In Vitro Radiosensitivity Study of Datura Sp Seeds for Increased Alkaloid-producing Mutant Lines

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ABSTRACT

The Daturas are tropane alkaloid producing plants from the Solanaceae family. These alkaloids are known to have very important pharmaceutical properties. Moreover, their chemical synthesis was found to be more expensive than their direct extraction from their natural source, the plant itself. In addition, their concentration in the whole plant remains low even among the most productive bodies, both when grown in artificial (in vitro hydroponics) or natural conditions. Hence, there is a rising interest for radiation-induced mutations in the perspective of producing higher extents of alkaloids through the natural process. In this context, three Algerian varieties of Datura seeds namely: Datura stramonium, Datura inoxia, and Tatula were gamma-irradiated by means of a Cobalt-60 source. The irradiation doses ranged from 5 to 80 Gy. Irradiated and scarified seeds were afterwards germinated in vitro on a MS-medium in a controlled growth chamber. The obtained results indicate that the Datura inoxia variety has good radiosensitivity compared to the two other varieties. Tropane alkaloids (atropine and scopolamine) of both Datura inoxia control and irradiated seeds were analysed by gas chromatography. This variety is expected to be used in an induced-mutation programme for the sake of obtaining mutant lines that will exhibit increased tropane alkaloid concentrations.

Key words: Tropane alkaloids, Datura, germination, in vitro, mutations, radiosensitivity, gamma rays, atropine, scopolamine.

Introduction

Datura genus belongs to the Solanaceae family. It contains 2500 species grouped in 90 genera of which more than half belong to a single genus which is: Solanum. The datura produces alkaloids such as atropine, scopalamine and hyoscyamine. These substances are known to cause various physiological responses to human beings because they interfere with neurotransmitters. At high doses, most alkaloids are very toxic whereas at low doses they have important pharmaceutical applications [14]. Tropane alkaloids or their extracts are commonly used in medical applications as muscle relaxants, analgesics, tranquilizers or even psychotropic drugs. They can also act as antispasmodic, sedative, anticholinergic and mydriatic substances as well [27]. Tropane alkaloids’ synthesis is found more expensive than extracting them from their natural source, the plant itself. Moreover, artificial cultivation (in vitro, hydroponics) of most alkaloids producing organs yields low alkaloid contents except for those which have undergone genetic transformation. Hence, there is growing interest to induce gamma irradiation mutations in the prospect of efficiently producing alkaloids in a natural way. Within the International Atomic Energy Agency database for such a field there exists up to present time no record for new datura mutant species. The aim of our work is to study in vitro seeds radiosensitivity from three
Algerian datura species; *Datura stramonium*, *Datura Datula*, *Datura inoxia*. Two models (scarification / irradiation and irradiation scarringification) are applied for the sake of selecting the useful dose range and to choose a single species to be used later in a mutation induction programme for obtaining rich tropane alkaloids Datura mutant lines. The alkaloids of investigated Datura species (control and irradiated) were analyzed by GC.

**Materials and Methods**

The investigated plant material is composed of the seeds of 03 species of Datura genus, which are: *Datura inoxia* Mill., *Datura stramonium* L. L. and *Datura Tatula* whose origins in Algeria are respectively: Djelid (Ain Defla), El Harrach and Tlemcen.

1 – *In vitro* Radiosensitivity Study:

Datura seeds are first separated to form homogeneous lots with 50 seeds per species and per dose. The seeds are then placed in glass tubes to be gamma irradiated making use of a Cobalt-60 source according to the two processes as follows:

Process 1- Irradiation / Scarification: the seeds are irradiated, scarified and then in vitro cultivated

Process 2- Scarification / Irradiation: the seeds were first scarified, irradiated and then in vitro cultivated.

