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Effect of different pre-treatments on seed germination of *Prosopis juliflora* and *Dalbergia sissoo*: a step towards mutation breeding

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Abstract: For improving the seed germination of *Prosopis juliflora* and *Dalbergia sissoo* different treatments were tested, including side cutting, abrasion, overnight soaking in boiling water, scalding in actively boiling water for 1 minute and immersion in 30%, 60% and 95% H_2SO_4 solution. Results showed that abrasion with sandpaper and side cutting were the most effective methods to break seed dormancy in both species, while scalding in actively boiling water for 1 minute, overnight soaking and different concentrations of H_2SO_4 gave low to zero seed germination. Based on the positive effects of scarification it was concluded that seed dormancy in both species was due to water impermeability of the seed coat. Mutation breeding involves the treatment of large quantities of seeds, therefore abrasion with sandpaper was the most efficient and less labour-intensive method; this method was subsequently used for determination of LD_{50} as it is a prerequisite in a mutation breeding program. Abrasion before irradiation had a positive effect on *P. juliflora* whereas it had a negative effect on *D. sissoo* seeds. Seeds of both species were exposed to different doses of gamma rays such as 0, 100, 200, 300, 400 and 500 Gy using a ^{60}Co source. The LD_{50} for *P. juliflora* was 651 Gy based on the rate of seed germination indicating that *P. juliflora* had tolerance to irradiation and low radiosensitivity to gamma ray. A high LD_{50} of 1097 Gy was observed for *D. sissoo*, suggesting high tolerance to irradiation and very low radio sensitivity. These findings will help to initiate a mutation breeding program in both species to obtain desirable mutants with desirable characteristics such as thornless genotypes, better tree form, disease resistance and increased genetic diversity.

Keywords: seed dormancy; gamma rays; genetic diversity; phenotypic variation

Pakistan inherited a small forest area of which much is being lost due to overutilization, land degradation, desertification, urbanization, climatic factors and diseases (Government of Pakistan 1991). Similarly, the area of Pakistan affected by waterlogging and salinity is about 7 million hectares, which hinders planting of agricultural crops and many tree species in those regions (Zaman, Ahmad 2009). Moreover, Pakistan's large area with arid and semi-arid climate has very little or no vegetation at all. Around 200 acres of Pakistan's fertile irrigated area go out of agricultural use per day because of salinity (Quraishi 2012).

Prosopis juliflora and *Dalbergia sissoo* are the two most important tree species planted in Pakistan. *P. juliflora* is an exotic species, whereas *D. sissoo*

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is an indigenous species. *P. juliflora* is the most important species of drylands and is considered as the jewel of desert ecosystem. It is widely used worldwide to control desertification in arid and semi-arid lands due to its high adaptability to arid conditions. It is a multipurpose tree which thrives well in dryland agroforestry systems and plays an important role in controlling soil erosion, stabilizing sand dunes, improving soil fertility, reducing soil salinity, providing fuel energy resources, supplying feed and forage for grazing animals, furnishing construction timber and furniture wood, supplementing food for humans, and promoting honey production. It is one of the most important species used for commercial coal production, thus offering a good source of income to local communities as well as fuel for the local industry. The major drawbacks of planting *P. juliflora* are its low biomass, poor stem form and presence of long thorns. Long thorns are a big impediment towards large-scale acceptance among local communities as it harms both animals and humans equally.

Dalbergia sissoo is one of the most important timber-producing species in Pakistan. It is a fast-growing, nitrogen-fixing, and very common species found on farmlands as well. It is a self-pollinated species mainly propagated both by seeds and stem cuttings. It also has good coppicing ability. Planting materials come from a fixed source, thus lacking genetic diversity which is one of the primary causes of dieback epidemic (Rajput et al. 2010). However due to the recent epidemic caused by dieback, a fungal disease caused widespread eradication of the species (Rajput et al. 2010). Biological and chemical controls are expensive and not practical for large-scale application. The long-term solution to control the epidemic is planting tolerant genotypes. As trees have a long optimal rotation age, conventional tree breeding is not a useful option, but mutation breeding can be used to induce desirable genetic variations (Abdul et al. 2010). Traditionally mutations are induced by physical (e.g. gamma radiation) or chemical (e.g. ethylmethane sulfonate) mutagens. Physical mutagens are most commonly used because they are easy to use and less hazardous than chemical mutagens. Mutation breeding requires the screening of a large population to find a useful mutant. Therefore, it is imperative to have a high rate of seed germination if a mutation breeding program has to be employed as both *Prosopis* and Shisham seeds exhibit dormancy mainly due to the hard seed coat (Fedrico Mollard 2009). The success of mutation breeding using the gamma rays depends on their dose. The low dose cannot cause mutation at a high rate, therefore there will be no changes in the genetic makeup of treated seeds. On the contrary, high gamma ray doses can result in the death of a large number of mutated seeds. Therefore, it is important to determine LD_{50}, a dose that causes 50% mortality of the seeds. Scarce studies have been carried out in tree mutation breeding. However, it has been determined in agricultural crops that LD_{50} varies between species and within a species (Kangarashu et al. 2014). This study was carried out to determine the best seed germination method and LD_{50} irradiation dose for seeds of *P. juliflora* and *D. sissoo*.

