-Multiplication stage: full MS salts + 5 mg L⁻¹ BAP + 30 g L⁻¹ Sucrose + 100 mg L⁻¹ Inositol + 6 g L⁻¹Agar.

3-Enlargement stage: full MS salts + 1 mg L^{-1} BAP + 30 g L^{-1} Sucrose + 100 mg L^{-1} Inositol + 6 g L^{-1} Agar.

4-Rooting stage: half MS salts $+ 2 \text{ mg } L^{-1} \text{ IBA} + 20 \text{ g } L^{-1} \text{ Sucrose} + 100 \text{ mg } L^{-1} \text{ Inositol} + 6 \text{ g } L^{-1} \text{ Agar.}$

2.1.3 Incubation of in vitro banana cultures

Explants were incubated at 25°C±2°C with light period of 16 hours and eight hours in dark and 2000 Lux of light intensity for multiplication stage and 27°C±2°C with 3000-4000 Lux for rooting stage.

2.1.4 Exposing *in vitro* banana plantlets to gamma radiation

Jars contained *in vitro* banana rooted plantlets (after four weeks on rooting medium) were exposed to ⁶⁰Co gamma source at the Middle East Regional Radioisotope Center for Arab Countries. The dose rate was 27.46 krad min⁻¹ to receive 0, 5, 10, 20, and 40 Gy. After exposure, plantlets were immediately transferred to fresh rooting medium and incubated at the same previously mentioned conditions. Sensitivity of banana plantlets to gamma irradiation were measured, after three weeks, as average plantlet length (cm), leaf number, leaf width (cm) as well as growth vigor and chlorophyll as scores (Pottino, 1981). 2.1.5 Acclimatization of *in vitro* banana plantlets

After four weeks from exposure and subculture, in

vitro banana rooted plantlets were transferred to adaptation room, washed thoroughly with tap water, quickly immersed in Rhizolix 50 WP (1.0 g L⁻¹) then cultured in pots (5 cm, in diameter) with sterilized medium including peat: sand (2:1 in volume). Cultured plantlets were rapidly covered with polypropylene bags to keep their humidity then kept under temperature of $27^{\circ}C\pm 2^{\circ}C$. After a week, plastic bags were punctured for decreasing humidity. After another week, bags were gradually removed. Average survival percentage, plantlet length (cm), leaf number, leaf width (cm) were measured after adaptation.

2.1.6 Transferring plants

After a month from successful acclimatization, plants were transferred to bigger pots (30 cm diameter) filled with 6 kg solarized sandy loam soil (1: 1 w w^{-1}) and kept for irrigation and fertilization for another month.

2.2 Nematode infection

Selected well growth plants (twelve plants/treatment) were placed on a greenhouse bench at $30^{\circ}C\pm 5^{\circ}C$. One week later, soil of six pots of each plant treatment was inoculated with two thousands of newly hatched second stage juveniles (J₂)/pot of *M. incognita* obtained from pure culture raised in glasshouse grown tomato (*Lycopersicum esculentum* cv. Peto UC 82), while the other six pots were left without inoculation. Six months later, three plants were uprooted to determine the following parameters and others were kept to the second season with regular irrigation and fertilization.

2.2.1 Morphological parameters after infection

Survival percentage, plant length (cm), leaf number, leaf width (cm) were measured. In addition, root length (cm), fresh and dry weight (g) were determined.

2.2.2 Nematode parameters

Observations of *M. incognita* parameters as numbers of juveniles (J2) in soil and both the developing larva stages {Third stage (L3) and Fourth stage (L4)}, females, eggs and number of galls in banana root system of irradiated and non-irradiated banana plants were recorded. The juveniles of nematode were extracted in soil samples of banana plants by sieving and decanting methods (Barker, 1985). In banana plant roots, samples were gently washed free of adhering soil and an aliquot of 5 g per plant was cut into 2-cm- long pieces then put in blended at 3×103 rpm for 3 minutes to extract number of L3, L4, females and eggs from roots. Rate of build-up was calculated according to the following formulae:

Rate of build-up = Total nematode populations in soil and roots (Pf) / Initial population of J2 at time application (Pi = 2000 J2).

