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Micropropagation potentiality and pigments content of *Hibiscus rosa-sinensis* L. as affected by gamma radiation

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Abstract: The present work was carried out at tissue culture laboratory of Ornamental Plants and Woody Trees Department to establish an *in vitro* propagation of *Hibiscus rosa-sinensis*. Shoot proliferation was induced on microcutting explants cultured on MS (Murashige and Skoog) medium (at full, ¾ and ½ strengths) containing different concentrations of BA (0, 1 and 2 mg/L). During multiplication stage, specific treatments were tested to enhance the rate of shoot multiplication by using different doses of cobalt 60 (^{60}Co) gamma rays (0, 5, 10, 15, 20 and 25Gy) for 30 minutes. Multiple shoot induction was present in the stem explants that were cultured on the MS medium (3/4 strength) supplemented with BA at the concentration of 2mg/l. Application of 5Gy resulted in100% of survival, the highest number of shootlets formation and the maximum number of leaves /explant. Exposure of shootlet explants to gamma irradiation at the dose of 5 or 10Gy increased the number of leaf primordial. However, the highest number of abscised leaves per explant was recorded with control (unirradiated shootlets).Using 10Gy gamma ray caused the highest values content of chlorophyll (a, b) and carotenoids.
Key words: Red hibiscus*, in vitro,* culture media, BA and gamma rays.

Introduction

Red Hibiscus (*Hibiscus rosa-sinensis*L.) is perennial ornamental shrubs widely planted in Egypt, a wellknown member of the family *Malvaceae*is native to East Africa[1]. It grows as an evergreen herbaceous plant. This plant is extensively cultivated as an ornamental plant. Various parts of this plant, like leaves, flowers and roots, have been known to possess medicinal properties like menorrhagia, oral contraceptive, laxative, etc[2]. Although the commercial value of *Hibiscus rosa-sisnensis* as cut flowers is somewhat limited also due to the fact that its flower blooms only few days, between one and three at the most, but it has high commercial value as a landscaping plant. In spite of its landscaping and symbolic value, *Hibiscus rosa- sinensis* has received very limited attention in the field of horticulture, especially in terms of its *in vitro* micropropagation. This may be attributed to the fact that *Hibiscus rosa-sisnensis*is a woody species, despite, tissue culture and micropropagation protocols having been described for a number of woody species[3], the rates of development *in vitro,* shoot proliferation and root induction of woody species have always been low and erratic[4]. The benefits of a successful tissue culture protocol would allow the deemed commercially valuable woody species such as *Hibiscus rosa-sinensis* to be propagated and thus commercialized, much more rapidly than other more traditional methods. However, very few studies exist on the *in vitro* regeneration of the *Malvaceae* family. The success procedures of consecutive micropropagotion of many woody plants could be influenced by various factors from which plant growth regulators, physical conditions and growing media are the most important ones. The effect of benzyladenine (BA) at different concentrations on shoot multiplication rate was recorded[5, 6, 7]on *Ruscus hypoglossum*, *Deutzia scabra* and *Paulownia kowakamii*).

Gamma radiation can be useful for the alteration of physiological characters[8]. The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals[9]. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose[10]. These effects include changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the anti-oxidative system, and accumulation of phenolic compounds[9, 11, 12, 13].

The experiment was then conducts to find out the effect of culture media strength, BA concentration and gamma radiation application through tissue culture technique on Micropropagation potentiality of shootlets and its pigments content of *Hibiscus rosa-sinensis*.

Materials and Methods

This work was carried out at tissue culture Laboratory of Ornamental Plants and Woody Trees Department, National Research Center (NRC), Egypt during years 2014 and 2015 to evaluate some morphological and chemical composition changes in shooting behavior of *in vitro Hibiscus rosa-sisnensis*shootlets treated with various gamma doses.

Plant Material and Explant Source:

The nodal segments were excised from stems of plants and used as source of explants for *in vitro* establishment of the plantlets. The nodal segments were cut into small pieces of approximately 1-2 cm long and washed under running tap water for 30 minutes. Under aseptic conditions, surface-sterilized was conducted by immersing the nodal segment in 20% commercial sodium hypochlorite solution and one drop of tween 20 (polyoxyethylene sorbiton monolaurate) for 20 min. The bottle was shaken continuously to increase the contact between the explants and the sodium hypochlorite solution then after 20 min the nodal segments were rinsed three successive times for 3 minutes with sterile distilled water.