Scarification is done manually using paper glass No. 80 according to Khelifi-Slaoui et al. [16] method, so as to identify embryos of the seed coat for *Datura stramonium* and *Datura Tatula* and in order to remove the seed coats with a nail clippers for *Datura inoxia* (Bensaid, 1991). The irradiation of Datura seeds was carried out in the Nuclear Applications Division of the Algerian Nuclear Research Centre at a rate of 0.623 Gy/mn. The doses are as follows: 5, 10, 20, 40, 60, 80 Gy which is in accordance with the recommendations of Gogebashvili et al. [13]. Scarified seeds were disinfected by bathing them in ethanol at 70 °C for 30 seconds followed by a soaking in sodium hypochlorite at 12 °C for 10 minutes. The seeds undergo three rinses by sterile distilled water and are then dried on sterile filter paper [16]. Disinfected seeds are sown in tubes containing 10 ml MS medium [20] and then placed in a growth chamber at 26 ± 1°C and a photoperiod of 16 hours. The same approach is applied for both tested processes. The measurements were made at 90 days after in vitro sowing for both processes. They concerned the following parameters:

- Germination rate: The germination percentage for each species and each dose of irradiation is estimated 90 days after the sowing phase.
- Stem and root length: They concern the entire length of the aerial part and the root length.
- Reduction or improvement in the average length of stems and roots: The rate can give an idea on the decrease or on the increase of the stems and the roots length depending on the radiation dose compared with the control set.

2 / Extraction and GC Analysis of Alkaloids:

The alkaloid content in the control set and in the irradiated seeds of *Datura inoxia* are extracted and concentrated according to the method described by Amdoun [4]. The analysis of the alkaloids is carried out by gas chromatography (GC) on a total of 65 samples all doses confused.

A statistical analysis of obtained results was performed using Statigraphics® software.

**Results and Discussion**

The effect of mutagenic treatments on unscarified seeds (process 1)

1.1 Germination rate:

For *D. Stramonium*, the highest rate is obtained for dose D1 (10 Gy) with 96.07%, while the lowest rate was noted for the D5 dose (60 Gy) with 85.24% (Fig.1A). Regarding, *D. inoxia*, we note from Figure 1-B that the germination rate varies with the radiation dose. The highest germination rate (90.19%) is achieved with dose D1 (10 Gy), whereas the D5 dose (60 Gy) yields the lowest rate of germination with 49.12%. For *D. Tatula*, the highest germination rate is observed in the control D0 with 18.86%. This rate decreased for other irradiation doses and reached to 5.35% at D5 dose (Fig. 1-C).

While studying the species vs. radiation dose issue, variance analysis showed a very highly significant effect of species on the germination rates for the used doses. Comparing averages two by two, brings into sight three homogeneous groups: Group A, consists of *Datura stramonium* with a significant germination rate of (90.93%), Group B consists of *Datura inoxia*, which has a germination rate of about 66.70% while Group C is composed of *Datura Tatula* whose germination rate is low (10.58%) (Fig. 2). For all combined species, Figure 2-B shows a rate of germination variation in different doses of irradiation. However, variance analysis shows no significant effect. The highest rate was recorded at the D5 dose (20 Gy) with 63.21%, while the lowest rate is mentioned for D5 (60 Gy) with 47.76%. In addition, no interaction effect namely
radiation doses vs. species, was observed on the germination of seeds. It thus proves that the germination rate alone is not a sufficient criterion for the selection of appropriate doses of radiation, hence the interest of studying other parameters.

1.2 Average stems’ length:

Figure 3-A shows that the largest average stem length is recorded with Datura stramonium. It is obtained with D, dose indicating a mean stem height of 8.42 cm and the lowest average length is noted for D2 dose with 4.93 cm. Variance analysis indicates a very highly significant effect of the radiation dose on medium length stems. The LSD test showed four homogeneous groups that are quite often overlapping (Fig. 3). The variation coefficient is high; however, it varies from 57 to 82%, which indicates the existence of a high variability in the response of the treated seeds. In Datura innoxia, the analysis shows four homogeneous groups with many overlapping (Fig. 3b).

The average stem’s height is achieved with a higher dose (D5) with 9.76 cm while the lowest average is observed for doses D1 and D2 respectively 5.40 cm and 5.48 cm. The radiation dose effect on the average stem length of Datura Tatula is not significant. However, the highest length is recorded for D2 dose (Fig.3-C).

Variance analysis (all doses combined) indicated a very highly significant species effect for the average stem length. Two homogeneous groups are identified using the LSD test. The first is represented by Datura stramonium and Datura innoxia, which have the shoot length quite high. The second group is composed of Datura Tatula with the shortest stems (Fig. 4-A). The dose effect (all species considered) is found very highly significant on mean shoot length. A two by two comparison of averages allows one to distinguish three homogeneous groups with single or multiple overlapping (Fig. 4 B). Variance analysis also shows the existence of an interaction effect ‘species vs. radiation dose’ on stems’ average length. The best stem lengths are obtained with doses D1, D2 and D3, combined with the two species of D. stramonium and D. innoxia (Fig. 5). This indicates a dose vs. species interaction effect on shoot length, indicating that the same dose does not necessarily have the same effect on the three Datura species.

