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Dosimetry and radio-stimulation in mesquite (*Neltuma laevigata* W.) seeds

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ABSTRACT

Objective: To carry out research focused on the germination response of mesquite (*Neltuma laevigata*) to different doses of gamma radiation (Cobalt 60), in order to obtain a higher germination response than with a non-irradiated seed.

Design/Methodology/Approach: Seeds had different collection times and identities. One set was collected in Durango (10 years) and another in Hidalgo (2 months). Both sets were exposed to sixteen different doses of gamma radiation and a control (non-irradiated); they were subsequently subjected to *in vitro* conditions using a Murashige and Skoog basal medium. They were monitored daily for two weeks in order to develop an accurate record of their germination.

Results: The best treatment for the radio-stimulation of germination in the Durango set was observed at 30 gray (12% higher than the control). Meanwhile, the Hidalgo set received 6 gray radiation (56% higher than the control).

Study Limitations/Implications: Only two different populations were evaluated for this study. Given the differences found between them, working with material from other origins would be ideal.

Findings/Conclusions: Low doses of gamma radiation cause an increase in the germination rate of seeds.

Keywords: Gamma radiation, germination, hormesis, *Neltuma laevigata*.

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INTRODUCTION

Mesquite (*Neltuma laevigata*) (Fabaceae) plays an outstanding ecological role and, according to Rodriguez *et al.* (2014); it is an excellent soil fixer, improves soil fertility, controls erosion, and prevents desertification. It is considered a valuable resource, because all its parts are put to a good use. In the Mezquital Valley (State of Hidalgo, Mexico), it is mainly used for firewood, although it is also used as a source of honey, flour, and cattle

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feed. The term *Prosopis laevigata* was proposed by Bentham (1842, 1875) and confirmed by Burkart in his 1976 monograph, but Catalano *et al.* (2008) recently confirmed that molecular phylogenies show that *Prosopis* is a polyphyletic genus and proposed the scientific name *Neltuma laevigata*. According to Gómez *et al.* (1970), mesquite trees are distributed mainly in Sonora, Chihuahua, Coahuila, Nuevo León, Durango, Zacatecas, Guanajuato, and Querétaro. The seeds of this species retain their viability for up to ten years after their collection (with the endocarp) and three years (without the endocarp). Their germination rate without the endocarp was of 80 to 90%. *In vitro* seed cultures provide nutrients to the seed that would be very difficult to obtain under normal conditions (Fay, 1992; Pierik, 1993). In this regard, hormesis is the result of the low-dose stimulation and high-dose inhibition of a physical or chemical agent. High doses result in a beneficial adaptability in the cell (Calabrese & Baldwin, 2007). According to Guerrero *et al.* (2019); radiation, heat, heavy metals, and antibiotics are the most relevant hormetic agents. Gamma radiation has been used to eliminate insects from the grains of *Coffea arabica* L. and to improve *Abies religiosa* with ≤300 Gy doses. There does not seem to be any documented antecedents of seeds of *Neltuma laevigata* treated with gamma radiation to improve their germination; therefore, the objective of this study was to evaluate the germination response of *Neltuma laevigata* seeds to different radiation doses, in order to determine the effect of radio-stimulation.

MATERIALS AND METHODS

After the seeds of *Neltuma laevigata* were collected (Table 1), the endocarp was removed with tweezers. They were then irradiated with Cobalt 60 (Co^{60}) using the Gammacell 220 irradiator (Figure 1) at the Instituto Nacional de Investigaciones Nucleares (ININ). The dose ratio was 18.360642 Gy/h (Table 2). The different irradiation doses were: 0, 2, 4, 6, 8, 10, 12, 15, 30, 50, 100, 150, 200, 250, 300, and 400 Gray (Gy); using 25 seeds per treatment.

Table 1. Records of *Neltuma laevigata* seeds.

Provenance	Municipality	Year of collection	Coordinates
Durango	Valle del Guadiana	2012	23.9908979° N, 104.5236664° N
Hidalgo	Ixmiquilpan	2022	20.41543° N, 99.21716° N

Table 2. Dose ratio (Gy) per treatment applied to the seeds.

