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Application of Mutagenic Radiation and Research the Optimal doses of Induction of bud break and Vegetative Growth in the Grapevine (*Vitis vinifera (L.*)

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***Abstract*— *The demand of grape in Morocco is fulfilled through import from foreign countries. The fruits of local cultivars of grapes offer a low yield. Development of seedless grape varieties having increased sweetness, higher yield with better nutritional quality is necessary to reduce the import dependency. The present research activities are the part of a grape improvement project. A pot experiment was conducted at the National Institute of Agronomic Research (INRA), Center Tangier (Morocco), during February to November 2017 to determine the suitable gamma irradiation doses on growth, leaf area and content of chlorophyll of grape seedling. One hundred vegetative bud cutting and three doses of gamma irradiation 20, 30, and 40 Gy were used as treatment. Different irradiation doses and vegetative cutting showed significant variations in respect of plant growth characters, leaf area and Chlorophyll Content. Higher doses of gamma irradiation had showed detrimental effect on grape saplings. Generally, increased in irradiation doses showed decreased and detrimental effects on most of the parameters under study. Maximum numbers of growing bud, leaf area and content of chlorophyll were found at 20 Gy irradiation dose. All parameters showed best results in 20 Gy with bud cutting initiation*.

Keywords*— Grape sapling, Gamma irradiation, Morphological Parameters, Leaf Area, Chlorophyll Content.

I. INTRODUCTION

Grape (*vitis vinifera L.*) belongs to the family Vitacae distributed widely all over the world (Olmo et al 1976) and originated in West Asia (Wang et al. 1999). Grape occupies the first position among the fruits in the world in terms of area and production. The world area occupied by grape is 7.63 million hectares producing 64.29 million tons per annum (FAO, 2001). But, in Morocco, the sector of vine of table is based primarily on the introduction of foreign varieties. Despite the fact that the traditional and local cultivars of grapes remain very appreciated by the consumers (Sbaghi, 2008), they do not express all their production potential and therefore offer a low yield. Grapevines may be propagated from seeds, cutting, layers, or grafts. Normally new vines grown from seeds differ markedly from the parent vigne and from each other. However, propagation, by cutting, layers, buds, or grafts, in contrast, produce vines identical with the parents in all varietal characteristics (Gulcan and Llter, 1975). Spontaneous somatic mutation has played a considerable role in improvement of vegetative propagated plants and many of the varieties under cultivation are of this origin, therefore the rate of spontaneous mutations is too low to be efficient means (Donini, 1993).

Mutant has improved cultivar with wide application. In grapevine several mutants, are now growing in preference to the original cultivar. There is great clonal variability among grapevine varieties and this is widely used by plant breeders to develop new varieties (Botta and Me, 1989; Alleweldt et al, 1990; Çoban, 1998). However, chemical and physical mutagenes were also used to increase the variability (Rathjen and Robinson, 1992). To determine the radio sensitivity of grapevine species, cultivars and clones are essential for assessing repair, recovery capacity of the vine from radiation injuries, to measure the influencing factors of these and the radio protective agents (Milosavljeviç and Mijajiloviç, 1965 ; Da Silva and Doazon, 1995). For the radio sensitivity determination the dose-effects in relation to the survival, seedling and plant heights, root shoot growth are measured most commonly (Shin et al., 1998; Hajdu et la, 1994; Körösi et al, 1995).

In order to evaluate gamma-ray (60Co) irradiation as a possible aid to increase the clonal variability, varietal responses to gamma-ray irradiation and cutting responses of each variety were studied. The aim of present study is to determine the radio sensibility of some cutting of the same variety of grapevine.

II. MATERIALS AND METHODS

The experiment was conducted out at the National Institute of Agronomic Research tangier (INRA), Morocco. Grapevine Doukkali was used as experimental crop in the study. There was total four lots of 100 bud cutting were irradiated separately by one of the following doses of radiation: 20 Grays (G1), 30 Grays (G2) and 40 Grays (G3). The control cuttings were untreated (G0). All cutting were sown under a greenhouse in plastic bags. The growth of cuttings was counted every 2 weeks during nine months since the date of planting. After irradiation by gamma radiation source, cutting was planted in each pot. Necessary data were recorded on the growth, morphological and biochemical parameters. Total leaf area of the plant was measured with auto matic electronic leaf area meter (model LI-3000, USA) and with imageJ software. Chlorophyll content of leaf was estimated by manual SPAD-502 Plus. The statistical analysis of variance was carried out by software SPSS ® version 20.0. The mean difference of the studies parameters among the treatments was adjusted by Ducan's Multiple Range Test (Gomez et al, 1984).

III. RESULTS AND DISCUSSION

1. Growth characteristics

The cumulative percentage of bud (CPB) followed a logarithmic progression for both control and irradiated buds (Table 1). The control showed a CPB (96%) higher than the irradiated buds. However, the irradiated buds showed decreasing CPB based on increasing doses of radiation. The lower CPB were noted for the high radiation doses; 2 and 3% for the radiation 40 Grays, 17% for the dose of 30 Grays and 55% for the dose of 20 Grays applied on buds on grapevine.

The correlation between radiation dose and cumulative percent of bud was r= -0.99***. The equation of related regression based on radiation dose is Y=79-2.07X (Fig. 5). Based on the minimum level of 50% of cuttings surviving compared to control, lead us to locate the optima radiation dose between 15 and 20 Grays for Grapevine cuttings. The yield of cuttings surviving of these treatments was satisfactory since the rate of survival was ranged from 17 to 55%.

