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Effect of Gamma Radiation and Gibberellic Acid on Germination and Alkaloid Production in *Atropa belladonna* l.

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Abstract: This investigation was carried out to improve the germination percentage and alkaloid production in M_1 seeds (irradiated seeds with 50, 80, 110 and 150 Gy) of *Atropa belladonna* L. by using and different concentrations of gibberellic acid (20,40,60,80,100 and 120 ppm). There was an increase in seed germination percentage by increasing gamma-ray doses until 150 Gy which gave decrease in seed germination percentage, and with regard to the effect of GA_3 on seed germination percentage, there was increasing in this percentage by increasing GA_3 concentration until 100 ppm. The best interaction was between 110 Gy and 100 ppm which was the highest percentage. At, M_2, a new mutation in seed colour (Red seeds) for almost mutants under study would be considered as a solution for genetic germination problems, that the germination percentage reached to 100 % . Three promising high alkaloids mutants were selected from M_2, M-11-1, M-11-2 and M-15-1.

Keywords: *Atropa belladonna*, germination, gamma rays, Gibbbrillic acid and total alkoids

INTRODUCTION

Medicinal plants are the most important source of life saving drugs for the majority of world's population, for thousands of years. Even today, the World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines. *Atropa belladonna* L. is valued for the use of alkaloids in the treatment of Parkinson's disease, anti-inflammatory properties for relief of bronchial asthma and motion sickness, ability to counteract toxic agents and for dilation of the pupils in optometry (Grieve *et al*., 1995).

Irradiated *Atropa belladonna* L. seeds with various doses of gamma rays can produce altered plant phenotypes having different alkaloidal content at 1^{st} and 2^{nd} mutagenic generation, M1 and M_2, (Ghiorghita *et al*., 1982). Irradiation can also increase the alkaloids percentage in the different organs of plant, particularly the leaves (Abo Elseud, 1983., El-Kholy, 1987., and Habba, 1989).

The aim of the present study was, Trace the seed of *Atropa belladonna* L. laboratory germination under the influence of gibberellic acid and gamma irradiation. Induce some *Atropa belladonna* L. mutants possessing high alkaloids contents

MATERIALS AND METHODS

This present investigation was carried out during three successive seasons at the greenhouse of Botany Department, National Research Centre to evaluate the first mutagenic generation (M_1) and second mutagenic generation (M_2), and (M_3).

Plant Material:

The plant material was *Atropa belladonna* L. seeds which kindly obtained from the Experimental station of Medicinal plants, Faculty of Pharmacy, Cairo University.

Seeds of *Atropa belladonna* L. were subjected to four doses of Gamma-rays (50, 80,110 and 150 Gy). Irradiation was carried out at National Center For Radiation Research and Technology, Cairo, Egypt.

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Germination Experiment:

Atropa belladonna L. Irradiated seeds of *Atropa* were soaked directly in six concentrations of Gibbrillic acid (GA_3) (20, 40, 60, 80, 100 and 120 mg/l) for ten hours in a comparable with control (un soaked) and then sown on filter paper in Petri dishes. All Petri dishes were incubated in a complete dark at 25 ±2ºC.

The percentage of seed (M_1) germination was determined after five and ten days from sowing.The two phenotypes seeds (Red and Black) of M_2 were soaked in the concentration 100 mg/l (GA_3) for ten hrs and sown on filter paper in Petri dishes. All Petri dishes were incubated in a complete dark at 25 ± 2ºC to determine seed germination percentage.

The percentage of seed (M_2) germination was determined after five and ten days from sowing.

Phytochemical Investigation:

Total alkaloids were estimated in dry leaves of twenty five M_1 plants at flowering stage according to Mahmoud (2004)

Statistical Analysis:

The experimental design was complete randomized blocks. L.S.D. values were estimated according to Sokal and Rohlf (1995).

