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Comparison of seed Germination Characteristics of *Albizia julibrissin* in Treated seeds with Gamma Rays and Control

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ABSTRACT

Aims: *Albizia julibrissin* is one of the ecologically valuable tree species in the Hyrcanian forest in the North of Iran that is endangered due to Fusarium wilt disease. Seeds of these trees have low germination because of their hard coat and dormancy. One of the most effective methods to improve the germination characteristics is seed irradiation during the pre-sowing processes. The present study was conducted to improve seed germination characteristics.

Materials & Methods: The experiment was arranged by a completely randomized design in four replications, five treatments, and one control. Seeds were radiated with 60 cobalt sources emitting gamma rays at the rate of 0.013 Gray/sec and different doses of gamma rays: 15, 25, 50, 100, and 150 Gray (Gy). All of the seeds were sowing in the moist stratification at 25°C.

Findings: Seed germination started simultaneously 28 days after sowing. Results showed a significant difference in germination characteristics between treated seeds and control ($p<0.05$). The highest value of seed germination was observed in the 15 Gy (86%), the highest seed vigor index was in 15 Gy treated seeds (266.6), the highest germination speed index was in 15 and 150 Gy (12.17 and 12.32 seed number day^{-1} respectively) and the highest mean daily germination were in 15, 50, 100 Gy (7.46, 7.32 and 7.34 number/day respectively).

Conclusion: Increasing seed germination characteristics was observed in seeds radiated by a low dose of gamma radiation. Hence, a lower dose (15 Gy) of irradiation treatment can be used to improve the *Albizia julibrissin* seed germination characteristics.

Keywords: Gamma irradiation, Seed germination, Mean Daily Germination, Seed Vigor Index, Seed, Forest.

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Introduction

Albizia julibrissin Durazz. (Persian silk tree - Family- Mimosaceae) known as a native tree to southwestern and eastern Asia [1], commonly used as an ornamental tree in the parks, gardens, and landscapes because of its appealing fragrance, showy flowers, and low maintenance requirement. It has escaped from the urban landscape and competes with native plants in disturbed habitats and occasionally in forested areas. *Albizia julibrissin* is a valuable tree species in the forest, which is endangered due to Fusarium disease. Fusarium wilt is a common disease of mimosa (*Albizia julibrissin*) caused by the fungus *Fusarium oxysporum* forma specialis *perniciosum*. So, *A. julibrissin* is one of the endangered tree species in Hyrcanian forests.

The seeds of *A. julibrissin* have physiological and physical dormancy. Seed dormancy is mainly due to the seed hard coat. Accordingly, seeds have low germination vigor. Seed germination of *A. julibrissin* should enhance by pre-sowing treatments [2]. Various methods were used to break the seed dormancy and stimulate their germination, including stratification, scarification, mechanical and chemical scratching, and placing the seeds in hot water [3].

One of the most effective treatments in seed germination is gamma irradiation. Gamma rays influence plant growth and development [4]. This ray is the most energetic form of electromagnetic radiation that has more penetrating than other types of radiation such as Alpha and Beta rays [5]. Gamma radiation has essential biological effects on human society through applications in medicine, agriculture, pharmaceutical, and other technological processes [6]. Gamma irradiation, one of the physical treatments, can be helpful for the alteration of its physiological characters and improving germination and seedling growth. Gamma irradiation is one of the standard practices to induce genetic variation in many plant and tree species [7,4] via advantageous mutations, which help introduce new, improved variants compared to the control plant [8]. The study of radiation effects on plants is a very complex and extended field [9,10]. Many studies have shown that physiological and biochemical processes in plants are significantly affected by gamma-irradiation stress [11,12,13,14]. The required energy for initial growth is already available inside seeds; gamma irradiation may increase the stimulation rate of cell division along with vegetation growth [15,16]. Earlier experiments have shown that gamma radiation could cause genetic effects, morphological modifications, and other effects on plants [17]. These effects depend on the intensity of radiation [11]. Utilization of lower gamma radiation doses has a positive effect on seed and increases the rate of germination [18, 19, 20] and mutagenic effects on living organisms [21], but germination has reduced at high doses of gamma radiation [6].

Although no specific explanation for the effects of low dose gamma radiation is available until now, following the results obtained by Wi et al. [16], there is a hypothesis that low dose radiation will induce growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti-oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth conditions [16].

