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Study of Radio-Sensitivity of Strawberry Runners *cv. Fortuna* under Moroccan Conditions

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Abstract

In recent years, strawberry is being successfully cultivated in Loukkos perimeter of Morocco and a wide range of varieties are being grown. The strawberry crop also generates 3 million working days per year. In fact, all plants are imported and prices are exorbitant. Varietal improvement is essential if a new impetus is to be given to the morocco strawberry. We have accordingly started a mutation breeding program by bud irradiation. Strawberry runners of *cv. Fortuna* were exposed to increasing doses of acute gamma rays. The physiological effects induced by irradiation were evaluated from cutting survival and growth. It has been determined that the doses of gamma and the varieties used are significant on the number of surviving plants and plant growth parameters. It was determined that low doses gamma irradiation respectively affected plant height, leaf length, leaf width and the number of leaves. These results allowed us, in the first instance, to fix the optimal dose range for cutting irradiated at 62 Grays.

Keywords

Strawberry, Radiosensitivity, Gamma Ray, Runner, Mutation

1. Introduction

Strawberries (*Fragaria* spp.) are a member of the Rosaceae family and native from Europe, which scattered almost all over the world [1]. Strawberry varieties which widely consumed and desired by the people community is a sweet, brightly colored, and large of strawberry variety. Strawberry fruits have short shelf life due to high perishability and are susceptible to mechanical injury, physiological disorders, water loss, and decay [2]. In Morocco but its cultivation started only towards the end of the Seventies, in the two irrigated perimeters of

Gharb-Loukkos and of Souss-Massa region. According to data of the ministry for Agriculture of Morocco, Area of this crop increased over the years, to reach approximately 3660 ha during the year 2016-2017, from which 80% are localized on the level of the area Tangier-Tetouan-Al Hoceima (Loukkos). The main destination for Moroccan strawberries remains the European Union, with 95% of the volume exported. Spain and France, Morocco's main partners, together with the United Kingdom account for almost 90% of the market [3]. The most cultivated varieties in Morocco are of California origin [4]. In morocco, producers are opening up to more tasteful varieties to meet a growing market demand. Overall, the varieties used are much the same as in the Huelva area (Spain), but with different propositions: Festival, Splendor, Fortuna, San Andreas, Sabrina, Ventana, Sabrossa, Lusa, Benicia and other varieties [5]. The increase in the number of hectares planted with strawberries (Camarosa, Festival, Splendor and Fortuna varieties) in Morocco has been more than significant in recent years. This increase is mainly due to the increased demand for the product. Fortuna is a cultivar of short duration published by the Strawberry Variety Development Program of the University of Florida. Selected for early maturity, fruit quality and productivity, the variety is adapted to high value-added production, fresh market and winter production areas [6]. It took less than 500 cold hours to develop and produce properly. It is advisable to cultivate in temperate and continental climates with winters.

The strawberry generates high economic value goods and employment. However, many growers deplore a limited varietal choice, the lack of a local repository on the behavior of the varieties and their production potential, the heavy dependence of producers of foreign nurserymen on supply of plants and access to high-performance varieties. The creation of new strawberry cultivars adapted to Moroccan soil can solve the country dependence on plant import, which can improve the country's economic balance.

Mutation improvement is an effective approach to producing varieties with improved traits [7], is a genetic improvement technique that can give birth, or contribute, to the development of varieties tolerant to biotic and abiotic stress. Although there are several techniques used for mutation breeding, gamma ray technique is one of the most physical mutagens used [8] [9]. Physical mutagens such as gamma rays are less risky to health than chemical mutagens because they require no application to remove mutagen from the material [10], because they are non-toxic and do not require detoxification after implementation [11]. Nowadays, the number of cultivars derived from mutation induction increases constantly [12] [13]. Several new cultivars have developed in Coriander [14] tomatoes [15] [16] Anthurium and mungbean [17] by using gamma rays. Gamma rays as an ionizing radiation affect plant growth and development by inducing cytological, biochemical, physiological and morphological changes in cells and tissues via producing free radicals in cells [18] [19] [20].

The most important point in mutation studies is to determine the appropriate

dose for the species. Regression in growth parameters in the seedling obtained from radiation-treated seeds is usually an indicator of what the plant was damaged genetically. The LD50 dose should be determined to detect the most appropriate mutation dose [21]. In order to determine the appropriate dose, the first generational seedlings (M1) and untreated seedlings are compared in terms of plant growth parameters and survival percentages [11]. This data is used to determine the dose of LD50.