RESULTS AND DISCUSSIONS

Effect of gamma-ray doses and GA_3 concentrations on seed germination percentage of *Atropa belladonna* L. at M_1 generation:

Table (1) and Fig. (1) showed the effect of gamma- ray doses (0, 50, 80, 110 and 150 Gy) and (GA_3) concentrations (0, 20, 40, 60, 80, 100 and 120 mg/l) on seed germination percentage in the first mutagenic generation (M_1) of *Atropa belladonna* L. There were highly significant differences between γ- ray doses and GA_3 concentrations and the interactions between them was also significant. Seed germination was increased by increasing of γ- ray doses, until 150 Gy of γ- ray, which resulted decrease of germination percentage in comparison to previous doses. These results confirmed with the main concept of γ- ray effects.

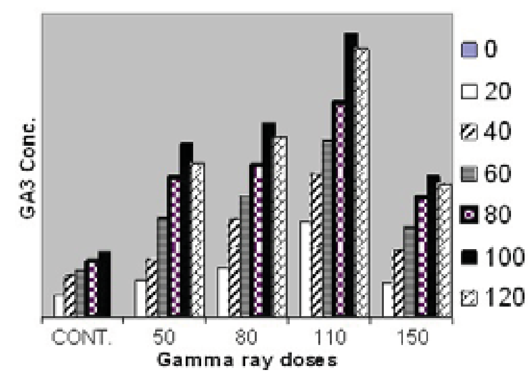

Fig. 1: Effect of interaction between gamma - ray doses and GA3 concentrations on seed germination % in M1 generation of belladonna plants.

Table 1: Effect of interaction between gamma – ray doses and GA_3 concentrations on seed germination % (M_1) of *Atropa belladonna* L.

	GA3 Conc. (mg/L)							Mean
	0	20	40	60	80	100	120	
Cont.	0	7.25	13.71	15.23	18.61	21.18	0	10.854
50 Gy	0	21.16	19.12	32.87	46.06	57.11	50.59	31.13
80 Gy	0	16.09	32.08	39.67	50.11	64.03	59.13	37.3
110 Gy	0	31.44	46.73	58.13	70.35	93.33	88.17	55.45
150 Gy	0	11.1	21.81	29.61	39.51	46.67	44.1	27.54
Mean	0	15.61	26.69	35.1	44.93	56.464	48.398	

L.S.D.	5%	1%
Gamma rays (γ)	2.502	3.322
GA_3 (G)	2.114	2.808
γ x G	5.594	7.429

Germinating seeds of *Atropa belladonna* L. on medium containing 0.0 ppm GA_3 (control) gave the zero germination percentage, while the highest of seed germination percentage (56.46%) was obtained for 100 ppm GA_3 as shown in Table (1). Also, there were highly significant differences between all treatments compared to the control. At 120 ppm GA_3 seed germination percentage reduced and recorded (48.40%) as compared to other concentration of gibberellic acid under study.

The increase or decrease in the germination percentage was found to attributed to gamma rays treatments. The stimulating effects of gamma ray on germination may be attributed to the activation of RNA synthesis (Kuzin *at al*., 1975) on coster bean, or protein synthesis (Kuzin *et al.,* 1976) which occurred during the early stage of germination after seeds irradiated with 4 K-rad. These results are in agreement with the findings of Grover and Dhanju, (1980) on *Papaver somniferum* and Donge *et al.,* (1986) on tea seeds. Habba (1989) who reported that increasing the dose of gamma rays up to 100 Gy, gradually increased the germination percentage, and then decreased gradually with increasing the gamma ray dose in the second season in *Hyoscyamus muticus*.

These results are in agreement with the findings of Hell *et al*., (1974) on *Phaseolus vulgaris*, who noticed that treating seeds with high rates of gamma radiation reduced germination. On the otherhand Abo Elsauod & Omran (1976) indicated that irradiation snap bean seeds with 50, 100 and 150 Gy resulted in greater percentage of germination than the control.

Regarding the effect of GA_3 on seed germination an increase in germination percentage was observed by increasing GA_3 concentration was in confirmity with Ruminska *et al.,* (1978). Who reported that the seed soaking, preceding the sowing, in solutions 500, 1000, 1500 and 2000 ppm of GA_3 improved germination ability of seven species of seeds, particularly good effects were achieved with *Lavandula vera* and *Atropa belladonna* where not only germination ability was not only increased but also accelerated and even shooting was obtained.