Dose (Gy)	Time (min)	Dose (Gy)	Time (min)
2	6.5	50	163.2
4	13.1	100	326.5
6	19.6	150	489.7
8	26.1	200	652.9
10	32.6	250	816.9
12	39.2	300	979.4
15	49.0	400	1307.1
30	97.9		

In order to evaluate the germination percentage of the seeds at different doses, an *in vitro* seeding was carried out at the Biotechnology laboratory of CENID-COMEF of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). A total of 5.1 L of MS medium (Murashige and Skoog, 1962) was prepared. Each liter contained 4.43 g of medium, 30 g of sucrose, and 8 g of agar, all with a 5.7 pH. In each (previously sterilized) test tube, 6 mL of the prepared medium were added. During the disinfection process, the seeds were washed and rinsed (with soap and water) four times; they were then immersed in 70% alcohol for 5 min; rinsed with sterile water, and left in 30% commercial chlorine for 20 min; and, finally, rinsed three times with sterile water in a laminar flow hood (Figure 1c). Petri dishes with (previously sterilized) paper were placed under the hood to remove excess water and ultimately to reduce the likelihood of contamination (Figure 1d). One seed was sown per test tube with medium and sealed hermetically with plastic wrap. In order to have a better control over each experimental unit, the tubes were labeled with the seed number and its corresponding treatment. To finalize this procedure, the seeds were kept in a conservation room at 35 °C ± with white light 24/7 (Figure 1f).

The seeds from two different origins were subjected to different doses of radiation. They were evaluated during a 2-week period (starting from their sowing), in order to identify their germination. Under the conditions of the study and following Agresti *et al.* (2015), the following statistical model was used for the analysis:

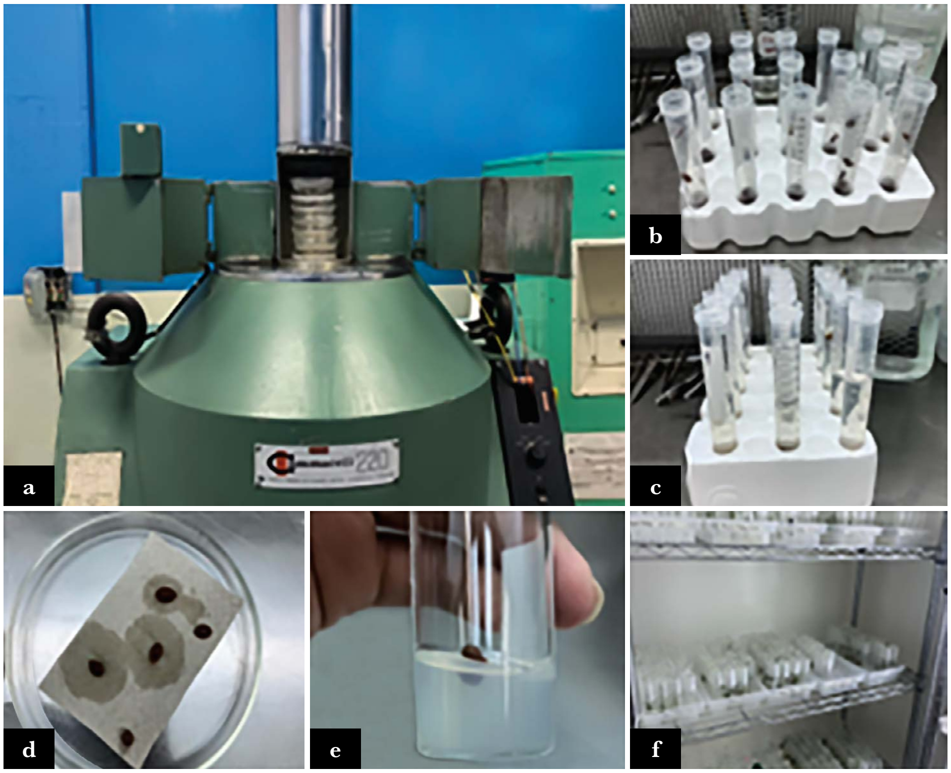

Figure 1. Radiation and *in vitro* seed establishment process. a: Gammacell 220 irradiator; b: washing with soap and water; c: rinsing with chlorine; d: seed drying; e: seed sowing; f: storage of sets.

$$\log\left(\frac{\pi_{ij}}{1-\pi_{ij}}\right)=\mu+P_i+\tau_i+P\tau_{ij}$$
$$Y_{ijk}\sim Binomial\left(25,\pi_{ij}\right)$$

Where: $Y_{ijk}=1$: germinated; $Y_{ijk}=0$ non-germinated; k=seed number (1, 2, 3...25); i=provenance (1 and 2); j=treatment (1, 2, 3...16).

The whole statistical analysis was carried out with PROC GLIMMIX (generalized linear mixed model) in the Statistical Analysis System statistical software (SAS 15.1).

RESULTS AND DISCUSSION

No significant differences were identified at the treatment level; however, there were significant differences in the provenance-locality interaction, considering an alpha of 0.57 (Table 3). The treatments responded differently in each of the localities evaluated (Durango and Hidalgo).