Some studies confirmed the effects of gamma rays on seed germination and noted that the higher exposures were inhibitory. In contrast, lower exposures were sometimes stimulatory the germination, growth, vigor index, and enhancement of plant metabolisms such as proline and phenolics content in *Terminalia arjuna* and *Ptrocarpus santalinus* [22,10]. Treatment of *Triticum aestivum* seeds with a low dosage of gamma radiation increased the speed and capacity of germination and development of the systems of the cultivated plants [19].

A. julibrissin is one of the critically endangered tree species in the temperate forests of Iran. Seed germination of *A. julibrissin* because of seed hard coat and dormancy is very low [2]. Missanjo et al. [23] have shown that seed size larger than 0.8 cm is better than other seed sizes for generation in *Albizia lebbeck* [23]. Others showed that germination in Albizia species without treatment is very poor [24]. Treatments such as hormone-priming increased the activities of glyoxylatecycle enzymes [25] and the other treatments like soaking the seed in cold or hot water [5], mechanical scarification like puncturing of the seed coat [26], filing and rubbing of the seed coat, hot water scarification [27] and acid scarification [24] have been recommended to improve the seed germination characteristics. Different responses have been obtained to different treatments for breaking *A. julibrissin* seed dormancy, but so far, no research has been done about the effect of gamma radiation as a solution to breaking the seed dormancy in this tree.

For this purpose and to complete the study of other researchers, the present study was conducted.

Despite the usefulness of ionizing radiation to increase the germination potential in crops, there are not many references in the literature on nuclear techniques about forest tree species. In this study, we examined the effects of gamma ray on seed germination characteristics of *A. julibrissin*. The specific aim of this study is the presentation of one approach to increase seed germination of *A. julibrissin.* Considering this aim, we hypothesized that: lower doses of gamma increase the seed germination than higher doses.

Materials and Methods

Material

The experiment was conducted at the Caspian Forest Seed Center in the North of Iran. Full ripen seeds were harvested from trees in Mazandaran province in the North of Iran (36°,38' N latitudes and 52°,16' E longitudes). Standard size seeds (not the biggest one or the smallest) were selected for seed extraction purposes. The selected seeds were dried to 14.3% water moisture content by vacuum drying in the seed laboratory.

Gamma irradiation

A total of 720 seeds were divided into six sub-samples (Five treatments and one control). One hundred twenty seeds were planted in each treatment and control with a randomly experimental design. Some seeds were radiated by γ–rays (15, 25, 50, 100, and 150 Gray (Gy) and other seeds non- radiated as control that only hot water as a routine treatment used for it. It was not any requirement for pre-treatment before gamma application. The time of irradiation was late February. The seeds were irradiated from the 60 Cobalt source at a dose rate of 0.013 $Gy.sec^{-1}$ in the Agricultural, Medical, and Industrial Research, Nuclear Science and Technology Research Institute, Karaj, Iran. Dose selection was based on previous studies such as Akshatha [28], Moussa [4], Melki, and Dahmani [29]. After irradiation, all of the seeds were sown in sandy Petri dishes at a room temperature of 25°C and humidity of 60-70% (Figure 1).

Effect of gamma irradiation on seed germination characteristics:

Germination percentage

The germinated seeds were evaluated initially

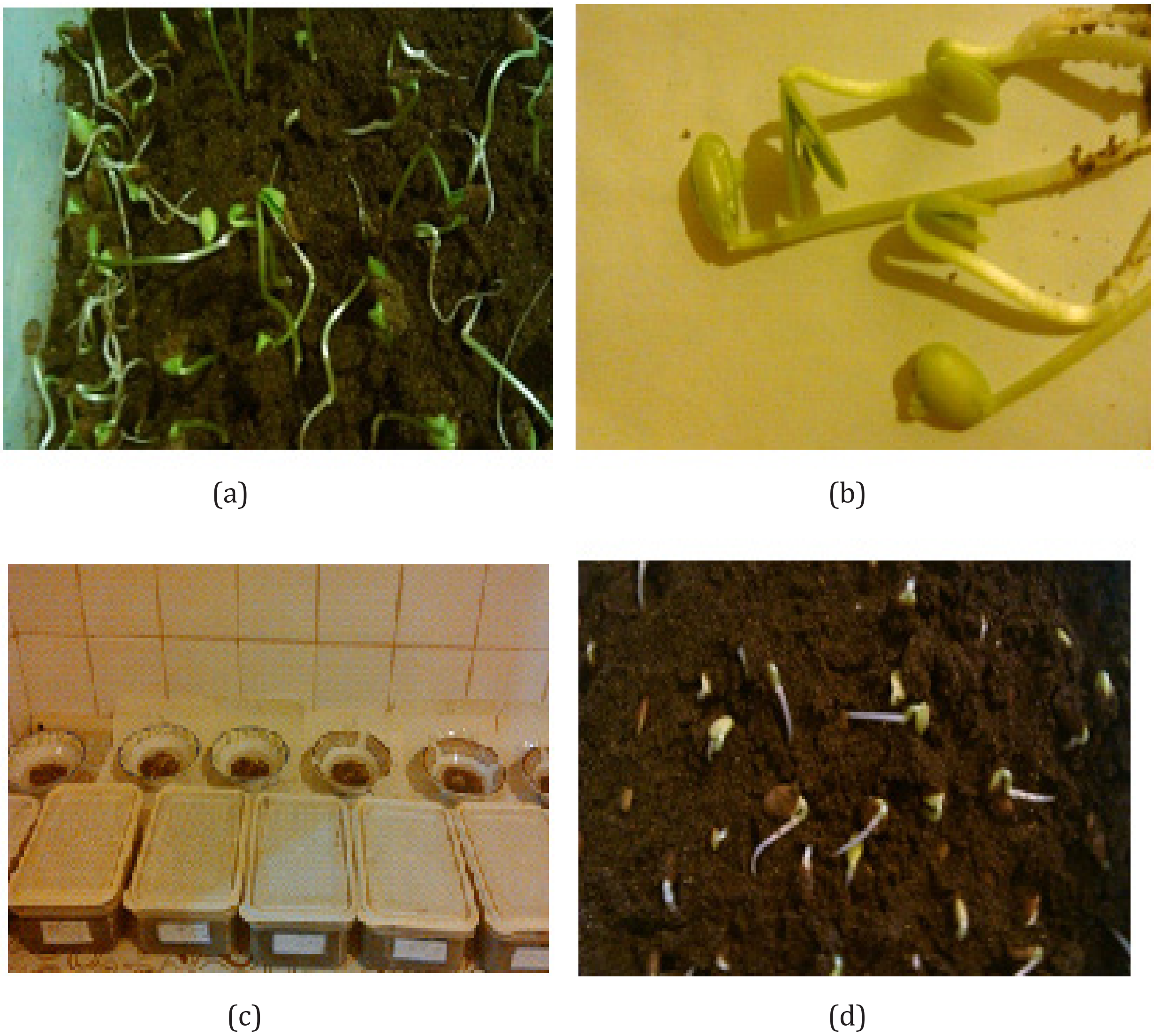

Figure 1) Seed germination stages of *Albizia julibrissin:* Sowing seed after irradiation (a) Starting germination (b), Emit germinated seed (c) and Completion germination (d)

at 28 days for normal, abnormal seedling, un-germinated and dead seeds. Germination percentage was given based on normal seedling only, and the final germination percentage (FGP) was calculated by the following equation [7].

$$FGP = \frac{N_T \cdot 100}{N}$$

N_T = Proportion of seed germinated in each treatment in the final measurement;
N = number of seeds used in the bioassay.

Seed vigor index

Ten normal seedlings were randomly taken from each replicate, and a linear scale measured the shoot and root lengths. Seed vigor index was calculated by the following equation[30].

Vigor index=Germination%×Seedling length (Root +Shoot)

Germination speed index

Germination speed index (GSI) was determined by dividing the number of normal seedlings germinating on each day by the number of days elapsed since germination test began, and then summing the quotients, following Maguire's formula [19]:

GSI=(G1/N1)+(G2/N2)+(G3/N3)+…+(Gn/Nn), where:

Table 1) Analysis of variance different doses of gamma radiation on seed characteristics (± standard deviation)

Radiation dose(Gy)	15	25	50	100	150	control	F value	sig
Mean daily germination	7.46 ±0.0806	7.11 ±0.0571	7.32 ±0.0163	7.34 ±0.0081	6.95 ±0.0565	6.7 ±0.0476	65.31	0.000
seed vigor index	266.6 ±0.9933	259.2 ±0.7483	232 ±0.8165	262.4 ±0.1414	254.2 ±0.2160	185.6 ±0.0725	84.22	0.000
germination speed index	12.17 ±0.0216	11.88 ±0.0697	11.52 ±0.0408	11.94 ±0.0432	12.32 ±0.0294	9.79 ±0.0294	27.73	0.000
germination	86 ± 0.1633	81 ±0.8165	80 ±0.8165	82 ±0.8165	82 ±0.8165	64 ±0.8165	80.32	0.000

GSI = Germination Speed Index;

G_1, G_2, G_3, …, Gn = number of normal seedlings computed in the first, second, third, and final count.