Where: Pf is number of J2 in soil and numbers of J2, L3, L4, females and eggs in roots.

2.3 Physiological and biochemical compound measurements

The colorimetric determination of free amino acids and proline were performed using the methods of Lee and Takahashi (1966) and Bates et al. (1973), respectively. While quaternary ammonium compounds (glycinebetaine (GB) and choline) were estimated using the method of Grieve and Grattan (1983). Total flavonoids content was analyzed according to Zhishen et al. (1999). Total phenols were determined using the Folin–Ciocalteu method used by Jindal and Singh (1975).

2.4 Statistical analysis

Treatments were arranged in a completely randomized block design. For *in vitro* exposure treatments, each treatment contains three replicates, each replicate involved three culture jars and each jar contained five plantlets. For greenhouse nematode infection, sixty plastic pots were arranged; twelve pots for each exposed treatment, six of them for inoculation with *Meloidogyne incognita* and other six pots remained uninoculated. Data were analyzed using LSD according to method described by Snedecor and Cochran (1989).

3 Results

3.1 Plant sensitivity to gamma radiation

3.1.1 Effect of gamma radiation exposure on banana *in vitro* plantlets

Data in Table 1 presents the sensitivity of *in vitro* banana plants to gamma radiation exposure as plantlet length (cm), leaf number, leaf width (cm), growth vigor, and chlorophyll (scores). It was observed that banana plantlets were affected negatively by gamma exposure as plantlet length, leaf width, growth vigor, and chlorophyll as they were dramatically decreased with increasing

doses. Meanwhile, the average leaf number did not

respond to the exposure.

| Growth Gamma dose (Gy) | Plantlet length (cm) | Leaf number | Leaf width (cm) | Growth vigor (score) | Chlorophyll (score) |
|---------------------------|----------------------|-------------|-----------------|-------------------------|------------------------|
| Control | 19.65a | 4.78a | 5.00a | 4.26a | 4.48a |
| 5 | 17.47b | 4.63a | 4.00b | 3.63b | 4.06b |
| 10 | 16.74c | 4.58a | 3.56c | 3.79b | 3.84b |
| 20 | 16.50c | 4.96a | 2.91d | 3.44b | 3.04c |
| 40 | 15.63d | 4.75a | 2.50e | 2.95c | 2.95c |

Table 1 Effect of gamma radiation exposure on banana in vitro plantlets

Note: Means with different letters within each column were significantly different at 5% level

3.1.2 Effect of gamma radiation exposure on banana acclimatized plants

Data in Table 2 presents the *in vitro* banana plants response to gamma radiation exposure after acclimatization as survival percent, plantlet length (cm), leaf number, and leaf width (cm).

 Table 2 Effect of gamma radiation exposure on banana

 acclimatized plants

| Growth Gamma dose (Gy) | Survival percent % | Plantlet length (cm) | Leaf number | Leaf width (cm) |
|------------------------------|--------------------------|----------------------------|----------------|-----------------------|
| Control | 95.65 | 21.85a | 5.70a | 4.56b |
| 5 | 88.75 | 19.77b | 5.64ab | 4.34b |
| 10 | 84.21 | 15.94c | 5.31b | 5.84a |
| 20 | 73.91 | 15.26d | 4.81c | 3.05c |
| 40 | 72.73 | 14.60e | 3.79d | 2.66d |

Note: Means with different letters within each column were significantly different at 5% level

Generally, banana plantlets were still affected negatively by gamma exposure. Growth parameters were dramatically decreased with the increasing doses compared with the control except for leaf width of exposure plantlets which did not differ or surpassed the control at 5 and 10 Gy doses, respectively. In addition, leaf number showed no significant differences between the control and the lowest dose.