2. Leaf area plant

A highly significant variation in leaf area plant was observed due to the influence gamma irradiation. The maximum leaf area plant (123.7 cm^2) was observed in G1 and the minimum leaf area (74.8 cm^2) was observed in G3 (Table 2). The leaf area plant increased in low doses of irradiation. The similar results were also obtained by others (Charbaji et al. 1999; Islam et al, 2015). The maximum and the minimum leaf area plant were found in G2 and G3 respectively was statically different from others. But the leaf area plant found in the G1 was not statically different from the control.

3. Chlorophyll Content

A highly significant variation in chlorophyll content found due to different gamma irradiation doses (Table 3). At the 16/05/2017, the maximum content chlorophyll (22.8 $mg.g^{-1}$) was observed in G1 while the minimum (7.4 $mg.g^{-1}$) was observed in G3 (Table 3). At the 16/06/2017, the maximum content of chlorophyll (26.03 $mg.g^{-1}$) was found in the G1 and the minimum in the G3. But at the 04/07/2017, the maximum content of chlorophyll (32.41 $mg.g^{-1}$) was found in the G2 and the minimum (24.5 $mg.g^{-1}$) was found in the G3. The effect gamma irradiation doses and different vegetative bud stages showed variation in content of chlorophyll. Increase in irradiation doses decreased the amount of chlorophyll content also agreed by others (Lima et al, 1995; Islam et al, 2015).

Conclusion

The irradiation by gamma radiation of buds is a classical method of varietal creation by induced mutagenesis. This method aims to improve or diversify, for one or a few characters, cultivars, which already have a good agronomic value. In this study, it was evident from the result that higher dose had detrimental effect on the plant morphological and biochemical parameters. Among the irradiation doses, 20Gy showed better morphological parameters in M1 generation, however, it is difficult to say at this stage which dose and stage will show maximum mutability. In M2 generation, the expression of mutagenicity will be observed. So, the research works done will push a step forward for further observation and selection of most desirable mutant in M2 and the following generations.

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Table.1: Cumulative rate of growing of grapevine and irradiated with increased doses (20-30-40 Grays)

Gamma irradiation	dates																		
	28/02	15/03	28/03	13/04	25/04	08/05	23/05	06/06	21/06	04/07	18/07	02/08	15/08	29/08	12/09	26/09	24/10	31/10	08/11
G0	0	25	36,67	61,67	86,67	93,33	96,67	96,67	96,67	96,67	96,67	96,67	96,67	96,67	96,67	96,67	96,67	96,67	96,67
G1	0	0	10	31	33	34	39	42	42	44	45	50	55	55	55	55	55	55	55
G2	0	0	0	1	2	4	6	11	16	17	17	17	17	17	17	17	17	17	17
G3	0	0	0	0	0	1	2	2	2	2	3	3	3	3	3	3	3	3	3

Table.2: Effect of gamma irradiation on leaf area plant of grape sapling

Gamma Irradiation	Leaf area (cm^2)
G0	109,6 ab
G1	103,7 ab
G2	98,7 a
G3	74,8 b
p (α = 0,05)	*0,028*

**the means do not differ significantly at 5% level according to Duncan test.*

Tableau 3: Effect of gamma irradiation on the content of chlorophyll of grape sapling

	Chlorophyll of leaf (mg.g-1)		
Gamma irradiation	16/05	16/06	04/07
G0	21,2 b	22,5 b	25,2 a
G1	22,8 b	26,0 b	28,3 b
G2	14,7 ab	24,2 b	32,4 c
G3	7,4 a	16,6 a	24,5 ab
p (α = 0,05)	*0,00*	*0,00*	*0,00*

**The means do not differ significantly at 5% level according to Duncan test.*

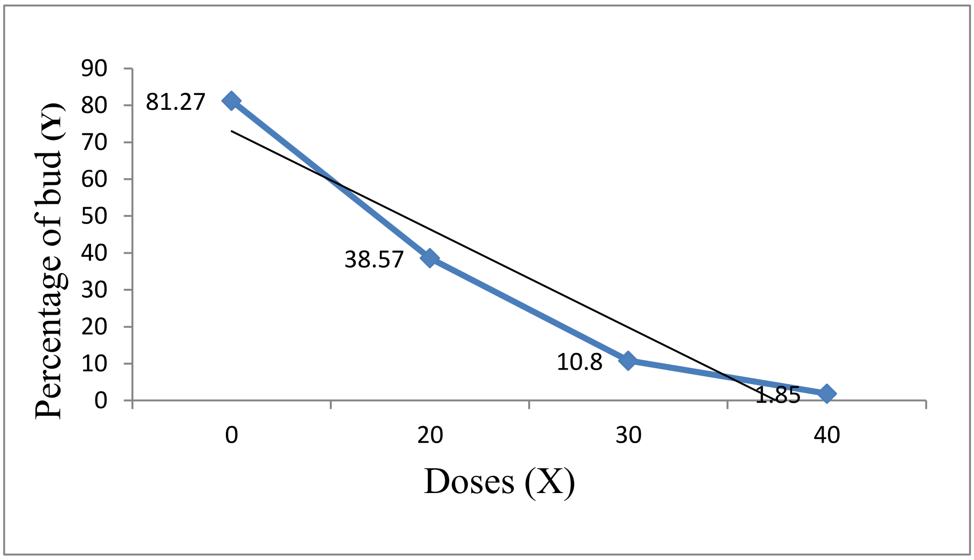

Fig.2: Average of the percentage of growing of the cuttings of the grapevine