MATERIAL AND METHODS

The research was carried out at the Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan. Seeds from open-pollinated trees of both species were obtained from Punjab Forest Research Institute (PFRI) Gatwala Faisalabad, Punjab, Pakistan. The experiment was arranged in a complete randomized design (CRD) with three replicates for each treatment and each treatment had twenty seeds. Seeds were germinated between the layers of filter paper and saturated with distilled water and incubated at a constant temperature of 25 °C.

Methods used to break seed dormancy

Manual cutting. Seeds were cut carefully from the side with the help of a manual cutter while protecting the embryos.

Abrasion with sandpaper. In this treatment, seeds were abraded with 80 grit sandpaper from both sides.

Treatment with sulphuric acid. Seeds were immersed in 30, 60 and 95% H_2SO_4 solution for 3 min.

Hot water treatment vs scalding.

In the case of scalding seeds were soaked in boiling water for short periods of time ranging from 2 second to 10 minutes. Soaking seeds in boiling water overnight is a standard technique used to break seed dormancy in tree species. Therefore seeds were soaked overnight in boiling water in the case of hot water treatment.

Gamma irradiation. Two methods of seed germination were tested for irradiation treatment in

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Table 1. Effect of different pre-treatments on seed germination of *P. juliflora* and *D. sissoo*

Source	Degrees of freedom	Mean Square	*F* - test
Prosopis juliflora			
Treatment	7	461.1	64.36***
Residuals	14	7.2	
Dalbergia sissoo			
Treatment	7	1 654	67.85***
Residuals	14	24.4	

***significant at $P < 0.001$

order to understand and compare the effect of irradiation on seed germination. By the first method, seeds were abraded before irradiation and by the second method seeds were abraded after irradiation. *P. juliflora* and *D. sissoo* seeds were irradiated by gamma rays from a ^{60}Co source at Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Punjab, Pakistan.

Data analysis. After pre-treatment, observations of seed germination lasted for 9 days.

The following equation was used for estimating the germination percentage (Equation 1):

$$(\%)\ \text{Germination} = \frac{\text{Seed germination}}{\text{Total number of seeds}} \times 100 \qquad (1)$$

The effect of examined germination pre-treatments was tested by analysis of variance (ANOVA) and mean comparisons were conducted by Duncan's Multiple Range Test (DMRT) incorporated into the *Agricolae* package (Mendiburu, 2019) in R programing language (R Development Core Team, 2018).

Determination of gamma ray LD50 for P. juliflora and D. sissoo seeds. LD_{50} was estimated by LD_{50} function of the *HelpersMG* package (Girondot, 2019) using R programming language (R Development Core Team, 2018). Both logistic and logit analyses were used. Both models are described as the following Equations (2–3).

$$\text{Logistic model: } y = 1/(1+ \exp(1/s) \times (p - d))) \qquad (2)$$

$$\text{Logit model: } y = 1/(1+ \exp(p + d \times s)) \qquad (3)$$

where:

s – vector with dead seeds after 9 days,

p – vector with seeds germinated after 9 days,

d – the gamma irradiation dose.