N_1, N_2, N_3, …, Nn = Number of days to first, second, third, and final count.

Mean daily germination

This is an index of daily germination speed and calculated by Abbasian and Moemeni [31]:

$MDG = FGP/d$

FGP: Final Germination Percent

d: test period

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the germination characteristics among the five treatments and control. The normality of the variables was checked by the Kolmogrov Smirnov test and Levene test to examine the equality of the variances. Duncan test was used for grouping the averages of the dependent variables, which were significantly affected by treatment. (Mean daily germination, germination percentage, seed vigor index and germination speed index) at $p < 0.05$ level. All statistical analyses were conducted using the SPSS 21.0 statistical software package, and drawing the charts was performed in excel Microsoft.

Results

There are significant differences in seed germination characteristics, including germination percentage, germination speed index, mean daily germination, and seed vigor index ($P<0.05$), as shown in Table 1.

Germination

Gamma irradiation had a significant effect on germination compared to control (Figure 2). The highest germination rates were observed in seeds treated with gamma radiation at a dose of 15 Gy (86%) ,whereas control seeds had the lowest germination (64%). The minimum rate of germination percentage was observed in the control.

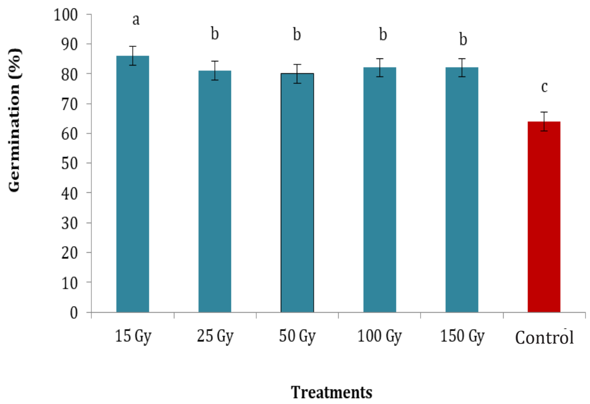

Figure 2) Effect of different doses of gamma irradiation on germination

Germination speed index

The germination speed index in treated seeds significantly increased by γ –irradiation. The highest values were observed in 150 and 15 (Gy) doses of gamma radiation (12.32, 12.17 seed number day^{-1},respectively), followed by 100, 25, and 50 Gy. The lowest germination speed index was recorded by control (9.79

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seed number day^{-1}) (Figure 3).

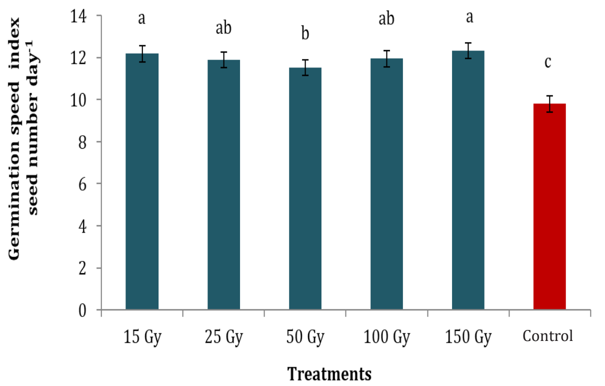

Figure 3) Effect of different doses of gamma irradiation on germination speed index.

Seed vigor index

The results of the vigor index showed that there was a significant difference between treated seeds and control. The highest vigor index was in seeds radiated by 15 Gy, followed by 100, 25, 150, and 50 Gy. In comparison, control seeds had the lowest vigor index (Figure 4).

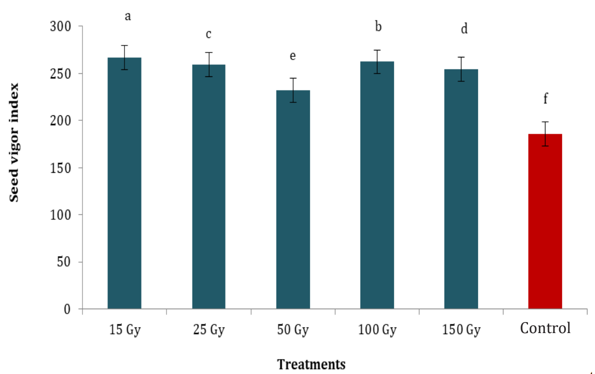

Figure 4) Effect of different doses of gamma irradiation on seed vigor index.

