P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.12

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Effects of Different Treatments on Seed Germination and Breaking Seed Dormancy in Wild *Luffa* Species

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Abstract

The wild species of *Luffa* require prolonged duration to germinate under normal environmental conditions due to prevalence of seed dormancy owing to variation in their seed size, degree of seed hardiness and variable lignin content in seed coat. Therefore, present study was conducted to identify best pre-sowing treatment for seed germination, seedling growth in two wild *Luffa* species namely *Luffa graveolens* Roxb and *L. echinata* Roxb. Seeds of four accessions of both the species (three of *L. graveolens* and one of *L. echinata*) were exposed to scarification, chemical treatment with gibberellic acid (GA_3) and potassium nitrate (KNO_3) at different concentrations. Among various applied treatments, scarification + GA_3 @ 200 ppm was found most effective in *L. graveolens* and scarification + KNO_3 @ 0.2% for *L. echinata* for breaking of seed dormancy in freshly harvested seed. The seedling vigor in terms of shoot length, root length, seedling dry weight were recorded maximum in scarification + GA_3 @ 200 ppm for *L. graveolens* and scarification + KNO_3 @ 0.2% for *L. echinata* for both fresh as well as one year old seed of same lot. It reflects the presence of hard seed coat and physiological dormancy in wild *Luffa* species, which can be broken to a greater extent with an additional pre-sowing treatment, to enhance seed germination and vigor for uniform plant stand.

Keywords: *Luffa* wild species, Dormancy, Seed priming, Scarification, Gibberellic acid, Potassium nitrate.

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Received: 12/06/2023 **Revised:** 12/07/2023

Accepted: 17/07/2023

How to cite this article: Nagar, A., Sureja, A.K., Pandey, C., Verma, M., Arya, L., Gupta, N. and Munshi, A.D. (2023). Effects of Different Treatments on Seed Germination and Breaking Seed Dormancy in Wild *Luffa* Species. Indian J. Plant Genetic Resources. 36(3), 429-433.

DOI: 10.61949/0976-1926.2023.v36i03.12

Introduction

Genus *Luffa* belongs to family Cucurbitaceae, which includes more than eight species, among them *Luffa aegyptica* Mill., *L. acutangula* (Roxb.) L., *L. hermaphrodita, L. echinata* Roxb., *L. graveolens* Roxb., and two debatable species, *L. umbellata* M. Roem and *L. tuberose* Roxb., found in India (Prakash *et al*., 2013). *L. cylindrica* (Syn. *L. aegyptica*) and *L. acutangula*, are widely grown for their edible fruit and sponge-like fibers, whereas *L. echinata* and *L. graveolens* are wild species and known as bitter *Luffa*. Wild *Luffa* are having rough texture fruit with a very bitter flavor which are native to South Asia and Southeast Asia and grows abundantly in India, Bangladesh, Pakistan and Northern Tropical Africa with Gujarat, Bihar, Rajasthan and Madhya Pradesh being the most common locations in India (Mondal *et al*., 2022). Wild *Luffa* species are used in traditional Ayurvedic medicines, as they have various medicinal properties such as analgesic, laxative, antidepressant, anti-inflammatory, anxiolytic, antibacterial, antifungal, antiepileptic, hepatoprotective, antiulcer, anticancer, anti-anthelminthic and anti-hepatic, hence used to treat bronchitis, piles, jaundice and vaginal discharge (Prakash *et al*., 2013). Fruits, bitter in flavor and highly fibrous, are used to treat chronic bronchitis, dropsy, biliary, intestinal colic, putrid fever, and jaundice (Patel and Ghane, 2021). The whole plant is also used in curing leprosy, diabetes, antihelminthic, nephritis, rheumatism, stomachic, nephritis and abortifacient (Patel and Ghane, 2021).

Normally, seeds are equipped with suitable mechanisms to germinate under favorable environmental conditions. However, when a seed provided with adequate water, optimal temperature and sufficient oxygen for normal aerobic metabolism within physiological limits does not germinate, it is termed as "dormant" (Berrie, 1984). In plants, seed dormancy is a key evolutionary component, ensuring their survival in unfavorable conditions and allowing them to germinate when the chances of survival for the young seedlings are at the greatest (Koornneef *et al*., 2002). Seeds of wild *Luffa* species exhibit some degree of dormancy expressed by low or no germination. This may be attributed to hard seed coats that prevent water and oxygen from entering the seed, making it difficult for the embryo to germinate. The germination of the seeds of wild *Luffa* species has continued to be problematic and the seeds may require different scarification methods and exogenous treatments with chemicals to break dormancy and enhance germination, as have been reported for other species (Tambari and Aminu, 2015; Al-Menaie, 2010). Studies on pre-treatment methods to break dormancy in wild *Luffa* seeds have not received desired attention. This study, therefore, intends to identify the suitable pre-sowing treatments for breaking seed dormancy of freshly harvested seed of wild *Luffa* species *L. echinata* and *L. graveolens* (Accessions IC-587288, KC/CSR/ 41, KP/RS/PKS-20) in view of the economic, medicinal and agricultural potentials of these species.