RESULTS

Examined pre-treatments showed a significant effect on the seed germination percentage in both species (Table 1). In *P. juliflora*, manual cutting of seeds and abrasion with sandpaper resulted in 100% germination (Table 2). However, scarification by immersing seeds in different concentrations of H_2SO_4 resulted in variable seed germination. A high germination rate (83%) was observed at low concentration and it declined with increasing concentration, whereby there was no germination at 95% sulphuric acid treatment. Similarly, seeds when soaked or scalded in boiling water overnight or for a short period of time did not show any germina-

Table 2. Germination percentage of *P. juliflora* and *D. sissoo* seeds from different pre-treatments

Treatment	*Prosopis juliflora*	*Dalbergia sissoo*
Control	0.0^{d}	44.85 ± 6.72^{b}
Side cutting	100 ± 6.02^{a}	90.4 ± 6.12^{a}
Abrasion with sandpaper	95 ± 6.14^{ab}	86.9 ± 6.25^{a}
H_2SO_4 30% solution	83 ± 7.63^{ab}	0.0^{c}
H_2SO_4 60% solution	28 ± 6.59^{c}	0.0^{c}
H_2SO_4 95% solution	0.0^{d}	0.0^{c}
Overnight soaking in boiling water	0.0^{d}	0.0^{c}
Scalding	0.0^{d}	0.0^{c}

Treatments sharing same letters are not significantly different

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tion (Table 2). Similar was the case with *D. sissoo*: both side cutting and abrasion proved to be more effective treatments than the control in enhancing the seed germination, whereas pre-treatments with overnight soaking, scalding and H_2SO_4 solution negatively affected seeds as there was observed no germination in these treatments (Table 2).

Effect of different doses of gamma irradiations on seeds

Data on seeds abraded before the gamma irradiation treatment with different doses of gamma rays are presented in Figure 1. *P. juliflora* seeds showed 100% seed germination when treated at 200 Gy followed by 93.33% at 300 Gy, 96.66% at 400 Gy and the lowest was 80% at 500 Gy. The highest germination of *D. sissoo* seeds was recorded after irradiation at 200 Gy (88.57%), followed by 300 Gy (85.71%), 500 Gy (82.85%) and control (82.85%). The lowest seed germination was observed at 400 Gy (74.28%). *D. sissoo* seeds showed higher sensitivity to seed treatment as well as different doses of irradiation (Figure 2).

Effect of different doses of gamma irradiations on seeds (abraded after irradiation)

Seeds when abraded after irradiation showed a significant effect on the germination of both species. In *P. juliflora*, maximum germination of 96.66% was observed at 200 Gy followed by 73.33% at 300 Gy, 76.66% at 400 Gy and the lowest 73.33% was observed at 500 Gy (Figure 1). *D. sissoo* seeds

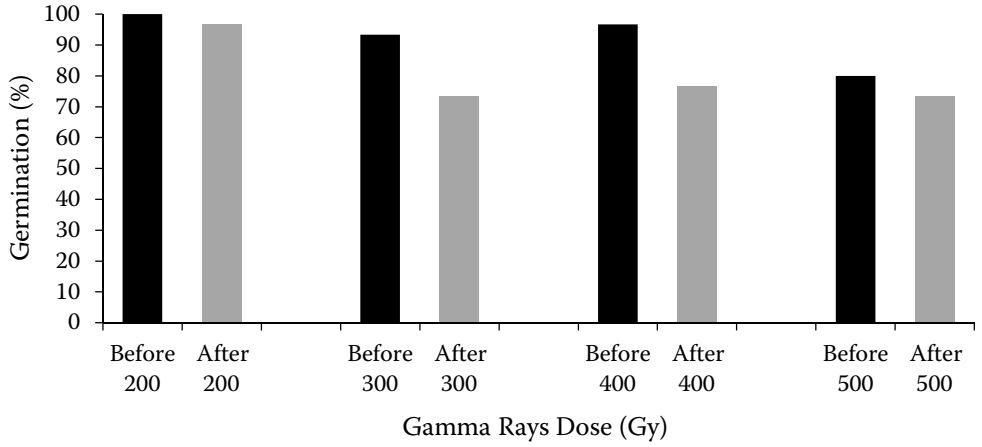

Figure 1. Germination of seeds pre-treated with abrasion before and after different doses of irradiation in *Prosopis juliflora*