3.2 Effect of γ -radiation (Gy) on banana plants after six months with or without root-knot nematode (*M. incogenita*) infection

3.2.1 Effect of γ -radiation on uninfected banana plants

Data in Table 3 showed that there were no differences between plants exposed to the dose of 5.0 Gy and the control (non-irradiated plants) in all growth parameters under investigation. The highest averages were presented with irradiated plants exposed to the dose of 5.0 Gy and the control in all parameters except fresh weight and leaf width as 10 Gy exposed plants showed the maximum values besides the balance in dry weight. Gamma dose at 40 Gy showed the lowest averages.

| Growth | Plant length | Leaf number | Leaf width | Survival % | Fresh weight | Dry weight (g) | |
|-----------------|--------------|-------------|------------|------------|--------------|----------------|--|
| Gamma dose (Gy) | (cm) | Leaf number | (cm) | | (g) | Dry weight (g) | |
| Control | 45.9a | 7.8a | 10.22b | 45.9a | 106.09b | 20.88a | |
| 5 | 46.0a | 7.6a | 10.25b | 46.0a | 107.32b | 19.78a | |
| 10 | 44.4b | 7.0b | 11.12a | 44.4b | 111.62a | 19.88a | |
| 20 | 34.90c | 6.75bc | 9.47c | 34.90c | 106.76b | 16.11b | |
| 40 | 32.25d | 6.60c | 9.03d | 32.25d | 90.90c | 10.09c | |

Table 3 Effect of γ -radiation (Gy) on uninfected banana plants after six months in greenhouse

Note: Means with different letters within each column were significantly different at 5% level.

3.2.2 Effect of y-radiation on infected banana plants

Plants exposed to gamma irradiation then infected with nematode responded differently. It seems that gamma irradiation could turn plants managing the infection as they tolerated nematode infection when exposed to 10 Gy. This response was clear as the highest average plant length, leaf number, leaf width, fresh and Table 4 Effect of a radiation (Cy) on infected banana plants with dry weight and kept the highest survival percent, when compared with the control (without exposure). Moreover, the dose of 5 Gy could also enhance plant length, leaf width, fresh and dry weight compared with the control. Meanwhile, it seemed that the highest exposing dose (40 Gy) retarded all growth parameters noticed (Table 4).

Table 4 Effect of γ -radiation (Gy) on infected banana plants with root-knot nematode (M. incognita) after six months in greenhouse

| Growth Gamma dose (Gy) | Plant length (cm) | Leaf number | Leaf width (cm) | Survival % | Fresh weight (g) | Dry weight (g) |
|---------------------------|----------------------|-------------|--------------------|------------|---------------------|----------------|
| Control | 42.00c | 7.6b | 10.38b | 100.0 | 80.67d | 8.82c |
| 5 | 44.71b | 7.57b | 10.97a | 100.0 | 95.21b | 9.26b |

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|------------|----|----------------|-------|--------|-------|---------|--------|--|
| | 10 | 48.50a | 8.00a | 11.34a | 100.0 | 103.92a | 10.59a | |
| | 20 | 37.85d | 7.1c | 9.25c | 80.0 | 85.12c | 9.53b | |
| | 40 | 34.90e | 6.74d | 9.09c | 75.0 | 64.96e | 7.59d | |

Note: Means with different letters within each column were significantly different at 5% level.

3.3 Nematode parameters

Data in Table 5 shows that the number of juveniles in soil, developmental stages, galls and egg masses in roots were significantly reduced gradually by increasing gamma doses up to 10 Gy, and even at dose of 40 Gy they were still lower than the control. Meanwhile, data in Table 6 revealed the effect of gamma radiation on reduction percentage of the root-knot nematode parameters in banana plants which all presented as follows:

3.3.1 Juveniles (J₂) number in soil

Results showed that the number of $J_2/200$ g soil with banana plants were in the range of 170 - 273 J₂ (gamma at doses of 5, 10, 20 and 40 Gy), compared to 450 J₂ with unexposed plants (Table 5). It was found that the highest reduction percentages of J₂ in soil (81.6%) was achieved by dose of 20 Gy followed by (62.2%) at dose of 5 Gy (Table 6).