Therefore, this study aims to determine the optimum dose of gamma rays for improving leaf length, leaf width, plant height and survival rate of strawberry runners cv *Fortuna* under Moroccan conditions.

2. Material and Methods

2.1. Study Site

The Larache experimental station of INRA Tangier is located in Larache city, Morocco. It is dedicated to supporting agricultural development in the irrigated area of Loukkos, Morocco. It mainly houses research on strawberry, small red berries and horticultural crops. This research station covers 4.8 ha including 0.8 ha of greenhouses. Strawberry cultivars are grown in greenhouses and were planted with 4 complete random blocks. Each elementary parcel is made up of a ridge 60 meters long and 7 meters wide with four rows spaced 60 cm. The space between two ridges is 70 cm.

2.2. Plant Material

The Fortuna cv strawberry was selected in this study. Plant runners of these strawberry cultivars were used as the experimental materials. Plants runner were taken from the strawberry field in mid-October 2018 and irradiated immediately by ^{60}Co-gamma ray in the National Institute of Agricultural Research Center of Tangier, morocco. The dosages were 0 Gy (non-irradiation), 20 Gy, 40 Gy, 60 Gy, 80 Gy and 100 Gy. There were 80 plant runners per treatment, and all treatments were repeated four times. After the irradiation treatment, plant runners were planted immediately in the field and conventionally managed. The death rate and the change of the leaf length, leaf width, plant height of the treated runner plants were investigated in mid-April 2019. The leaf length, leaf width and plant height were measured according to description and data standard of strawberry germplasm resources [22]. The death rate of the plant runners was expressed by the number of the dead plant runners divided by the number of the treated runner plants. The experiment was conducted in a randomized complete block design (RCBD). Each box containing 20 plants was considered as a single experimental unit that was further replicated four times.

2.3. Statistical Analysis

Statistical analysis of variance was carried out by software SPSS ® version 20.0. The mean difference of the study parameters among the treatments was adjusted

by Duncan's Multiple Range Test [23].

3. Results and Discussion

3.1. Effects on Death Rate of Plant Runners

The death rate of the runner plant is presented in Table 1. Gamma-rays in different doses affected the vegetative growth parameters in different ways. Rate of surviving plants reduced when dose rate increased. Radio-sensitivity of runners on the same cultivar was different. Results show that the death rate of the runner plant of Fortuna strawberry cultivar was higher remarkably in control treatment than in all treatment, and the death rate was between 27.85% and 74.84% (Table 1). There was significant difference in death rate of the runner plants irradiated by four different doses and there was no significant difference in the dose 40 Gy and 60 Gy. However, the death rate of the plant runners of Fortuna strawberry cultivar was higher remarkably with 20 Gy, 40 Gy and 60 Gy than in 80 Gy, and there was no significant difference between 40 Gy and 60 Gy. The death rate of runner plants of Fortuna strawberry cultivar was low with 100 Gy.

The correlation between radiation dose and cumulative percent of bud was r = −0.96 (with P *value* = 0.002). The equation of related regression based on radiation dose is $Y = 90.21 - 0.59X$ (Figure 1). Based on the minimum level of 50% of runner surviving compared to control, lead us to locate the optima radiation dose between 60 and 65 Gy for the Fortuna strawberry cultivar. The yield of runner surviving of these treatments was satisfactory since the rate of survival was ranged from 27.85% to 87.5%.

As a result, GR 50 dose for the application of mutation breeding of Fortuna strawberry cultivar under Moroccan condition was determined as 62 Gy. The strawberry is an herbaceous fruit tree; the complete strawberry runner plant consists of root, stem and leaf. It has no obvious branch and bud, its bud hides in the stem. Thus, the stem of strawberry runner plants is similar to the branch of woody fruit tree [24]. [25] [26] found GR50 dose to be 20 - 25 Gy on woody cutting of grape variety.

Table 1. Effect of gamma ray irradiation to strawberry cultivar on death rate (%) of runner plant.

Dosage (Gray)	Death Rate (%)
0	87.50 *a*
20	74.64 *a*
40	71.07 *ab*
60	64.64 *ab*
80	38.92 *cd*
100	27.85 *d*
p ($\alpha = 0.05$)	0.003

Significant differences within the same column and means followed by the same letter do not differ at Pα ≤ 0.05 according to Duncan test.