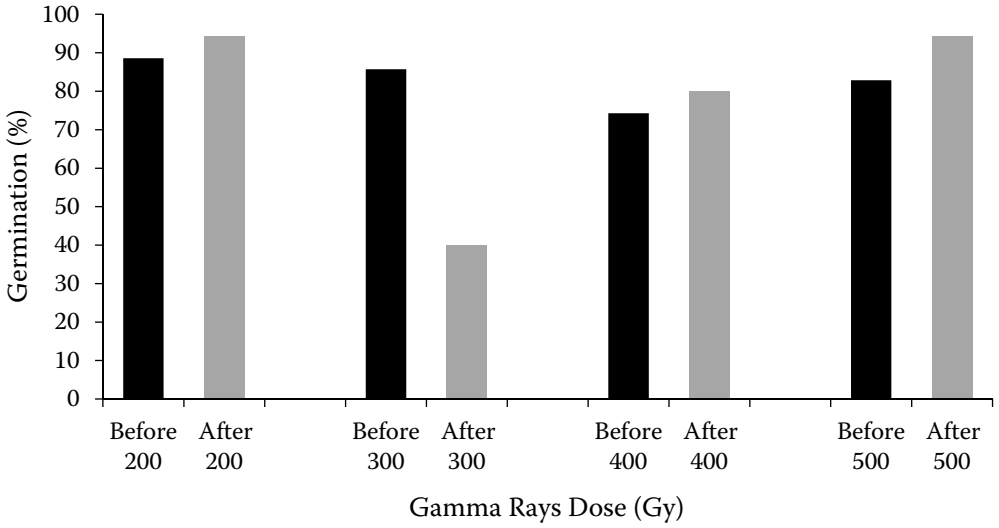

Figure 2. Germination of seeds pre-treated with abrasion before and after different doses of irradiation in *Dalbergia sissoo*

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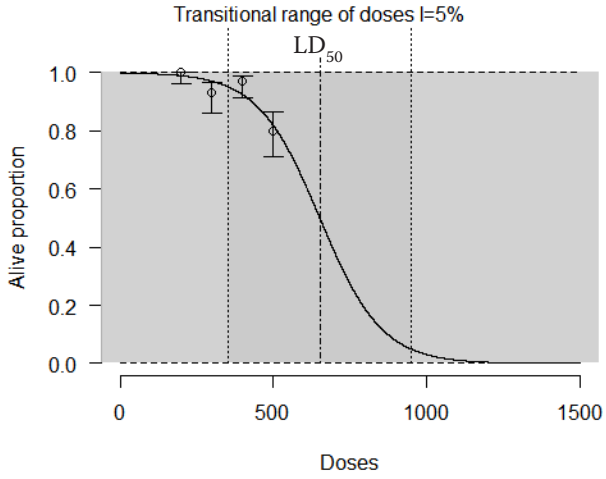

Figure 3. LD_{50} of *Prosopis juliflora* seeds based on the relationship between seed germination rate and irradiation doses

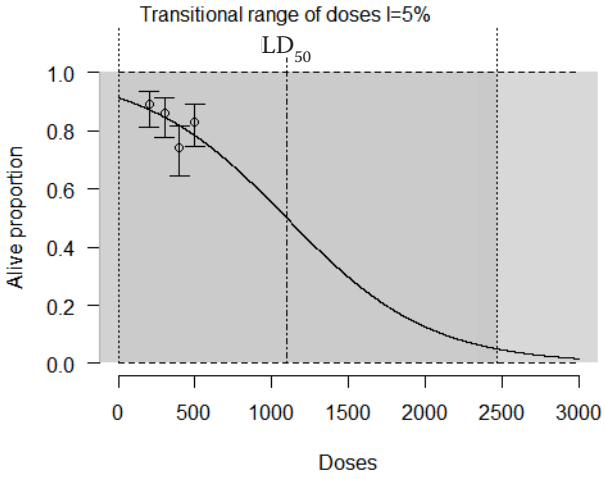

Figure 4. LD_{50} of *Dalbergia sissoo* seeds based on the relationship between seed germination rate and irradiation doses

showed maximum germination of 94.28% at 200 Gy followed by 40% at 300 Gy, 80% at 400 Gy and 94.28% at 500 Gy (Figure 2). Comparison of germination when seeds were abraded before and after irradiation showed variable responses. Seeds when abraded before irradiation showed a very consistent high germination rate whereas it was very erratic when seeds were abraded after irradiation treatment (Figures 1 and 2).

Assessment of median lethal dose (LD_{50})

LD_{50} was determined based on the relationship between germination rate and gamma ray doses. For the determination of LD_{50} the data on the germination rate when seeds were abraded before irradiation was used as it was observed to be consistent in both species. For *P. juliflora* seeds the LD_{50} based on seed viability was 651 Gy (Figure 3) and for *D. sisoo* it was 1097 Gy (Figure 4).