1.3 Average root length:

Variance analysis indicated a highly significant effect of radiation dose on the average length of Datura stramonium root. The LSD test revealed two homogeneous groups (Fig. 6-A). Group A is presented by D3 and group B by the other doses. The coefficient of variation is about 71.48%, which indicates the existence of a wide variability in response of seeds irradiated. In Datura innoxia, variance analysis shows always a dose of radiation very highly significant on the roots length. Comparing two by two averages allows homogeneous groups with a coefficient of variation equal to 73.13% (Figure 12-B, Appendix 11). In the case of Datura Tatula, variance analysis indicated a significant dose effect on the average roots length. The LSD test will show two homogeneous groups with overlapping single (Fig. 6-C).

Variance analysis showed a very highly significant species effect on the average roots length. Three homogeneous groups are identified using the LSD test, the first is presented by D. stramonium which shows higher root length of the second by D. innoxia, while the third is presented by D. Tatula (Fig.7-A). Variance analysis shows another dose effect of irradiation on the average length of the root and the LSD test allows distinguishing two homogeneous groups (Fig. 7-B). Group A represented by the dose D6 with the smallest average root length and group B including all the other doses. There is also an effective interaction ‘species vs. radiation dose’ on the average root length. A multi-factorial variance analysis shows that both aforementioned factors affect significantly this criterion (root length) (Fig. 8).

1.4 Reduction / improvement in the stems and roots length:

1.4.1. Reduction / improvement in stem length:

For the Datura stramonium species and instead of a reduction in the stem length, it is observed an improvement at intermediate doses: D1, D3 and D4 unlike for extreme doses: D1, D5 and D6 which with there is a more or less significant decrease (maximum: -30.03% with D6) (Fig.9-A). In Datura innoxia, Figure 9-B shows an increase in stem length with all doses except for D2 and D6 respectively where reductions of 19.40% and 18.21% where obtained. For Datura Tatula a significant decrease in stem length was obtained with all doses (Fig. 9-C).

1.4.2 Reduction / improvement of root length:

With Datura stramonium, Figure 16-A shows a decrease in root length for all doses except for D3 for which there is an increase. The largest reduction rate is observed for D6 dose (-45.92%). In Datura innoxia, there is an increase in root length for doses D1 and D2. However, other doses showed a decrease in root length compared to the control D0. The reduction rate is achieved with the most important dose D5 (-34.49%) (Fig. 10-B). In Datura Tatula, Figure 10 C shows a significant decrease in root length with all doses of radiation. The most
important root length decrease in this species is recorded for doses D$_2$, D$_3$ and D$_6$.

2. Effect of mutagenic treatments on seed scarification process (2):

2.1. Germination:

From figure 11-A, we note that the rate of germination varies with the irradiation dose. The highest rate was recorded for dose D$_1$ at 91.83% while the lowest rate was noted at 72.55% for D$_6$. In the case of *Datura innoxia*, the highest germination rate is recorded with the control set at 72.55%, the rate decreases for other doses of irradiation to reach 24.07% for D$_6$ dose (Fig. 11-B). For *Datura Tatula*, the germination rate is very low for the six studied doses and the control (Fig. 11-C).

Variance analysis showed a very highly significant effect of species upon the irradiated seeds’ germination. The LSD test could enable the identification of three homogeneous groups: Group A with a important germination rate of *Datura stramonium* at (84.94%), Group B for *Datura innoxia*, which has a germination rate of the order of (45.05%) and Group C were the rate of germination is very low (4.32%) for *Datura Tatula* (Figure 12-A). The radiation dose has no effect on the rate of seed’s germination and variance analysis indicates no dose effect on the germination rate for all species. However, Figure 12-B, shows some variation of germination rates for different radiation doses, the rate is the highest in the control with 54.75% and decreases for other irradiation doses to reach 33.4% at dose D$_6$. As it was the case for the first process, there is no interaction effect of irradiation dose and species on seed’s germination.