3.3.2 Developmental stages in roots

The developmental stages (J_2 , L_3 , L_4 and females) in roots of banana plants were in the range of 795-3033 stages/plant with treatments of the gamma irradiation at 5, 10, 20 and 40 Gy doses, compared to 5060 stages in unexposed plants (Table 5). The highest reduction percentage of developmental stages in roots was achieved by the dose of 10 Gy (84.3%) followed by the doses of 20, 5, and 40 Gy, respectively (Table 6). 3.3.3 Number of galls in roots

The number of galls in roots of banana plants was in the range of 8-32 galls/plant with gamma exposure at doses of 5, 10, 20 and 40 Gy, compared to 68 galls in unexposed plants (Table 5). The highest reduction percentage of galls in roots was achieved by dose of 20 Gy (88.2%) followed by 5 and 10 Gy, respectively (Table 6).

3.3.4 Number of eggs in roots

Numbers of eggs in roots of banana plants were in the range of 1160-3260 eggs/plant with exposure to gamma at doses of 5, 10, 20, and 40 Gy, compared to 6820 eggs in unexposed plants (Table 5). The highest reduction percentage of eggs in roots was achieved by dose of 20 Gy (83.0%) followed by 5 and 10 Gy (Table 6).

3.3.5 Total nematode population and rate of build-up

Results also showed that gamma exposure at doses 5, 10, 20 and 40 Gy significantly reduced total nematode populations and rates of build-up (Pf/Pi) of *M. incognita* in irradiated plants, compared to non-irradiated plants, after six months (Table 5). Dose of 10 Gy highly reduced total nematode population and rate of build–up (1.16) followed by dose of 20 Gy (1.23), compared to 6.17 in non-irradiated plants (Table 5). The highest reduction percentages of total nematode populations and rates of build-up were achieved at dose 10 Gy (Table 6).

| Nematode | | Г | Developmental | stages in roots | | | | | | |
|------------|------------------------|--------------------------|---------------|-----------------|--------|-------|--------|-------------|------------------|----------|
| parameters | J ₂ in soil | | (5 | (g) | | Total | No. of | No. of eggs | Total nematode | Rate of |
| Gamma dose | (200g) | Second (J ₂) | Third (L3) | Fourth (L4) | Female | Total | galls | No. of eggs | populations (Pf) | build-up |
| (Gy) | | | | | | | | | | |
| 0 | 450 a | 2430 a | 1600a | 490a | 540 a | 5060a | 68 a | 6820 a | 12330 a | 6.17 a |
| 5 | 170 c | 953 c | 546c | 266c | 293 bc | 2058c | 18 c | 1367 c | 3595c | 1.80 c |
| 10 | 200 c | 66 e | 293d | 153d | 283 c | 795e | 32 b | 1327 d | 2322 d | 1.16 d |
| 20 | 83 d | 633 d | 153e | 157d | 280 c | 1223d | 8 d | 1160 e | 2466 d | 1.23 d |
| 40 | 273 b | 1433 b | 907b | 380b | 313 b | 3033b | 21 c | 3260 b | 6566 b | 3.28 b |

Table 5 Effect of irradiation (Gy) on the root-knot nematode parameters infecting banana plants

Note: Means with different letters within each column were significantly different at 5% level.

Table 6 Reduction percent of the root-knot nematode parameters with irradiated banana plants (0-40 Gy)

| Nematode paramete | rs | Devel | opmental s | tages in ro | oots | | | | | |
|-------------------|-------------------------------|--------------------------|-------------|-------------|---------|--------|--------------|----------------|----------------------------|---------------------|
| Gamma dose | J ₂ in soil (200g) | | (5 g |) | | TotalN | lo. of galls | No. of eggsTot | tal nematode populations (| Pf)Rate of build-up |
| (Gy) | 5 | Second (J ₂) |)Third (L3) | Fourth (L | 4)Femal | e | | | | |
| 5 | 62.2 | 60.8 | 65.9 | 24.9 | 45.7 | 59.3 | 73.5 | 80.0 | 70.8 | 70.8 |
| 10 | 55.6 | 97.3 | 81.7 | 68.8 | 47.6 | 84.3 | 52.9 | 80.5 | 81.2 | 81.2 |