DISCUSSION

Seed germination depends on the potential of embryo growth or the potential of growth inhibitors (Koorneef et al. 2002). These potentials depend particularly on the seed structure that surrounds the embryo, i.e. endosperm, pericarp, glume and seed coat. Other factors like hormones and environmental factors also affect embryo growth (Benech et al. 1998; Mares 2005). *P. juliflora* and *D. sissoo* seeds were subjected to different seed treatments. The best pre-treatment to enhance the germination was the side cutting of seeds and seed abrasion with sandpaper as it gave a higher germination percentage in both species compared to the other examined pre-treatments e.g. immersion in different acid concentrations, overnight soaking in boiling water and scalding. It seemed that the seed coat is the main inhibitor of germination in both species as cutting and abrasion made the seed coat permeable to water. This finding is in agreement with results of Uzen and Aydin (2004) and Soyler and Khawar (2006). Vanstone (1978) believed that scarification of *Tilia* seeds was necessary to obtain maximum germination. Similarly, Cox et al. (1945) concluded that removal of the seed coat in some cabbage varieties could lead to a decrease in inhibitory factors and help in promoting seed germination. *D. sissoo* seeds with thin seed coat are more delicate than those of *P. juliflora*. Thus, soaking in water overnight or treatment with different concentrations of H_2SO_4 caused serious damage to the embryo. This observation demonstrated that above-mentioned treatments had deleterious effects on the embryo. Rincon-Rosales et al. (2003) also reported that seed soaking in hot water improved seed germination but an increase of seed immersion time in hot water led to a decline in seed germination percentage. However, it was not the case with *P. juliflora*, as excellent seed germination was observed in this species after its seeds were cut from the side, abraded, soaked overnight in water or treated with lower concentrations of H_2SO_4. Soaking in hot water overnight and scalding proved to be harsh and resulted in the embryo death as there was no germination. Scalding showed improved germination in *P. juliflora* in the study of Pasiecznik et al. (1998), which may be attributed to the physiological condition

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or water content. Mahmood et al. (2015) treated *Acacia victoriae* seeds with 98% H_2SO_4 solution for 30 min, which resulted in 78% germination. However, there was no seed germination at higher sulphuric acid concentrations in either species in our study, but *P. juliflora* tolerated lower concentrations and *D. sissoo* did not tolerate the pre-treatment with any of the sulphuric acid concentrations. The germination percentage after side cutting and abrasion was successful because the seed coat was ruptured, and the water intake capability was enhanced. So seeds of *P. juliflora* gave a nearly 100% germination rate and *D. sisso* around 90%. A similar study conducted by Mawahib (2004) also showed a 92.5% germination rate when scarification treatment opposite the micropyle was applied to seeds of *Delonix regia*. Moreover, Pasiecznik et al. (1998) studied the side-cutting and abrasion treatments on *P. juliflora* seeds and recorded germination percentages of 97% and 96%, respectively. Similar findings were reported by Zare et al. (2011) in *P. koelziana* and *P. juliflora* seeds. They also reported that mechanical scarification of the seed coat resulted in an increase of the seed germination percentage in *P. juliflora* and *D. sissoo*. It was concluded that seed dormancy in both *P. juliflora* and *D. sissoo* is due to the exogenous factors, i.e. seed coat. This kind of dormancy happens when factors like water and gas cannot penetrate to the seed interior from outside, so imbibition does not occur and consequently results in a decline of seed germination (Bewley 1997). In this state, the pressure force resulting from water absorption and the radicle growth are not sufficient to break dormancy of seeds (Asif et al. 2001). In addition, water and gas impermeability of seeds is caused by physical and biochemical obstacles of the seed coat (Bewley 1997). On the other hand, existence of inhibitory materials in the seed coat could also be considered as the reason for this kind of dormancy (Black, Bewley 2000).