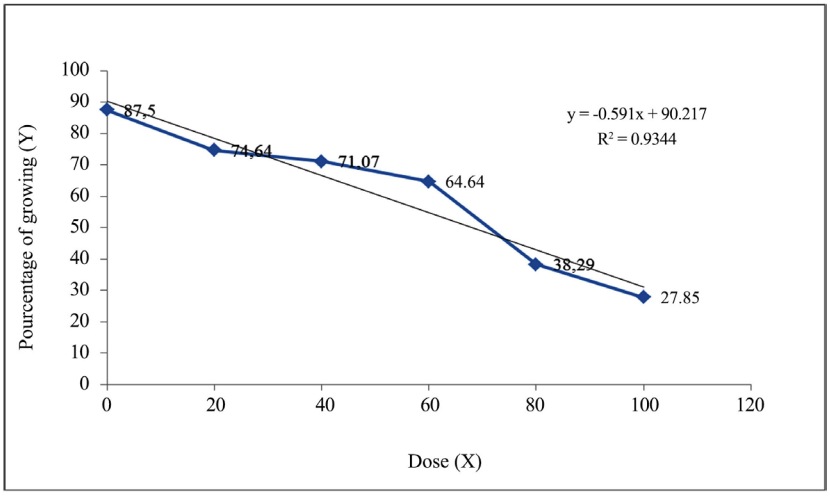

Figure 1. Average of the percentage of growing of the runner plant Fortuna strawberry cultivars.

[27] observed a higher percentage of survival at lower doses and poor survival at higher doses in all the generations of *Calendula officinalis*. [28] found the reduction survival of plants after exposure to gamma rays with the increase of dose in *Rudbeckia laciniata*. A similar trend has also been reported by [29]. [30] also reported the decrease in ability to produce shoots on increasing the radiation dose in *Lonicera* species. [31] reported the inhibitory effects of higher doses of gamma rays on strawberry cultures.

The application of high doses of gamma rays negatively influenced plant development, as in many studies previously done in different species [32] [33] [34] [35].

3.2. Effects on Plant Runners Growth

The survival of plant runners grew further after they were planted in the field, but the plant height, leaf length, leaf width and the number of leaves of the survival of plant runners had changed compared with the control (**Figure 2**). However, the change was different according to irradiation dosage (**Table 2**). The dose 20 Gy recorded significantly maximum plant height (11.61 cm). The minimum plant height (2.03 cm) was recorded by the dose of 100 Gy. The height plant was higher significantly similar in dose 20 Gy and 40 Gy, and significantly lower similar in dose 60 Gy and 80 Gy. The dose 100 Gy shows the very less height of plant.

The increased plant vigor caused by irradiation could be explained by stimulation of biosynthesis of some amino acids like Lysine and phenylalanine [36], modification of some enzymes activity such Polyphenol oxidase, catalases and peroxidases which are great in the leaf of treated plants [37] [38] [39] [40] for increase of primary biochemical processes, uptake of mineral nutrients [41] and photosynthesis [42]. [31] reported the inhibitory effects of higher doses of gamma rays on strawberry plant. He found that the number of roots per shoot decreased at higher gamma dosage (10 KR and 15 KR).

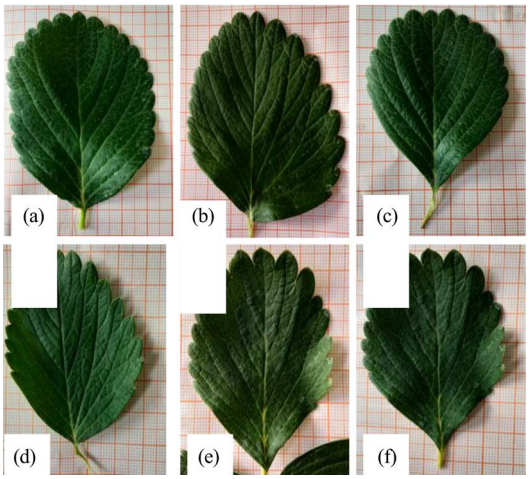

Figure 2. Shape of leaf edges. ((a) 0 Gray; (b) 20 Gray; (c) 40 Gray; (d) 60 Gray; (e) 80 Gray; (f) 100 Gray).

Table 2. Effect of gamma ray irradiation to Fortuna strawberry cultivar on growth of plant runners.