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|-----|----------|------|---|------|------|------|------|------|------|------|---------------|
| | 20 | 81.6 | 74.0 | 90.4 | 68.0 | 48.1 | 75.8 | 88.2 | 83.0 | 80.0 | 80.0 |
| | 40 | 39.3 | 41.0 | 43.3 | 22.4 | 42.0 | 40.1 | 69.1 | 52.2 | 46.7 | 46.7 |

3.4 Physiological and biochemical response

In uninfected plants (Table 7), free amino acids and proline contents increased in the leaves of gamma exposed plants relative to control (unexposed plants), and the increase was dose dependent. The highest content of free amino acids and proline were at 40 Gy dose. Similar results were obtained regarding to the effect of γ irradiation on glycinebetaine, choline, phenols, and flavonoids contents. It was found that plants showed increases in these contents with irradiation compared with non-irradiated plants. The highest values were obtained at 5 Gy with glycinebetaine and choline contents while, phenols and flavonoids values were the highest at 10 and 20 Gy compared with other irradiated and non-irradiated plants.

With respect to the nematodes infected plants (Table 8), data showed the increases in free amino acids, proline, phenols, and flavonoids contents in the leaves with gamma exposure compared to the control (non-irradiated plants), and the increase was dose dependent. Meanwhile, all the investigated doses caused decrease in glycinebetaine and choline contents compared with the control.

| γ- dose (Gy) | Free A.A. μgg ⁻¹ f.wt | Proline µmoleg ⁻¹ f.wt | Glycinebataine μmoleg ⁻¹ d.wt | Choline µmoleg ⁻¹ d.wt | Phenols µgg ⁻¹ f.wt | Flavonoids µgg ⁻¹ f.wt |
|-----------------|-------------------------------------|--------------------------------------|---|--------------------------------------|-----------------------------------|--------------------------------------|
| 0 | 798c | 61d | 0.29d | 0.48d | 124d | 13d |
| 5 | 789c | 89c | 0.75a | 0.78a | 440c | 17c |
| 10 | 714d | 102b | 0.65b | 0.77a | 732b | 31a |
| 20 | 1230b | 111b | 0.33d | 0.54c | 1294a | 32a |
| 40 | 1523a | 117a | 0.54c | 0.60b | 632b | 24b |

Note: Means with different letters within each column were significantly different at 5% level.

Table 8 Physiological and biochemical compounds of irradiated banana plants with nematode infection (M. incognita)

| γ- dose (Gy) | Free A.A. µgg ⁻¹ f.wt | Proline µmoleg ⁻¹ f.wt | Glycinebataine µmoleg ⁻¹ d.wt | Choline µmoleg ⁻¹ d.wt | Phenols µgg ⁻¹ f.wt | Flavonoids µgg ⁻¹ f.wt |
|-----------------|-------------------------------------|--------------------------------------|---|--------------------------------------|-----------------------------------|--------------------------------------|
| 0 | 637d | 66 d | 0.83a | 0.63a | 634d | 14b |
| 5 | 828c | 73 d | 0.48b | 0.58b | 979c | 13b |
| 10 | 981b | 114b | 0.25d | 0.53c | 1123b | 21a |
| 20 | 1008b | 144a | 0.28c | 0.53c | 1220b | 23a |
| 40 | 1648a | 98 c | 0.28c | 0.49d | 1330a | 9c |

Note: Means with different letters within each column were significantly different at 5% level.