High doses of gamma rays affected seed germination and germinated seeds could not grow well and died at later stages. However, very low doses may not be effective to induce favourable mutations. This could be the result of a destructive effect of radiation on various cellular compartments or because of chromosomal damage (Kiong et al. 2008). Therefore it is very important to determine the LD_{50} value prior to inducing mutation. The LD_{50} is one of the parameters to predict a radiosensitivity level in plants (Kumar et al. 2013). The LD_{50} is a dose that causes 50% mortality of the seeds. The higher the LD_{50}, the lower the plant radiosensitivity. Response to different gamma irradiation doses was also tested by seed germination of *P. juliflora* and *D. sissoo*. *P. juliflora* seeds showed a high germination rate when seeds were abraded and then irradiated. *P. juliflora* seeds are small but they have the hard seed coat, abrasion followed by irradiation seemed to have a synergistic effect on seed germination. However, it was not the case in *D. sissoo* where higher seed mortality was observed when seeds were abraded and then irradiated as compared to seeds abraded after irradiation. *D. sissoo* seeds are thin, flat and the seed coat is not hard as compared to *P. juliflora*. Therefore, it seemed that exposure of embryos to abrasion and their injuries reduced their germination rate. It has been reported that the radiosensitivity varies from species to species and also from genotype to genotype, depending on the size, seed coat thickness and water content inside the seeds.

The seeds which contain a high amount of water and oxygen are more sensitive to gamma rays because gamma rays can interact with atoms or molecules in the cell, particularly water, producing free radicals (Kovacs, Keresztes 2002). It was observed that imbibed papaya (*Carica papaya*) seeds were more sensitive to gamma rays (LD_{50} = 50–87 Gy) than the dry ones (LD_{50} = 300 Gy), (Chan 2009). Similarly, the LD_{50} of two different plants, i.e. of the physic nut (*Jatropha curcas* L.) is relatively lower (425 Gy) than that of the long bean (*Vigna sesquipedalis*; 800 Gy) (Kon et al. 2007; Songsri et al. 2011). One of the methods in plant breeding to improve the properties or acquire high yielding varieties is through induced mutation using gamma rays. This is particularly desirable in tree breeding due to difficulties involved in performing hybridization and long reproductive cycle. The first step in mutation breeding is to determine the lethal dose (LD_{50}) that varies between species and varieties. In this study the most efficient pre-treatment was developed for both *P. juliflora* and *D. sissoo*. LD_{50} was determined by using the best pre-treatment, which is scarification of seed by abrasion with sandpaper. These methods will be used in a future mutation breeding program to induce genetic variations in both species for finding *P. juliflora* without thorns, with better tree form and high yield and dieback-tolerant *D. sissoo* genotypes.

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CONCLUSION

Two methods, i.e. side cutting and abrasion with sandpaper, proved the most efficient pre-treatments to break dormancy and enhance the seed germination in *P. juliflora* and *D. sissoo*. However, for the sake of ease and efficiency abrasion with sandpaper is used and is recommended for future applications as compared to side cutting. The LD_{50} of *P. juliflora* based on the rate of seed germination was determined to be 651 Gy, which indicated that *P. juliflora* had low radiosensitivity to gamma rays. On the other hand, the LD_{50} of *D. sissoo* was 1,097 Gy, an indication of very high radiosensitivity to gamma rays. It was observed that abrasion in *P. juliflora* coupled with irradiation had a positive effect on the seed germination, however there was a negative effect of this treatment on *D. sissoo*. This research will help to develop desirable mutants with increased genetic diversity, better tree form, high yield and tolerance to different diseases.

REFERENCES

Asif M.J., Mak C., Othman R. (2001): In vitro zygotic embryo culture of wild *Musa acuminata ssp. malaccensis* and factors affecting germination and seedling growth. Plant Cell Tissue and Organ Culture, 67: 267–270.

Benech R.L., Giallorenzi M., Frank J., Rodriguez V. (1998): Termination of hull-imposed dormancy in developing barley grains is correlated with changes in embryonic ABA levels and sensitivity. Seed Science Research, 9: 39–47.

Bewley J.D. (1997): Seed germination and dormancy. Plant Cell, 9: 1055–1066.

Black M., Bewley J.D. (2000): Seed technology and its biological basis. Sheffield, CRC Press: 419.

Chan Y.K. (2009): Radiation-induced mutation breeding of papaya. In: Jain M.S., Spencer M.M. (eds.): Induced mutation in tropical fruit trees. IAEA Technical document, 1615. Austria, International Atomic Energy Agency: 170.

Cox C.G., Munger H.M., Smith E.A. (1945): A germination inhibitor in the seed coats of certain varieties of cabbage. Plant Physiology, 20: 289–294.