Culture Medium:

MS basal medium (at full, 3/4 or1/2 strengths) supplemented with30g/L sucrose, and solidified with 0.7% agar at pH 5.7[14].

In vitro mass propagation

Microcutting explants (5±2 mm length) were excised from *in vitro* plantlets and cultured on MS medium supplemented with different concentrations of 6-Benzyladenine (BA) (0.0, 1.0 and 2.0mg/l).

Irradiation treatments

The irradiation treatments were carried out at the Middle Eastern Regional Radioisotope Centre for the Arab Countries (Dokki). Gamma irradiation was conducted using Co^{60} gamma source for 30 min. The *in vitro* plantlets were subjected to various doses of gamma rays (0, 5,10,15,20 or 25Gy).

Incubation conditions

The culture jars were kept in growth chamber under incubation conditions, 25ºC and 16h/8h light/dark photoperiod supplied with fluorescent light tubes with a photosynthetic photon flux of approximately 3k lux to promote plantlets development.

Each treatment consists of 25 replicates, four – five weeks after culturing, the following data were recorded:

1. Vegetative characters:

Number of shootletss /explant, number of leaves/shootlet, number of abscised leaves/shootlet and number of leaf primordia /shootlet.

2. Chemical analysis:

Determination of pigments content (mg per 100 g F.W) of chlorophyll A, B and carotenoids was carried out according to the method described[15].

Statistical analysis:

All recorded data were subjected to analysis of variance procedures and treatments. Means were compared using L.S.D test at 5% as the method described[16].

Results and Discussion

1. Effect of culture media strength and BA concentration on *In vitro* Multiplication

The effect of different of culture media strength (full, ¾ and ½ strength) and BA concentrations (0.0, 1.0 and2.0mg/L) on the *in vitro* shootlets formation of *Hibiscus rosa-sisnensis* is shown in Table (1). Data indicated that, supplementation of the¾ strength of MS culture medium by 2.0 mg/L of BA favoured shootlets multiplication compared with control. It can be noticed that this treatment gave the best results of shootlets number per explant (3.0) and number of leaves formed per shootlets (11.33). The ability of explants to produce shoots and was studied[17]. They concluded that optimum shoot proliferation was obtained in full-strength MS salts. Also, it is clear that the addition of BA with to the medium increased the shootlets and enhanced their vigorously. These results could be explained by that cytokinins have important physiological effects, as they have been shown to stimulate cell division as well as cell elongation, to activate RNA synthesis and to stimulate protein synthesis and enzyme activity, as was reviewed[18]. The use of high cytokinin levels was one of the most effective methods to reduce shoot and leaf growth and promote the formation of meristematic clusters[19].

Table (1): Effect of culture media and benzyladenine (BA) concentrations on *in vitro* multiplication of *Hibiscus rosa-sisnensis*.

Treatment	Mean number of shootlets/ explant	Mean number of leaves /explant
Control (Full strength MS)	2.00	8.00
Full strength MS + 1mg BA	2.67	6.33
Full strength MS + 2mg BA	1.83	6.50
¾ strength MS + 1mg BA	2.33	5.67
¾ strength MS + 2mg BA	3.00	11.33
½ strength MS + 1mg BA	2.17	5.17
½ strength MS + 2mg BA	2.33	6.00
L.S.D 5%	0.86	2.90

2. Effect of gamma irradiation on *in vitro* Multiplication

2.1. Survival percentage

Data in Fig. (1) indicate the survival percentage of *Hibiscus rosa-sisnensis* subjected to various doses of gamma rays (0, 5,10,15, 20 and 25Gy). Application of 5Gy recorded 100% of survival. Increasing doses up to 15Gy decreased survival percentage to lowest values as compared to control (0Gy) and caused plant leaves to become yellow, then died within 25 days[20, 21] on *Dracaena Surculosa*. They concluded that low doses of gamma ray stimulated survival (%), but raising irradiation doses led to a progressive decrease in such parameter. Furthermore, explained lethality of explants at high doses of gamma rays by breakdown of meristimatic cells and cell nuclear damage[9].