2.2. Average stems length:

For *D. stramonium*, variance analysis showed no dose effect on the average stems’ length. However, figure 13-A shows that the largest average length is recorded for D$_3$ with 5.5 cm while the lowest recorded average length is that of dose D$_6$ with 2.79 cm. For *Datura innoxia*, variance analysis showed a very highly significant dose effect on the average stem length. The LSD test revealed four homogenous groups (Fig 19-B). The average stem length is obtained at high dose D$_4$ with 4.10 cm while the lowest average was recorded at doses D$_6$ with 1.28 cm. As it was the case for the first species, the variance analysis indicates no significant radiation dose effect on the stem length for *Datura Tatula* (Fig. 13-C).

Variance analysis indicates a very highly significant species effect for the parameter mean stems length. The LSD test identified three homogeneous groups. The first group consist of *Datura stramonium* and *Datura innoxia*. The third group by *Datura Tatula* (Fig. 14-A). The dose effect on the average stems length is very significant, and the multiple range tests identify three homogeneous groups often overlapping (Fig. 14-B). The best stem lengths are obtained at D$_0$ and D$_1$ doses. The ANOVA also shows the existence of an interaction effect species x dose radiation on the average stems length, the LSD test can show four homogeneous groups often overlapping (Fig. 15).

2.3. Average roots length:

Variance analysis for *Datura stramonium* and *Datura Tatula*, shows no significant effect of radiation dose on the average root length. However, Figures 16 and 16-A-C exhibit for dose D$_1$ a certain improvement in the root length compared to the control. For *Datura innoxia* variance analysis showed a significant effect of radiation dose on the same parameter. The two by two comparisons of averages allows one to identify three homogeneous groups (Fig. 16-B). D$_1$ dose exhibits the highest root length average, while the lowest average dose is obtained with D$_6$.

The irradiation effect is thus found to be highly significant regarding the average root length parameter. Three homogeneous groups were identified by means of the LSD test. The first is depicted by *Datura stramonium*, the second one by *Datura innoxia* while the third one is represented by *Datura Tatula* (Figure 23-A). However, variance analysis showed no dose effect (Fig. 17-B). Moreover, no interaction of species vs. dose effect on the average length of roots.

2.4. Reduction / improvement in the length of stems and roots:

2.4.1 Reduction / improvement in stem length:

In *Datura stramonium*, the results show an improvement (at doses D$_4$ and D$_5$), and a reduction of stem length (for the remaining doses) (Fig. 18-A). D$_6$ dose exhibits the highest reduction rate reaching -30.44%. In *Datura innoxia*, all irradiation doses showed a reduction of stem length except for D$_1$, D$_2$ doses for which a slight increase is obtained. The largest observed reduction rate is that for D$_6$ dose reaching -42.87% (Fig.18-B). A decrease in stem length for *Datura Tatula* is observed at doses D$_1$, D$_2$, while stem length increased at the remaining doses: D$_3$, D$_4$, D$_5$, and D$_6$ (Fig 18-C).

2.4.2 Reduction rate / improvement of root length:

In *Datura stramonium*, we noted a reduction in the roots’ length at all doses except for D$_1$ and D$_3$ for which an improvement is noted. D$_6$ dose exhibits
the highest reduction rate: -22.42% (Fig. 19-A). In *Datura innoxia*, Figure 25-B shows a root length reduction at all doses except for D2 where there is an increase. The most important reduction rate in root length (~50.49%) was noted for D2. For *Datura Tatula*, increased root length was noted at all doses except for D2 that induces a reduction in root length equal to -93.23% (Fig. 19-C).

**Study of tropane alkaloids:**

Analysis of alkaloids was performed by means of GC on both irradiated and control seeds sets of the selected variety *Datura innoxia*. The results show that the largest amount for atropine and hyoscyamine is observed for D2 (60 Gy) with respectively 1.34 and 1.19g/g dry matter compared to the control set whose quantities are around 0.64 and 0.73 g/g dry matter respectively. (Fig. 20)

**Discussion:**

1. **Seed germination:**

Seed germination is observed in all three species and for the 7 irradiation doses (0, 5, 10, 20, 40, 60 and 80 Gy) that were used and in both processes. *D. stramonium* germination rate is the most important in both processes followed by *D. innoxia*, and finally *D. Tatula* with a very low percentage of germination. According to Benslimane and Khelifi [8] *D. Tatula* exhibits *in vivo* germination rate ranging from low to nil. This is indeed in agreement with our results. In effect for *D. Tatula* the germination rate is very low with both processes and even in the control sets. This poor germination may be attributed to the seeds heterogeneity since they are of the same origin. Nevertheless, no significant differences were observed between the germination rate from one dose to another and for all species together. Our results show an increase and a percentage of reduction in germination compared to the control. These results corroborate those of Thapa [26] on Pinus spp. According to Melki and Dahmani [17] low doses cause an increase in the rate of germination. Germination stimulation can be attributed to the elimination through irradiation of some pests that affect seed germination [10]. On the other hand, the decrease in germination by high doses can be attributed to the inhibition of mitotic divisions [5].