Dosage (Gray)	Plant Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Number of Leaves
0	12.28 *a*	5.11 *a*	4.34 *a*	22.92 *a*
20	11.61 *a*	5.03 *a*	4.26 *a*	20.11 *ab*
40	10.68 *a*	4.58 *a*	3.96 *a*	16.95 *bc*
60	8.63 *b*	4.08 *ab*	3.34 *b*	14.77 *c*
80	7.59 *b*	3.51 *b*	2.84 *b*	18.21 *bc*
100	2.03 *c*	0.99 *c*	0.72 *c*	5.78 *d*
$p\,(\alpha = 0.05)$	0.000	0.000	0.000	0.000

The same letter within the same column and means do not differ at Pα > 0.05 according to Duncan test.

Leaf number is important for photosynthesis and it differed significantly ranging from 5.78 to 20.11. The dose 20 Gy shows the highest number of leaves (20.11) and lower number for leaves was at 100 Gy doses. The doses 40 Gy and 80 Gy were not significantly different but in the number of leaves were significantly different in other doses (Table 2).

Leaf length and leaf width from each irradiated plant were studied (Table 2). In the leaf length and leaf width the dose 20 Gy show the higher value 5.03 cm and 4.26 cm respectively, the lowest value for these two parameters was observed for 100 Gy dose. In leaf width, we distinguish three groups significantly different; the dose 20 Gy and 40 Gy are not significantly different also from the dose 60 and 80 Gy they are not significantly different, only the dose 100 Gy was significantly different from all the doses. The leaf length only the dose 20 Gy and 40 Gy they are significantly not different. In this study, the gamma ray influences the leaf length and leaf width (Figure 2).

Numerous investigators [43] [44] [45] [46] have presented results which indicate that cells are more vulnerable to radiation at certain stages in mitosis. [47]

obtained the number of leaves vitro plant varying from 60.52 to 49.00 in different germplasm, which was higher to the findings of the present study. But the finding about leaves plant reported by [48] was lower than the present study. They found that the number of leaves ranged from 3.33 to 6.33 plants under irrigation in semi-arid region of Punjab, which might be due to the genetic as well as the environmental effect. [49] obtained a variation in leaf number among cultivars during the later part of the season and found that cv. "Bolero" and "Everest" produced the highest and "Elsanta" the least number of leaves plant. Another study revealed a significant variation in leaves plant among strawberry germplasm and recorded 4.6 to 9.8 leaves plant at 86 days after planting in Cambridge.

Stimulatory effects of low doses of gamma rays on strawberry can be related to hypothesis that the low dose irradiation induce growth stimulation by changing the hormonal signaling network in plant cells or increase the anti-oxidative stress capacity of the cells to easily overcome the stress factors such as fluctuations of light intensity and temperatures in the growth condition [50]. In contrast, the high dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome [51].

Since it is possible to alter growth rate without altering radio-sensitivity at the same time, it is no longer sufficient to say that an organism is more susceptible because it is growing rapidly or because it contains cells in mitosis. It would appear that growth, as defined, is the result of a condition which determines radio-sensitivity, rather than the cause of it. It is probably more nearly correct to say that growth activity and radio-sensitivity depend on a common physiological condition in the organism-one that remains unchanged although growth may be stopped by lowering temperature.

Stems of strawberry plants were observed (**Figure 3**). The strawberry plant from doses 20 Gy 40 Gy and 60 Gy show a higher number of stems in a plant like plant control. But in the plant irradiated with 80 Gy and 100 Gy, the number of stems

Figure 3. Stems of strawberry plants.

in a plant was lower. The character of plant robustness was influenced by the anatomical structure of the stem. This is due to the fact that strawberries are a dicotyledonous plant whose stems are subjected to secondary growth by having a lateral meristem. Additionally, the clustering life from of strawberries also supports the robustness of their stems. [52] stated that phenotypic appearances could be controlled by the genetic properties of the plant itself, which are the responses to the interaction with environmental factors.

4. Conclusion

In this study, it was aimed to determine the morphological changes occurring in strawberry runners cv Fortuna exposed to gamma irradiation (60 Co) and to determine the proper irradiation dose for mutation breeding studies. Results show that the doses applied in the study do not affect growth in strawberry runners cv Fortuna but they cause significant differences in the number of surviving plants. In this study, it was also found that the optimal irradiation dose for strawberry runners cv Fotuna was 62 Gray under Moroccan condition. Finally, results show that the radiation sensitivity of the cultivars is different by examining the surviving plants and plant growth parameters. This has once again proved that it is necessary to carry out dose determination study before starting large-scale studies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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