Fedrico P. O., Mollard P. (2009): Breaking *Setaria parviflora* seed dormancy by nitrates and light is part of a mechanism that detects a draw down period after flooding. Aquatic Botany, 91: 54–60.

Government of Pakistan (1991): The Pakistan National ConServation Strategy: where we are, where we should be, and how to get there. Karachi, The World Conservation Union, IUCN: 396.

Kangarasu S., Ganeshram S., Joel A.J. (2014): Determination of lethal dose for gamma rays and ethyl methane sulphonate induced mutagenesis in cassava (*Manihot esculenta Crantz*.). International Journal of Scientific Research, 3: 3–6.

Kiong A.L.P., Lai A.G., Hussein S., Harun A.R. (2008): Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. American-Eurasian Journal of Sustainable Agriculture, 2: 135–149.

Kon E., Ahmed O.H., Samin S., Majid N.M.A. (2007): Gamma radio-sensitivity study on long bean (*Vigna esquipedalis*). American Journal of Applied Sciences, 4: 1090–1093.

Kumar D.P., Chaturvedi A., Sreedhar M., Aparna M., Venu-Babu P., Singhal R.K. (2013): Gamma radio sensitivity study on rice (*Oryza sativa* L.). Asian Journal of Plant Science & Research, 3: 54–68.

Kovacs E., Keresztes A. (2002): Effect of gamma and UV-B/C radiation on plant cells. Micron, 33: 199–210.

Koorneef M.L., Bentsink H.H. (2002): Seed dormancy and germination. Plant Biology, 5: 33–36.

Mares D.J. (2005): Quarterly reports on plant growth regulation and activities of the PGRSA. Plant Growth Regulation Society of America, 33: 78–89.

Mahmood B., Shanta I.A.E., Hisham N.A. (2015): Germination responses of *Acacia cyclops* and *Acacia victoriae* seeds to different scarification treatments. In: 3rd International Conference on Biological, Chemical & Environmental Sciences (BCES-2015), Kuala Lumpur, Sept 21–22: 119–124.

Mawahib A., EL-Khalifa A. (2004): Effect of seed scarification methods on germination and seed vigour of poinciana (*Delonix regia*). [M.Sc (Hons.) Thesis.] Khartoum, University of Khartoum: 62.

Pasiecznik N.M., Harris P.J.C., Tavares J.P., Cassama M. (1998): Pre-treatment of *Prosopis* seeds to break dormancy. International Tree Crops Journal, 9: 187–193.

Quraishi M.A.A. (2012): Basics of Forestry and Allied sciences, Vol. 1. Faisalabad, University of Agriculture: 310.

R Core Team (2018): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajput N.A., Pathan M.A., Rajput A.Q., Jiskani M.M., Lodhi A.M., Rajput S.A., Khaskhali M.I. (2010): Isolation of fungi associated with Shisham trees and their effect on seed germination and seedling mortality. Pakistan Journal of Botany, 42: 369–374.

Rincon-Rosales R., Culbero-Espinosa N.R., Gutierrez-Miceli F.A., Dedoren l. (2003): Scarification of seeds of *Acacia angustissima* and its effect on germination. Seed Science and Technology, 31: 301–307.

Songsri P., Suriharn B., Sanitchon J., Srisawangwong S., Kesmala T. (2011): Effects of gamma radiation on germination

https://doi.org/10.17221/64/2019-JFS

and growth characteristics of physic nut (*Jatropha curcas* L.). Journal of Biological Sciences, 11: 268–274.

Soyler D., Khawar K.M. (2006): Effects of pre-chilling, scarification, incubation temperature, photoperiod, KNO_3 and GA_3 treatments on germination of caper (*Capparis Ovata Desf. Var. Palaestina Zoh*.) seeds. Propagation of Ornamental Plants, 6: 159–164.

Uzen F., Aydin I. (2004): Improving germination rate of Medicago and Trifolium species. Asian Journal of Plant Science, 6: 714–717.

Vanstone D. E. (1978): Basswood (*Tilia americansl*) seed germination. Combined Proceedings of the International Plant Propagatols Society, 28: 566–570.

Zaman S. B., Ahmad S. (2009): Salinity and waterlogging in the Indus basin of Pakistan: Economic loss to agricultural economy. A series of NRD Research Briefing, PARC, Islamabad: 21–26.

Zare S., Tavili A. M., Darini J. (2011): Effects of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis juliflora*. Journal of Forestry Research, 22: 35–38.

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