Materials and Methods

Seeds of wild genotypes of *L. graveolens* and *L. echinata* (Table 1) used in this study were obtained from ICAR-National Bureau of Plant Genetic Resources, New Delhi. The present investigation was carried out at Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi, during spring-summer and *Kharif* seasons of the year 2021-2022.

Luffa seeds (fresh and one year old seeds; 50 seeds of each) were subjected to different physical and chemical treatments for breaking seed dormancy (Table 2). A total of nine treatments (Table 2) were applied on the fresh seed of wild *Luffa* for breaking dormancy, whereas best treatments, including control were applied on one year old seed.

For seed germination studies standard germination test was conducted following ISTA rules (Anonymous, 2019). Other observations were recorded on ten random seedlings from each replication. Data on rate of seed germination was recorded regularly up to fourteen days and at the time of final count root and shoot length is taken with the help of a scale and expressed in "cm". Dry weight of ten random seedlings was recorded and further subjected to calculation of vigor indices. Seed vigor indices (SVI I and SVI II) were calculated using the following formula suggested by Abdul-Baki and Anderson (1973).

Vigor Index-I = Standard Germination (%) × Seedling length (cm)

Vigor Index-II = Standard Germination (%) × Seedling dry weight (g).

Germination percentage was calculated on the basis of fresh seeds, un-germinated fresh seeds and hard seeds on daily basis. The seedlings used for recording root: shoot ratio (RSR) were oven-dried at 70˚C for 48 hours after removing the cotyledon and seedling dry weight was expressed in gram per seedling as per the protocol of Kleyer *et al*. (2008). Germination rate was calculated as follows: Germination Rate = Σ(Gt/Dt), where Gt shows number of germinated seeds on day *'t'* and Dt is the number of days after placing seeds in germination paper when the germinated seeds were counted.

The experiment was conducted following CRBD, using three replicates, and subjected to ANOVA. ANOVA of each trait, including the mean, range, and coefficient of variation (CV%), were estimated using SPSS software 7.0. The data as percentage were transformed to arc sine values prior to statistical analysis.

Table 1: Source and identity of materials used in the experiment

Species	*Identity*	*Source*
L. graveolens	KP/RS/PKS/4 (IC-587288)	ICAR-NBPGR, New Delhi
	KC/CSR/ 41	
	KP/RS/PKS-20	
L. echinata	KP/SS/2871 (IC-618051)	

Table 2: Different treatment combinations used for breaking seed dormancy

S.N.	*Treatments (Fresh seed)*	*Treatments (One year old seed)*
1	Dry Seed	Dry Seed
2	Hydro-priming	Hydro-priming
3	GA_3 @ 200 ppm	Scarification
4	GA_3 @ 400 ppm	Scarification + GA_3 @ 200 ppm
5	KNO_3 @ 0.2%	Scarification + KNO_3 @ 0.2%
6	KNO_3 @ 0.4%	
7	Scarification	
8	Scarification + GA_3 @200 ppm	

Results and Discussion

In fresh seeds of *Luffa* genotypes germination percentage ranged from 0 to 88% (Figure 1) in nine different treatment combinations. Among all treatment combinations scarification + GA_3 @ 200 ppm was found superior to improve seed germination. In one year old dry *Luffa* seeds minimum germination of 36% was recorded in *L. echinata* which was improved up to 92% with scarification of seeds followed by chemical treatment with KNO_3 @ 0.2% (Figure 2). These findings are in line with Chaodumrikul *et al*. (2016) in *L. cylindrica* and Adhikari *et al*. (2021) in bitter gourd.

Germination percentage was negligible in freshly extracted wild *Luffa* seeds possibly due to higher levels of Abscisic Acid (ABA) which was lowered considerably on storage of seeds for one year or treatment of seeds either with GA_3/ KNO_3 (Hernández *et al*., 2022). In both fresh and one year old seeds of wild species *viz. L. echinata, L. graveolens* presence of hard seed coat hampered seed germination as its scrubbing resulted considerable improvement in seed germination. Hence, it can be interpreted that two types of dormancy exist in *Luffa i.e.* physical dormancy due to hard seed coat and another is physiological dormancy due to higher levels of dormancy inducing growth regulator. ABA is a key growth regulator known for its crucial role in dormancy induction in developing seeds or in maintenance of dormancy at time of seed imbibition, while gibberellic acid is widely documented for its key role in breaking of seed dormancy (Miransari and Smith, 2014). These interpretations are in line with those documented by Nambara *et al*., 2010 and Miransari and Smith, 2014.