4 Discussion

From previous data, we can conclude that the more gamma radiation exposed, the less growth parameters occurred with banana *in vitro* plants. Similar results were obtained in breeding grapevine (*Vitis vinifera*) rootstocks using gamma radiation. *In vitro* derived Plantlets of Fercal and Gravesac variaties were subjected to gamma radiation at rates from 10 to 60 Gy. Increasing irradiation doses decreased the rate of survival, rhizogenesis and shoot development of *in vitro* derived plants; at 20 Gy and upwards (Lima da Silva and Doazan, 1995). Similarly, Harb et al. (2005) studied the response of micropropagated banana plant to gamma irradiation at 0, 10, 20, 30, 40, and 60 Gy. It was found that, increasing the exposure doses led to increase in plant sensitivity as survival rate, shoot length, leaf number, root number,

fresh and dry weight. Treatments with gamma radiation at the lower doses (10 and 20 Gy) markedly increased shoot length compared with unexposed plants. In addition, using gamma radiation at 0.1 to 1 kGy on maize dry seeds negatively affected plant germination, growth and development. Increasing the irradiation dose suppressed germination percentage and its index, root and shoot lengths. Moreover, higher doses (≤ 5 kGy) led to plants death after 10 days from germination (Marcu et al., 2013). Our observations proved that chlorophyll negatively responded to increased exposure doses. Photosynthetic content revealed an inversely proporti onal relationship to doses of exposure as chlorophyll a, b and total as well as carotenoids (Marcu et al., 2013; Harb et al., 2005). However, our study indicated that leaf number was not affected when plantlets exposed to 5 Gy dose compared

with unexposed plantlets (control). Moreover, leaf width was positively affected by gamma radiation up to 10 Gy, compared with the control and the higher doses used. Similarly, Ilyas and Naz (2014) assured that gamma irradiated plants of *Curcuma longa* L. had the highest length, average number of leaves and leaf width lower doses while higher doses had inhibitory effect.

With respect to nematode response, number of juveniles in soil, developmental stages, galls and egg masses in roots were significantly reduced with all gamma radiation doses, compared to unexposed treatment. The 10 and 20 Gy doses caused the highest reduction for total nematode populations and rate of build-up. These results are similar to that of Abdel-Fattah et al. (2008) who mentioned that number of galls and egg masses on sugar beet roots were reduced gradually by increasing gamma doses. Damianova et al. (2015) investigated various doses of gamma radiation (90, 700 and 1800 mGy) on tomato plant growth and root-knot nematode (Meloidogyne arenaria). Irradiated tomato seeds by the lowest dose (90 mGy) showed increasing plants development while, the highest experimental dose (1800 mGy) prevented the females' development of M. arenaria (J₄) to mature forms.

After infection with nematodes, exposed and unexposed plantlets with or without infection were presented to some biochemical analysis. With respect to uninfected plants, it was found that free amino acids, proline, glycinebetaine (GB), choline contents as well as phenols and flavonoids were increased in leaves of exposed plants compared to unexposed plants. GB and proline are two main organic osmolytes that accumulated in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. Many studies have indicated a positive relationship between accumulation of GB and proline and plant stress tolerance (Ashraf and Foolad, 2007). However, Ghadi et al. (2015) found that, gamma irradiation at 0.5 and 1kGy showed no significant differences on the amount of phenolic content in dates, while at 2.5 kGy, total phenolic content was increased. Furthermore, gamma irradiation doses significantly

increased total flavonoids content in Dill Herb (Said-Al Ahl et al., 2015)

With respect to infected plants with nematodes, all biochemical compounds measured increased with gamma radiation exposure except for glycine betaine and choline contents which decreased compared with the control. Root -knot nematode (Meloidogyne javanica) induced distinct physiological and biochemical changes in mungbean plants. The results suggested that plants responded to the nematode by adopting biochemical strategies (increasing total phenols and decreasing protein content) to withstand the adverse effects of infection (Ahmed et al., 2009). We can observe that gamma radiation has a rule in enhancing plant biochemical response to infection. Irradiation modified some biochemical contents in infected plants leading to withstand nematode infection as shown in all investigated growth parameters.

5 Conclusion

Irradiated banana plants might be resisted/tolerated nematode infection. Irradiation with gamma caused changes in growth parameters, physiological and biochemical contents in banana plants. It increased some chemical compounds that enhanced the response to stresses and increased resistance to nematode infection. These substances may be released from roots to inhibit growth and reproduction of nematode in soil.

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