Furthermore, the interaction between gamma-ray doses and gibberellic acid concentrations was highly significant in comparable to the control (Table 1). Results in (Table 1 and Figure 1) indicated that highly significant increase in seed germination percentage was observed between all gamma-ray doses and 100 ppm GA_3 concentration. However, the interaction between the dose 110 Gy and 100 ppm GA_3 recorded the highest seed germination percentage (93.33 %).

Data presented in Table (2) illustrated the effect of days number (5 and 10 days) on seed germination percentage in the second mutagenic generation (M_2) of two different phenotypes, i.e., black and red colour seeds (black seeds as a common colour seeds of *Atropa belladonna* L., while red colour seeds consider as a new type of colour mutants which became stable at M_2 and M_3 generations and gave higher germination percentage than the origin seeds (black seeds) at 100 ppm GA_3. The data showed highly significant effect between red and black seeds of mutants. However, there were highly significant differences between all mutants as compared to control. The mutant M-11-1 recorded the highest percentage (79.38%) while M-5 and M-8 the lowest one (50%).

Concerning the effect of seeds phenotypes (red and black) on seed germination percentage, there were highly significant percentage of seed germination. Red seeds gave the largest response on seed germination (100%) at 5 and 10 daya, while black seeds scorded (22.09%) at 5 days and (27.71%) at 10 days.

Also, data obtained in Table (2) indicated that there was highly significant increase in percentage of seed germination by increasing days number. At 10 days, the highest percentage of seed germination (63.86%) was obtained, while at 5 days seed percentage scorded (61.05%).

Furthermore, the interaction between gamma-ray doses, days number and different phenotypes was highly significant. Results in Table (2) indicated that highly significant increase in seed germination percentage was observed between all mutants and red seeds at 5 and 10 days (100 %).

The interaction between mutants and black seeds, M-11-1 and black seeds at 10 days recorded the highest percentage of seed germination (81.25 %).These results confirm the results obtained by Habba (1989) who reported that increasing the dose of gamma rays up to 100 Gy, gradually increased the germination percentage, and then decreased gradually with increasing the gamma rays dose in the second season in *Hyoscyamus muticus*.

In conclusion, the new type of seed colour (red seeds) for almost mutants under study, will be considered as a genetic solution for germination problems in *Atropa belladonna* L.

Total Alkaloids:

High Alkaloids Promising Mutant Lines at M_1 and M_2 Generations:

These three mutants were selected from 25 morphological apparent genotypes than the control, or the basis high alkaloid contents at M_1 and M_2 generation (Table 3).

Table 2: The response of new mutants (red and black seeds) for germination % at 5 and 10 days under 100 ppm GA_3 at M_2 generation.

Treatments	5 days		10 days		Mean
	Red	Black	Red	Black	
Cont.	100	20.00	100	35.00	63.75
M-5	100	0	100	0	50
M-8	100	0	100	0	50
M-11-1	100	77. 51	0	81. 25	79.38
M-11-2	100	0	100	0	50
M-15-1	100	35.00	100	50.00	71.25
Mean	100	22.09	100	27.71	

L.S.D.	5%	1%
Gamma rays (γ)	6.436	8.586
Phenotype (Ph)	4.071	5.430
Days (D)	1.071	5.430
γ x Ph x D	12.873	17.1

Table 3: The average mean of total alkaloids of selected mutants at M_1 and M_2 generation.

Treatments	Total alkaloid (mg/g)		Total alkaloid (mg / plant)	
	M_1	M_2	M_1	M_2
Control	1.96	2.03	48.84	53.41
M-11-1	4.80	4.01	128.54	109.67
M-11-2	4.28	3.43	113.56	96.33
M-15-1	3.31	3.79	89.24	108.05

The highest of total alkaloids appeared in almost all mutants than the control, they possessed twice values than the control, especially M-11-1, which gave 4.01 mg/g and 109.67 mg/plant at M_2 generation by comparison, 2.03 mg/g and 53.41 mg/plant for the control.

These mutants require more detailed studies on the kinds of alkaloids and fractionation of them and testing of stability until M_5 generation. These results confirmed with several investigators on *Atropa belladonna* L. (Helmy (1984), Habba (1989) ,mahmoud(2004)) and fayed *etal*. (2007)

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