In fresh *Luffa* seeds rate of seed germination varied between 0 to 6.88 (Table 3). Among all treatment combinations, scarification + GA3 @ 200 ppm significantly enhanced the seed germination rate over control (no treatment of seeds). Similarly, for one year old seeds rate of seed germination ranged from 1.8 to 5.48 (Table 4). Seed scarification followed by GA_3 treatment @ 200 ppm was found best for all three genotypes of *L. graveolens* included in this study. On the other hand, seeds of *L. echinata* genotype showed improvement in seed germination on exposure of its seeds to scarification, followed by chemical treatment with KNO_3 @ 0.2%. These findings are in line with Chaodumrikul *et al*. (2016) in *L. cylindrica*. Findings of this experiment pointed toward the presence of morphological barrier *i.e.* hard seed coat in wild *Luffa* spp. as scrubbing of hard seed coat with sandpaper showed considerable improvement in rate of seed germination. Seed dormancy is the most common adaptive mechanism prevalent in wild flora for survival under unfavorable environmental conditions (Baskin and Baskin, 2004). The beneficial effects of chemicals like KNO_3 were documented in bitter gourd (Renuga Devi and Jacqueline, 1995). Puncturing seed coat or scarification, seed treatment with KNO_3 is reported to reduce seed dormancy in cucurbits (Devi and Selvaraj, 1994).

For fresh seeds seedling length was recorded between 0 to 18.17 cm (Table 3). The control treatment showed no germination, while treatment of seeds with GA_3 @ 400 ppm recorded maximum seedling length in *L. echinata* genotype. GA_3 at higher concentration *i.e.* 400 ppm enhanced seedling length considerably but the seedlings became lanky and unfit for transplantation under open field conditions. Seedling length was recorded for one year old seeds between 10.84 and 15.40 cm (Table 4). Seed scarification followed by GA_3 treatment @ 200 ppm enhanced seedling length significantly over control and

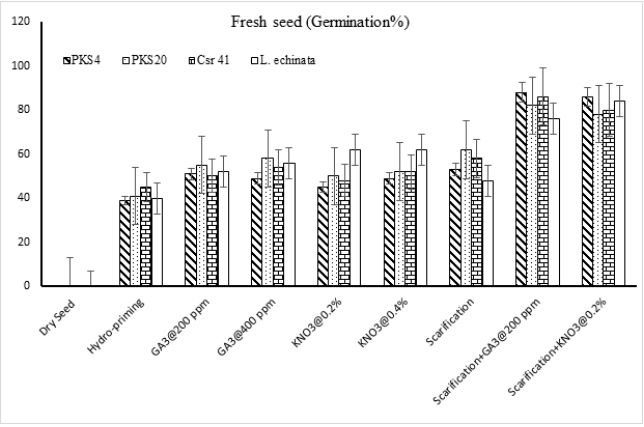

Figure 1: Effect of physical and chemical seed treatments on seed germination of fresh seeds of wild *Luffa* species

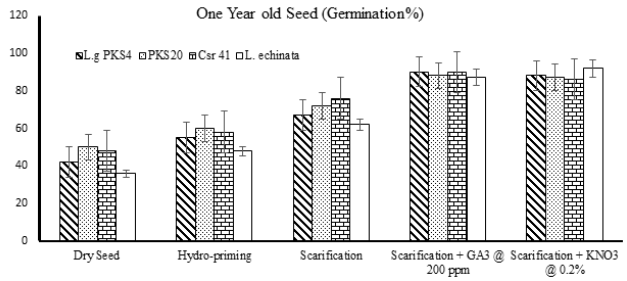

Figure 2: Effect of physical and chemical seed treatments on seed germination of one year old seeds of wild *Luffa* species

Table 3: Effect of seed dormancy breaking treatments (physical and chemical) on seed quality of freshly harvested seed of wild *Luffa* species