In the first method, germination rate increases with an increasing radiation dose, and this is the case up to 60 Gy for *D. stramonium* and up to 40 Gy for *D. innoxia*. Those were the limits of doses beyond which seed’s germination begins to decline as compared to the control. These results are in accordance with those obtained by Abdel Hady [2] who studied the effect of both gamma rays and gibberellin on seeds’ germination of *Atropa belladonna*. The author showed that the germination rate starts to decrease beyond the 100 Gy dose limit. For the second process, the number of seeds germinated progressively decreases with an increasing irradiation dose for both *D. innoxia* and *D. stramonium*, except for D2 dose which rather shows an increase. This phenomenon is not new as it has been reported by many authors, on okra, peanuts and corn [18], on tomato [6], on onion [5] and on bean [15]. The two processes showed different behaviors for the irradiated seeds. The observed decrease in germination with the second method and the three studied species was due to the scarification of seeds before irradiation. With this second method, the effect of gamma rays is more pronounced and has a greater penetrating power. The removal of seeds’ coat is in fact found to be detrimental to germination. This is due to the high rate of O2 in the seeds after skin removal. We must not forget to remind that the seed coat is an impermeable barrier to gases’ diffusion [8].

According to Conger et al. [12], the effect of gamma irradiation increases when the O2 rate is high during and after radio-mutagenesis. This is confirmed in several species at different radiosensitivity (barley, rice, cucumber, fescue, lettuce, alfalfa, radish and onion).

2. **Average stems and roots length:**

The effect of irradiation dose on the stems and roots length average is significant. However, this parameter varies depending on the studied species (*Datura stramonium*, *Datura innoxia* and *Datura Tatula*). For the same species it varies depending on the dose of irradiation. This is clearly indicated by the number of homogeneous groups that were observed. An increase in stems’ length and roots’ length is found for relatively low doses and conversely a decrease is noted at high doses. According to Seung et al. [24] plants that are exposed to low doses of gamma rays develop normally or may show a growth’s stimulation, whereas exposure of these plants to high dose (50 Gy) inhibits their growth. These findings are also consistent with ours.

The growth stimulation by low doses of gamma radiation is the hormesis. This is reported and confirmed by several authors [25,17]. Plant cells that are exposed to low doses increase in volume. A parallel increase in the DNA and proteins amount [22] that could be due to synthesis activation of growth hormones is also noted [10]. Moreover, the reduced growth by increasing the irradiation dosage is attributed to the mitotic inhibition and to chromosomal damage and induction of physiological changes such as a decrease in the amount of
endogenous growth regulators: auxin and cytokinin caused by the high irradiation doses [19,22]. Stems and roots lengths in the first process are lower than those obtained in the second process for the three species. This is mainly due to the seed coat which diminishes the irradiation effect in the first method. Our results show that the seeds are more radiosensitive when irradiation is performed after scarification (method 2). This is true for low radiation doses. At high doses, the germination percentage is notably affected. The significance of the variation coefficient for stems and roots length in the different species indicates a considerable variability. This means as reported by Bilquez [10] that the genetic constitution affects radiosensitivity. At the genotype level, specific mechanisms are involved in the damaging and the repair of the gamma radiation effects on the body.

3. Reduction / improvement rate:

The radiation dose effect on the rate of reduction / improvement of stem lengths is not significant regardless of the species or of the monitoring process. However, the results we obtained indicated that at low doses, an improvement rate has been reported for *Datura stramonium* and *Datura innoxia*. This improved growth of stems and roots may be due to the stimulation of cellular division or cellular elongation [6,21]. However, at higher doses a reduction rate is recorded. This decrease is due to the alteration of metabolic processes resulting from the nucleic acids disruption that lead to the disruption of the hormonal system. The phenomenon has been reported for other species. It can be explained by the mitotic cells reduction in the apical meristem [23,1]. Similar findings were made for the effect of radiation dose on the rate of reduction / improvement in the roots’ length. However, these effects are more significant for the reduction / improvement of the aerial part at the root system level. The highest reduction rates are observed depending on the species, at high doses greater than 40 Gy. Fluctuations recorded for the stems and the roots improvement / reduction rates with increasing doses have been reported by other authors [6]. The stimulation effect is not found to follow a regular pattern.