Mean daily germination

Gamma radiation impacts a significant effect on mean daily germination. A significant (P<0.05) difference in mean daily germination in treated and control seeds. The highest value (7.45 number day^{-1}) was observed at 15 Gy, followed by 100, 50, 25, and 150 Gy, respectively. The seeds in control had the lowest mean daily germination (6.7 number day^{-1}) (Figure 5).

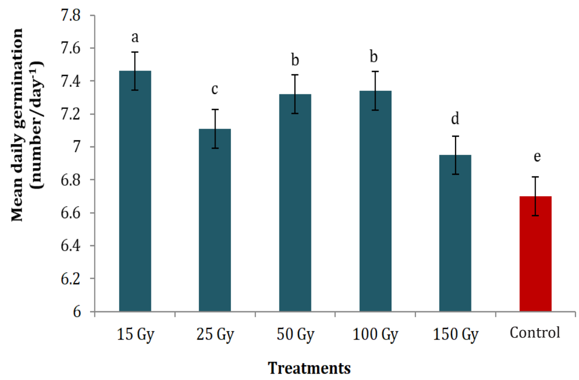

Figure 5) Effect of different doses of gamma irradiation on Mean daily germination.

Discussion

In this study, gamma irradiation as a safe method was used for improving the seed germination characteristics and breaking seed dormancy. Our results indicated that irradiation of *A. julibrissin* seeds with gamma rays significantly affects seed germination characteristics compared to control treatment. A maximum decrease in germination percentage was observed with control. Earlier studies showed that usage of gamma radiation had different effects on seed biochemical and physiological characteristics and germination [21,7,32,15,33].

It is known that various radiation doses of gamma rays have positive or negative effects on germination. According to the results, the differences among all gamma treatments and control seeds were statistically significant (p<0.01). These results were in disagreement with Melki and Marouani [29] and Camilo et al. [34], there was no significant difference in germination, but they were in agreement with Akshatha et al. [22] (*Terminalia arjuna*), Akshatha [28](*Ptrocarpus santalinus*).

The energy required for initial growth is already available in seeds. A low dose of gamma irradiation may improve the germination characteristics [34,2,25,21,28,9,32,16,4]. Similar results were observed in our study on *A. julibrissin* that usage of low gamma ray significantly increased germination parameters compared to control.

Seed germination test after gamma radiation revealed that mean daily germination has increased compared to control. This result was following earlier studies [17,19].

Some studies have been conducted about the usefulness of low dose gamma irradiation for improving plant vigor index [15, 33]. Similar observations were observed on *A. julibrissin* in our study so that seeds with a low dose of gamma ray had the highest seed vigor index. It may be concluded that the low dose of gamma irradiation treatment may help to trigger the germination, which was probably because short-wave photons (i.e., gamma-rays) were more energetic than visible light photons (>400 nm), hence, had a more substantial effect on the plant cells surface. This caused the ultimate breakdown of the seed coating allowing the germination to accrue [11,22]. Germination percentage varied among different pre-treatment methods. High seed germination percentage for nicked seeds suggests that gamma radiation is the best method to be applied before sowing *A. julibrisi*n seeds. The reported results in this study agree with others in the literature [35,30,26,36].

Considering that most germination characteristics have been improved at the dose of 15 Gy and due to the cost of irradiation at higher doses, it is recommended that irradiation be performed at lower doses. Our results support the hypothesis that lower doses of gamma can improve the seed germination characteristics than higher doses.

Conclusion

Albizia julibrissin seed has dormancy because of the hard seed coat, so it needs treatment for germination. The present study is the first report on the effect of gamma irradiation on *A. julibrissin*, where a positive effect was found with radiation treatment to increase the germination percentage. Hence, this technique could improve the speed and seedling quality to support the seedling procurement for establishing plantation forests. This study recommends that lower doses of radiation treatment increase germination and break *Albizia julibrissin* seed dormancy.

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Ethical permissions: The authors of this study approve to send it to Ecopersia journal and declare that this study is not under revision in any other scholarly journals at the time of submission to the journal and will not be sent to any other scholarly journal during the revision at the journal until the definite answer about it. The authors choose Dr. Leila Vatani (one of the authors) as the corresponding author and delegate all the responsibility of the article to her regarding following the relation with ECOPERSIA.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this study.

Authors' Contribution: Leila Vatani (First author), Introduction author/Methodologist/ Original researcher/Discussion author (35%); Seyed Mohsen Hosseini (Second author), Introduction author/ Methodologist/ Discussion author (35%); Seyed Saeed Shamsi (Third author), Introduction author/ Methodologist (30%).

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