	Rate of seed germination				*Seedling length (cm)*				*Vigor index-I*				*Vigor index-II*			
Treatments	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Dry Seed	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00
Hydro-priming	1.5	2	1.33	0.96	9.48	10.02	9.46	6.88	369.72	410.82	425.7	275.2	0.55	0.74	0.92	0.81
GA_3 @ 200 ppm	2.25	3.41	3.48	1.44	12.38	12.32	11.92	10.35	631.38	677.6	596	538.2	0.78	1.19	1.47	1.16
GA_3 @ 400 ppm	2.28	3.81	3.52	1.42	15.38	15.52	14.91	18.17	753.62	900.16	805.14	1017.52	0.80	1.11	1.67	1.30
KNO_3 @ 0.2%	2.89	3.45	2.85	1.68	11.32	15.05	12.16	11.52	509.4	752.5	583.68	714.24	0.72	0.84	1.32	1.27
KNO_3 @ 0.4%	3.15	3.5	2.68	1.65	16.74	16.18	15.5	17.83	820.26	841.36	806	1105.46	0.84	0.82	1.48	1.49
Scarification	3.45	3.68	3.44	1.5	13.22	12.77	10.68	7.8	700.66	791.74	619.44	374.4	1.04	1.33	1.28	0.98
Scarification+GA_3@ 200 ppm	6.88	5.83	4.19	3.21	14.51	13.52	12.6	10.84	1276.88	1108.64	1083.6	823.84	1.85	1.84	2.34	1.79
Scarification+KNO_3@ 0.2%	4.33	5.77	3.58	3.6	13.99	13.8	11.5	11.85	1203.14	1076.4	920	995.4	1.64	1.67	2.03	2.03
CD @ 5%	0.6	0.51	0.48	0.62	1.12	1.38	0.98	1.02	81.2	77.6	70.8	84.7	0.01	0.02	0.02	0.01
CV	3.8	4.4	3.1	2.9	4.6	2.8	3.6	3	7.2	5.5	4.9	5.7	2.30	4.20	1.90	2.80

(A=*L.g*.KP/RS/PKS/4, B=*L.g*.KC/CSR/41, C=*L.g*.KP/RS/PKS-20, D=*L.e*.KP/SS/2871)

Table 4: Effect of seed dormancy breaking treatments (physical and chemical) on seed quality of one year old seed of wild *Luffa* species

	Rate of seed germination				*Seedling length (cm)*				*Vigor index-I*				*Vigor index-II*			
Treatments	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Dry Seed	2.55	2.4	1.8	2.2	11.54	12.56	12.2	10.84	484.68	628	585.6	390.24	0.66	0.81	0.88	0.69
Hydro-priming	3.85	3.2	2.2	2.5	12.58	12.88	13.42	11.92	691.9	772.8	778.36	572.16	0.92	1.05	1.09	1.11
Scarification	4.2	4.4	4.2	3.84	13.11	13.2	13.41	11.72	878.37	950.4	1019.16	726.64	1.20	1.24	1.49	1.33
Scarification + GA_3 @ 200 ppm	5.48	5.1	4.8	4.2	15.48	14.40	14.42	11.74	1386	1267.2	1297.8	1021.38	1.77	1.92	1.94	2.07
Scarification + KNO_3 @ 0.2%	5.18	4.8	4.84	4.4	15.21	14.32	13.94	12.84	1338.48	1245.84	1198.84	1181.28	1.72	1.74	1.70	2.30
CD @ 5%	0.57	0.69	0.72	0.49	1.44	0.89	1.16	1.09	67.5	71.5	56.8	65.1	0.01	0.02	0.02	0.01
CV	4.1	3.9	2.9	4.8	3.2	2.9	2.2	1.8	6.1	8.2	7.0	6.6	3.10	2.80	2.10	1.80

(A=*L.g*.KP/RS/PKS/4, B=*L.g*. KP/RS/PKS-20, C= *L.g*. KC/CSR/41, D=*L.e*.KP/SS/2871)

seedlings were uniformly healthy. These findings are in line with Tapfumaneyi *et al.* (2023) in Amaranthus. The major benefit of any physical or chemical seed treatment procedure is to increase germination and to ensure uniform seedling emergence. Overall, seed treatment to break seed dormancy leads to improved plant population and thus, higher productivity (Sridhar *et al*., 2013).

In fresh *Luffa* seeds vigor index-I valued between 0 to 1276.88 (Table 3) and for fresh seeds, seed vigor index-I for one year old seed was recorded between 390.24 to 1386 as shown in Table 4. The genotypes of *L. graveolens* exhibited a positive response to scarification followed by chemical treatment with GA_3 @ 200 ppm. In *L. echinata* seed scarification, chemical treatment with KNO_3 @ 0.2% was found most promising over other treatment combinations. These findings are in line with Tapfumaneyi *et al.* (2023) in Amaranthus. For fresh *Luffa* seeds values of seed vigor index-II were recorded between 0 to 2.34 (Table 3). In one year old seeds seed vigor index ranged from 0.66 to 2.30 (Table 4). Among nine different treatments, scarification followed by chemical treatment with either GA_3/KNO_3 was best suited in the enhancement of seed vigor indices and other growth parameters included in this study. These findings are in line with Tapfumaneyi *et al.* (2023) in Amaranthus.

The wild species of *Luffa* have hard seed coat and varied levels of physiological seed dormancy in freshly harvested and one-year-old seeds. Scarification and chemical seed priming can provide the necessary threshold to seed germination in all four accessions of wild *Luffa* spp. *viz. L. graveolens* and *L. echinata*. Seed germination treatments are quite helpful in improving seedling vigor and ensuring uniform crop stand immediately after seed extraction.

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