4. Species effect:

For *D. stramonium* our results show that the most important stem and root growth reduction rate is obtained for dose D₆ for both processes. In *D. innoxia*, D₄ and D₅ are the doses that induce the most important reduction rates compared to the control for the first process whereas it is the D₄ and D₅ in the second process. Finally, for *D. Tatula* completely different results are observed as the germination rate is very low even for the control.

5. Useful Doses Determination:

To determine the useful doses, two criteria are taken into account: the LD 50 and the stems and roots length reduction rate. According to Alikamangola [3]; Abdul et al. [1], the selected doses are those that induce a survival percentage of about 50% of the total sown seeds. This LD 50 corresponds to the dose that kills 50% of seeds (inhibited germination). In our study, *D. innoxia* has a germination rate of 53.38% and 49.12% respectively at doses 40 Gy and 60 Gy in the first process, and 47.14% at a dose of 60 Gy in the second process. Considering the obtained results, *Datura innoxia* is a species that is sensitive to radiation doses and its useful range is between 40 and 60 Gy. In the future, trials should be performed at a dose of 50 Gy for this species. For the *D. stramonium* seed germination exceeds 50% whatever the dose. Therefore, it would be useful to consider increased irradiation doses that would enable one to reach the LD 50. *D. Tatula* germination rates are very low even among the control. No reliable conclusion can thus be drawn. One needs first to be sure of the real germination of the seed sets before going forward with mutagenesis.

There exists another criterion for choosing the appropriate dose based on the choice of doses causing 30-50% reduction in stem and root lengths compared to the control. The reduction of these two lengths with increasing doses of irradiation has been observed in several species: peanuts [9], okra, peanuts and corn [18]. In our case, *Datura stramonium* first process indicates 30.03% and 46.76% reduction rate on stem and root lengths respectively for the 80 Gy dose. In the second process, the stems reduction rate is 30.44% at 80 Gy. This shows that *D. stramonium* seeds are radiosensitive at 80 Gy. The latter could be adopted as a useful reference dose before testing higher doses. *D. innoxia* has a rate of 33.52% root length reduction at a dose 60 Gy with the first process. In the second process, this species exhibits a rate of 35.98% reduction and 42.87% at 40 Gy and 80 Gy respectively. The observed roots’ reduction rates are 31.66%, 33.82% and 50.49% for 20 Gy, 40 Gy and 80 Gy doses respectively. Then the useful dose would therefore lie between 20 and 80 Gy. The 80 Gy leads to a high mortality rate, it cannot thus be accepted. The dose of 40 Gy would be useful for this species. Considering the results obtained for *D. Tatula* one can state that no reliable conclusion can yet be drawn.
Fig. 1: Variation of seeds germination of three Daturas’ species depending on the dose of radiation.

Fig. 2: Change in rate of germination depending on the species (A: all doses combined) and irradiation's dose (B: all species combined).

Fig. 3: Change in average length of the stem of three species according to the dose of irradiation.

Fig. 4: Change of the stem length depending on the species (A: all doses combined) and on the dose of irradiation (B: all species combined).
Fig. 5: Variation of the average stem length depending on the species and the dose of radiation.

A: *Datura stramonium*

B: *Datura innoxia*

C: *Datura tatula*

Fig. 6: Variation of the average roots length of three species according to the dose of irradiation.

Fig. 7: Variation of the root length depending on species (A: all doses combined) and the dose of irradiation (B: all species combined).

Fig. 8: Variation in the average root length depending on species and the dose of radiation.
Fig. 9: Variation of the reduction and/or improvement of three species stem length of Datura in relation to the dose of radiation.

Fig. 10: Variation of the reduction or enhancement of the root length of three species of Datura in relation to the dose of radiation

Fig. 11: Variation of seeds germination of Datura species depending on the dose of irradiation.
Fig. 12: Variation of germination depending on the species (A: all doses combined) and the dose of irradiation (B: all species).