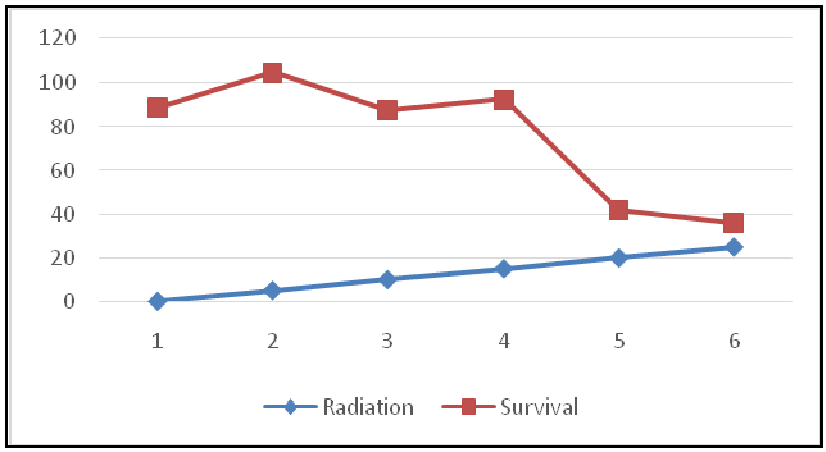

Fig.1. Effect of different doses of gamma radiation on survival percentages of *in vitro* hibiscus plantlets.

2.2. Effect of gamma irradiation on *in vitro* shooting behavior of *Hibiscus rosa-sisnensis*

Table (2) showed that the highest number of shootlets formation (1.50) and the maximum number of leaves /explant (2.33) were recorded with irradiating the shootles with the dose of 5Gy, respectively as compared to control and other treatments which were the cause of declining number of both shootlets and leaves on explants drastically. However, the highest number of abscised leaves (1.33) per explant was recorded with control (unirradiated shootlets) followed by those irradiated by15Gy.The low doses of gamma ray stimulated the growth patterns of *Pelargonium graveolenus*[22]. The obtained data showed that exposure of explants to gamma irradiation at the dose of 5 or 10Gy increased the number leaf primordia (2.67)/ explant, as shown in Table (2). The quality of leaves produced on shoots would be an indicator of shooting ability for micropropagation. Healthy profuse leaves without any malformation are the standard indicator forviable shoot induction. Other standards include low turnover of leaves due to senescence, low numbers of leaves and leaf primodia abscission and high counts of leaves and leaf primodia on the explants[23].It therefore can be concluded that increasing the dose of gamma irradiation, resulted in the decrease in the number of shootlets, leaves and leaf primordia on explants and an increase in the number of leaf abscissions, subsequently affect the shootlet ability for micropropagation.

Table (2): Effect of different doses of gamma radiation on*in vitro* shooting behavior of *Hibiscusrosa-sisnensis*.

Radiation dose (Gy)	Mean number of shootlets/ explant	Mean number of leaves /explant	Mean number abscised leaves	Mean number of leaf primodia/ explant
0 (Control)	0.50	1.67	1.33	0.67
5	1.50	2.33	0.33	2.67
10	0.83	0.67	0.67	2.67
15	0.50	0.33	1.0	1.0
20	0.50	0	0	0.67
25	0.50	0	0	0
L.S.D 5%	0.51	1.51	N.S	1.94

2.3. Effect of gamma irradiation on *in vitro* shootlets pigments content

Data illustrated in table (3) indicted that using 10Gy gamma ray caused the highest values content of chlorophyll (a, b) and carotenoids (81.92, 51.60 and 74.45 mg per 100 g F.W., respectively). However, the lowest value of chlorophyll a, b and carotenoids content was determined as result of 25Gy treatment. The obtained results shown that chlorophyll is virtually insensitive to low doses gamma irradiation. Also the irradiation of *in vitro* Dracaena plant gamma with rays at 20Gy decreased the chlorophylls (a, b) content[11.24]. On *Dracaena Surculosa* found that the highest contents of chlorophylls (a, b) and carotenoids (mg per 100 g F.W) were determined from application of 10Gy as compared with irradiation of 15Gy[21].

Table 3: Effect of different gamma rays at different doses on chlorophyll a,b and carotenoids contents (mg per 100 g F.W) in leaves in *in vitro Hibiscus rosa-sisnensis*.

Radiation dose (Gy)	Chl.a	Chl.b	Carotenoid
0 (Control)	79.44	26.58	64.55
5	47.36	38.65	49.25
10	81.92	51.60	74.45
15	32.60	20.60	50.56
20	29.80	13.64	30.80
25	22.80	13.11	22.35
L.S.D 5%	6.89	0.93	1.08

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