Comparison of seed germination with the different localities

According to the model used, the treatment with the best germination response for the seeds from Durango was 30 Gy, while the least effective treatments were 6 and 150 Gy. For the set from Hidalgo, the best treatment was 6 Gy and the least effective was the control (Figure 2).

Germination response in seeds from Durango

In total, 317 seeds from Durango germinated. The treatment with the greatest hormetic effect for this set was reported at 30 Gy, with a 96% germination rate, while the control treatment (0 Gy) recorded a 48% germination. Starting from the control treatment, the second-best treatments were 0 and 2 Gy with 82% germination, while 50, 250, 300, and 400 Gy only recorded a 72% germination (Figure 3). The treatments with the lowest germination rate were 6, 10, and 150 Gy, with a 36.3% average germination rate.

Germination response in seeds from Hidalgo

In total, 117 seeds from Hidalgo germinated. The treatment with the greatest hormetic effect for this set was 6 Gy, with a 64% germination rate. Compared with the 8% germination

Table 3. Type III fixed effects test.

Effect	DF N	DF D	Value F	Pr>F
Origin	1	768	81.65	<.0001
Treatment	15	768	1.65	0.0570
Provenance * Treatment	15	768	4.39	<.0001

DF N: degrees of freedom of the numerator.
DF D: degrees of freedom of denominator.

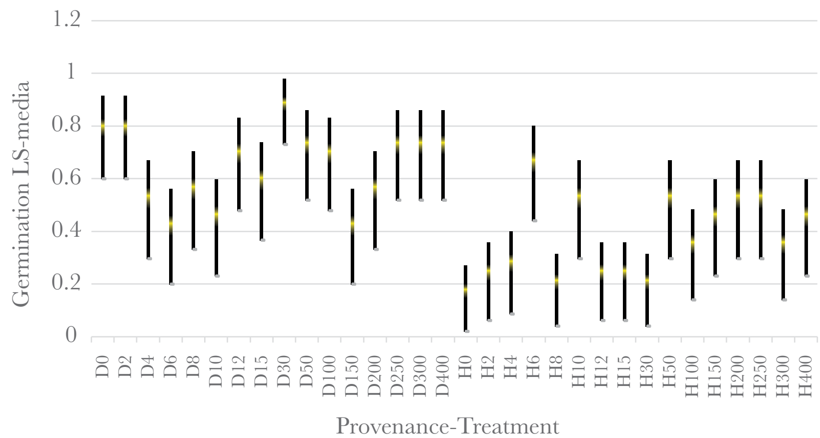

Figure 2. Comparison of germination response. Behavior of the different treatments and different origins (Durango and Hidalgo), where D represents the seeds from Durango and H represents the seeds from Hidalgo.

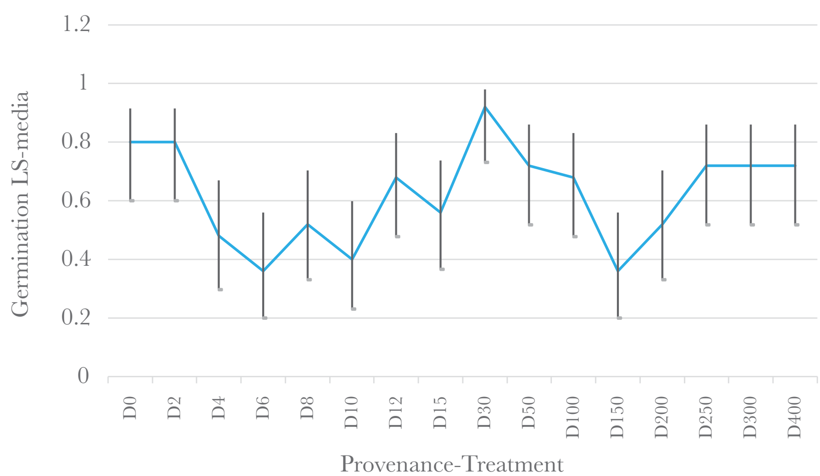

Figure 3. Comparison of germination response in seeds from Durango.

reported for the control treatment (0 Gy), the second-best treatments were 10, 200, and 250 Gy (48% average germination rate) and the third-best treatment was 400 Gy (39% germination) (Figure 4). The treatments with the lowest germination rate were 8 and 30 Gy, which reported a similar behaviour, with a 12% germination.

Some of the treatments behave similarly, which helps to decide which dose to use on the seeds, since a higher the dose increases the time required in the irradiator and consequently the economic cost (Figure 5).

The different treatments react differently in each locality

Regarding radio-stimulation, the treatment with a 30-Gy dose was evidently the most effective in the Durango set (germination percentage: 96%). These results match the findings of different studies, including: Chaomei and Yanlin (1993), who found that high doses of gamma radiation decrease the probability of germination; Salomón Díaz *et al.* (2017), who discovered that 20 Gy was the best dose to stimulate germination in potato

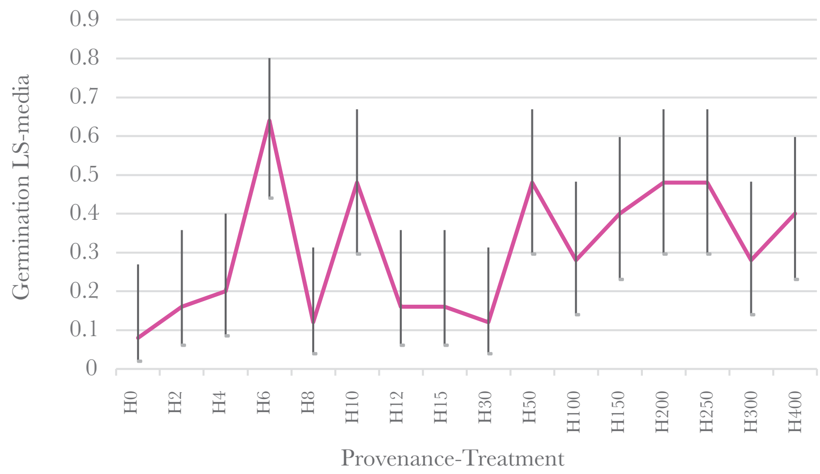

Figure 4. Comparison of germination response in seeds from Hidalgo with the different treatments.

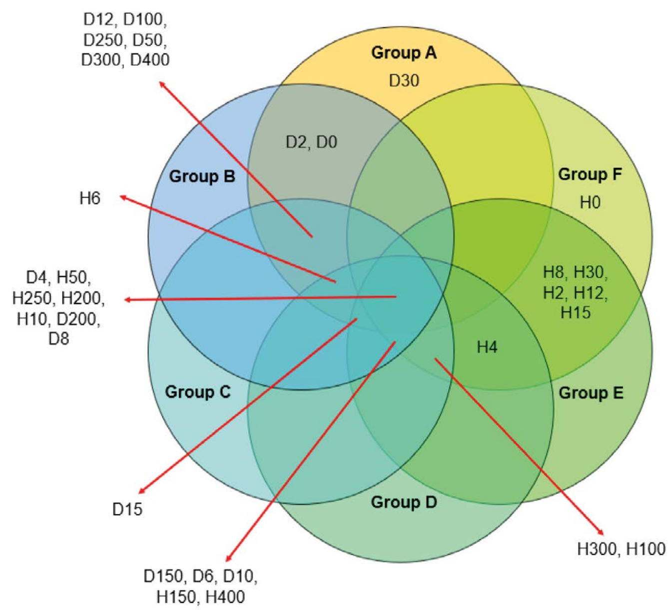

Figure 5. Grouping diagram. Treatments from different localities have similar behavior (D: Durango; H: Hidalgo).

(*Solanum tuberosum* L.); Melki *et al.* (2010) who reported that 20 Gy is the ideal dose to stimulate germination in durum wheat (*Triticum durum*) seeds; Gutierrez *et al.* (2021), who found evidence of germination in eucalyptus (*Eucalyptus nitens*) with lower doses of gamma rays; and finally Ferreira *et al.* (1980), who reported positive effects on the germination of black pine (*Pinus nigra*) seeds with a 10-Gy dose. In another study, Beyaz *et al.* (s/f) reported that, under *in vitro* conditions, 150 Gy is a dose with good germination response in Araguaney (*Lathyrus chrysanthus*) seeds, but that it decreased significantly with higher doses. Avendaño *et al.* (2021) reported that, under field conditions, coffee (*Coffea arabica* L.) seeds recorded a lower germination response with doses of 10 and 50 Gy than their control and that, at a dose of 300 Gy, seed germination was almost completely disabled. Ramirez *et al.* (2006) mentioned that radiation is a precursor of seed germination. Low radiation doses are a precursor of germination in seeds, since, as suggested by Akshatha and Chandrashekar, (2014), they activate metabolic processes. This would explain the effect of germination stimulation. Another possible cause is the composition of the seed coat. García *et al.* (2022) reported that the mesquite seed has a four-layered coat made of cuticle, epidermis, hypodermis, and parenchyma. Together, these four layers have an approximately 300-μ thickness.

CONCLUSIONS

Low doses of gamma irradiation induce radio-stimulation, increasing the germination rate in *Neltuma laevigata* with different responses, depending on their origin (6 Gy for Hidalgo, and 30 Gy for Durango), increasing germination in seeds from Durango and from Hidalgo by 12% and 56%, respectively, compared with the control treatment of each origin.

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