A: Datura stramonium  B: Datura inoxia  C: Datura tatula

Fig. 13: Variation of stem average length of the three species of Datura in relation to the irradiation's dose.

Fig. 14: Variation of stem length depending on the species (A: all doses combined) and the dose of irradiation (B: all species combined).

Fig. 15: Variation of the average stems length depending on the species and the dose of irradiation

Fig. 16: Change in average root length of the three species according to the radiation dose

A : *Datura stramonium*
F=0.60; df(6;343); P=0.728; NS

B : *Datura innoxia*
F=2.45; df(6;343); P=0.0249; *

C : *Datura tatula*
F=0.74; df(6;343); P=0.6160; NS

Fig. 17: Change in root length depending on species (A: all doses combined) and the dose of irradiation (B: all species combined).

A : *Datura stramonium*

B : *Datura innoxia*

C : *Datura tatula*

Fig. 18: Variation of the reduction or improvement of the stem length species of Datura with respect to irradiation dose

A : *Datura stramonium*

B : *Datura innoxia*

C : *Datura tatula*

Fig. 19: Reduction or enhancement variation in root length of species of Datura as a function of the irradiation dose.
Fig. 20: Tropane alkaloid content in the control and in the irradiated seeds of *Datura innoxia*

6 - Study of tropane alkaloids:

For the sake of better characterizing *D. innoxia* species alkaloids and with regard to its positive radiosensitivity response we performed an analysis of major alkaloids (atropine and scopolamine) by means of GC on both irradiated and control seeds. Our results showed that the rate of scopolamine in the control seeds is 0.73 mg/g of DM, which is in agreement with the results obtained by Houmani and Cosson, 2000 for *D. innoxia* (0.76± 0.20 mg/g of DM). The doses proposed by Gogebashvili et al. [13] (i.e. 20, 40, 60 and 80 Gy) on the species *D. stramonium* correspond to the most important alkaloids’ contents as compared to the control with the exception of dose 40 Gy for atropine (0.67 mg/g of DM ). In the case of *D. innoxia* species and referring to the joint IAEA-FAO database which provides information on worldwide radiomutagenesis investigations, no such work has been recorded to date. This gives hence more importance and interest to our results for *D. innoxia* mutation induction research.

Conclusion:

From the results obtained for the studied parameters namely the rate of germination and stems and roots lengths reduction it has been concluded that: when irradiation is applied before seed’s scarification, germination rate decreases for *D. innoxia* at doses 40 Gy and 60 Gy and reaches LD 50, whereas for *D. stramonium* it decreases only at 60 Gy without reaching the LD 50. When irradiation of seeds is carried out after scarification germination rate decreases sharply for all doses in *D. innoxia* and LD 50 is attained at 60 Gy, while for *D. stramonium* a slight decrease in germination rate was found for all doses except for 20 Gy and no LD 50 is reached. *D. Tatula* has a very low germination rate in both processes including the control. The results obtained for this case cannot be validated because of low germination percentages. The degree of sensitivity is different for the same dose and the same species from one process to another. The seeds are more radiosensitive when scarified and then irradiated because the penetration and the effect of gamma rays is more important. There is also a significant difference in sensitivity from a species to another. *D. stramonium* becomes radiosensitive only at 80 Gy while *Datura innoxia* has a radiosensitivity at doses beyond 20 Gy. *Datura Tatula* shows a high radiosensitivity starting from 10 Gy. The selection of the useful doses is based on the stem and root lengths reduction rate in the range of 30% to 50%. The study of in vitro seeds’ radiosensitivity of Datura genus, was used to study the effects of different doses of cobalt-60 gamma rays on germination and growth of stems and roots of three species. It allowed us to determine the useful doses: 40 Gy for *Datura innoxia*, at least 80 Gy for *Datura stramonium*. However, no useful dose has been identified yet for *Datura Tatula*. In addition, post-scarification seeds irradiation seems more interesting for future use on Datura genus *Datura innoxia* and especially in the dose range 40-80 Gy, which mainly generates the most important alkaloid contents (atropine and scopolamine). As a prospect for this investigation, it would be useful to confirm in the field (in vivo) the in vitro results obtained for *Datura innoxia*, by inducing mutations then following-up with a survey over the subsequent generations to select the tropane richest mutant lines.

References:


