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EFFECT OF PRE-TREATMENTS ON SEED GERMINATION AND SEEDLING VIGOR OF *EMBLICAOFFICINALIS*GAERTN

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ABSTRACT

Emblica officinalis Gaertn. is an important medicinal plant, which is well known in bringing better socio-economic conditions worldwide, so also in north-east India. Improvement of germination rate and percentage may enable commercial propagation of this plant from seed instead of cuttings. However, the seeds of *E. officinalis* do not germinate easily thereby necessitating pre-treatments to overcome dormancy.This study investigates the effect of different pretreatments *viz*., tap water for 24 hours, thiourea (2%) for24 hours, GA_3 500ppm for 12 hours, GA_3 500ppm for 24 hours, Stratification at 5°C for 10days, Acid (conc. HCl) scarification for 30 seconds and Control on the germination of *Emblica* seeds. Chemical treatment of *Emblicaofficinalis* seed exposed to $GA_3$500ppm for 24hous resulted better germination than other treatments. Imbibition percent increased in treated seeds upto 90 % in contrast to 70% in non-treated seeds (control). The study shows treatment of seeds with GA_3 500ppm and thiourea were effective in breaking seed coat dormancy in the present study.

Keywords: Germination, scarification, dormancy, pretreatments, *Emblica officinalis*

1. INTRODUCTION

The Amla or Indian gooseberry (*Emblica officinalis* Gaertn.) belongs to the family Euphorbiaceace, native to tropical South–East Asia, particularly the central and south India since the ancient times [1]. The plant is also found abundantly grown in the home gardens of Manipur, Mizoram and other north eastern states of India. In India, it is called by various names such as Aonla, Nelli, Amla, Amlika, Dhotri, Emblica, Usuri and Heikru in Manipur. In the recent past growing of superior varieties of aonla is highly remunerative. This species is highly valued for its medicinal properties, and is the most common ingredient of many medicines and tonics in traditional Indian health practices. *Amla* fruits are also used in the preparation of pickles, jams and juices, in addition to their use in the preparation of cosmetics such as hair dyes and shampoos and thus important in improving rural economy [2,3,4,5]. This species is highly valued for its medicinal properties, and is the most common ingredient of many medicines and tonics in traditional Indian health practices [6].

The fruit of Alma is fleshy and drupaceous and the seeds are found within the hardened endocarp of the fruit known as stone[7]. The seeds do not germinate easily owing to seed coat related dormancy[8] and the germination rate is also very

GLOBAL JOURNAL OF ADVANCED RESEARCH
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poor, necessitating pretreatments before sowing, however, the seeds can remain viable for a longer period under natural conditions [9]. Although the plant could be propagated through vegetative means [10] and in-vitro shoot proliferation [11], the former is a very slow process while the later is costly and cannot be implemented through sexual propagation [8]. In order to improve the rates of germination and to facilitate farmers in adopting proper techniques, trials have been made to find out the best pretreatment on seed germination in this paper.

2. MATERIALS AND METHODS

The present study was conducted in the laboratory of Department of Forestry, Mizoram University, India. The mature drupes were harvested from twenty selected *Emblica officinalis* trees. Collection was done annually in the last month of February 2015 from the Ngariyan hill of Senapati district near Bisempur district of Manipur for seed dormancy analysis. Prior to being pretreatment the mesocarp of the drupes was cut open and completely removed (de pulped) using a knife and the stones were left to air dry for 24 hours. The stone were split opened by applying a light pressure longitudinally. The extracted seeds were air dried for 24 hours. The extracted seeds were kept in a jar containing water and were subjected to float test to determine seed viability. The seeds that sank to the bottom were selected for pretreatments. Prior to sowing, all the equipment (i.e. forceps, scalper and 2ml syringe) used in sowing seeds was disinfected using 1% Clorox solution (The Clorox Company, Oakland, Canada).The seeds were subjected to 7 treatment combinations (Tap water for 24 hours, thiourea (2%) for 24 hours, GA_3 500 ppm for 12 hours and 24 hours separately, Stratification at 5°C for 10 days, Acid scarification for 30 seconds and Control); each treatment replicated five times (each replicate involving 50 seeds sown in Petri plate) thus there were 7 treatments x 5 replicates x 50 seeds i.e 1750 seeds for whole treatments . Germination was assessed every day for 30 days to determine the effect of seed treatments on germination rate. During the course of the experiment, the Petri dishes were kept moist by adding tap water every 24 hours. The seeds were kept under the germinator. A seed was considered germinated when a radicle could be seen with the naked eye [12].

The germination was recorded daily from the date of sowing and continued till the germination ceased. Daily observations were made on radicle emergence. Seed germination percentage was calculated using the following formula [13]. The percentage of germinated seeds after 30 days, imbibition period per day, germination value and germination energy were calculated for each treatment [14]. These parameters are indicative of germination percentage, speed and uniformity, respectively. Vigour index of the seedlings was calculated according to Abdul-Baki and Anderson [15]as germination percent X seedling total length i.e. total shoot and root length.

The effect of various treatments on germination and initial growth characters were analyzed using Analysis of variance (ANOVA one way, fixed effects model) and Lease significant difference using the SPSS program Version.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

3. RESULTS AND DISCUSSION

Seed dormancy occurs in many tropical fruit species to varying degrees. While various pretreatment methods have been adopted to reduce dormancy and hasten germination [16,17] no single pretreatment technique has been found to be equally effective in all seed species [18]. Semi-orthodox nature of the seeds of *Emblica* coupled with long-term dormancy [8] necessitates appropriate pretreatment techniques in breaking seed dormancy and facilitating germination in this species. Among the seven treatments, acid scarification (conc HCl) with 30 seconds could not stimulate growth of dormant seeds during the experiment of 30 days. However those seeds that were subjected to pretreatment like tap water for 24 hours, thiourea (2%) for 24 hours, GA_3 500ppm, for 12 hours and GA_3 500ppm for 24 hours, germinated exactly after 5 days of pretreatment with a germination percentage of 84%, 3.2%, 60% and 86% respectively. But the seeds subjected to stratification at 5 ºC for 10 days were germinated exactly after 6 (six) days with a germination percentage of 73.6% and those seeds subjected to control was germinated exactly after 8(eight) days with a germination percentage of 81.2%. Chemical treatment of *Emblica officinalis* seed exposed to GA_3 500ppm for 24 hours resulted in better germination. During 30days of experiment in warm, dry laboratory conditions, germination percentage in all test except acid scarification and thiourea treatment tended to increase and there was less difference between treated seeds and untreated controls. The highest germination value 18.88 was found in Tap water 24 hrs treatment followed by the value of 17.43 in GA_3 500ppm for 24 hrs and 11.40 in control but in stratification at 5°C for 10 days, GA_3 500ppm for 12 hours and thiourea (2%) for 24 hrs treatments, having low germination values like 4.63, 2.00 and 0.11 respectively. The longest imbibitions period 27 days was seen in the stratification at 5°C for 10 days treatment but the shortest imbibition period of *Emblica* seed 6 days was seen in two treatments viz. thiourea (2%) for 24 hrs treatments and GA_3 500ppm for 12 hours treatment. The highest total germinated period 20 days and highest germination speed 483.66 was seen in the Tap water 24 hrs treatment but the shortest germinated period 6 days was encountered in two treatments like thiourea (2%) for 24 hrs treatments and GA_3 500ppm for 12 hrs treatment but the shortest germination speed 2.64 was seen only in the thiourea (2%) for 24 hrs treatments (Table1). The longest average shoot length 3.94cm was found in the Control followed by the value of 3.43cm, 3.37cm, 2.96cm and 2.60cm in Tap water 24hrs treatment, thiourea (2%) for 24 hrs treatments, GA_3 500ppm for 24 hrs and stratification at 5°C for 10 days respectively. But the shortest average length 1.71cm is found in GA_3 500 ppm for 12 hrs. The longest root length 3.09cm is found in Tap water 24hrs treatment and the shortest root length 1.49 cm is found in GA_3 500ppm for 24 hrs. The maximum fresh weight 5.69g, dry weight 2.01g and biomass 3.68g is found in control but the minimum fresh weight 0.31g, dry weight 0.01g and biomass 0.19g is found in thiourea (2%) for 24 hrs treatments. (Table2).

Different approaches of breaking seed dormancy, in order to enhance germination rate and to increase germination process were argued by many authors[16,17].There are a number of reasons for variation in the timing of seed germination within a species, and in seeds from the same parent plant. Among these possibilities are: the position of the seed on the parent plant, environment and health of the parent plant during seed maturation [19, 20].Different methods used in breaking seed dormancy include physical scarification of seed coat by nicking; filling with needles, knife and or abrasion paper[16, 17, 21]. In addition, methods such as acid treatment, GA_3 treatment stratification at 5ºC and tap water

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

treatment can be used to overcome physical seed dormancy. Hossain et al. [22] reported that seeds with hard, solid, impermeable seed coat were noted to establish germination after pre-sowing treatments. Acid treatments have been found to give highest germination in *Afzella africana* within shortest time [18] indicating quicker the rapture of seed coat faster is the germination. However; breaking of seed dormancy varies from species to species. Therefore, it is very important to determine which method and condition is suitable for each plant species.

In the present study, *Emblica officinalis* seeds which were treated with GA_3 500 ppm for 24hrs produced the highest percentage of germination. This may due to fact that GA3 might have helped in physically breaching, thereby removing the physiological barriers associated with the impermeable seed coats that cause seed dormancy[23]. This might have exposed the lumens of the macro-sclereids cells permitting imbibitions of water which triggers germination [24]. The fertilized ovaries of Aonla (*Emblica officinalis* Gaertn.), unlike those in other plants, remain dormant for about 3-5 months and resume growth preceding divisions in endosperm and zygote nuclei [25]. The dormancy mechanism of the fruit appears to be auxin dependent. It is reported that the concentration of auxin increased in the fruit with the onset of dormancy and decreased to a low level prior to dormancy break [26] and exposure to GA_3 500 ppm for 24hrs could have counter downed the Auxin concentration. Besides, the impermeable layer in seed coat allowed water and oxygen to enter the seed and permitted the embryo to overcome the mechanical restriction of surrounding tissue by providing uniform germination when immersed in tap water for 24hrs. It also showed that nicking resulted in breaking the seed coat of *Emblica officinalis* and bringing an improvement in germination. The longest shoot length displayed only on control (without any treatment) and longest root length was attained only on tap water 24hrs treatment. The seed subjected to control treatment shows 81.20% germination percentage and longest seedling length (6.56cm). The seed treated with hydrochloric acid for a short period of 30 sec did not germinate; probably the action of the acid during this short period was not enough in breaking the hard coat and facilitating entry of water and gases required for germination. Release from dormancy by cold stratification nevertheless is indicative of physiological dormancy [27]. The highest imbibition period (27 day) of *Emblica* was attained in the cold stratification at 5ºC. The variable responses of *Emblica* seeds to cold stratification in this experiment illustrated differences in the degree of dormancy among seeds within the same lot.

Multiple mechanisms are likely to have been responsible for dormancy and irregular germination in *Emblica officinalis*. Data from tap water treatment, thiourea treatment, GA3, cold stratification, Chemical scarification and control treatment suggest the presence of physiological dormancy in this species. The seedling vigor, however, was in the order of T1>T7>T4>T5>T3>T2.Ourstudy suggests that inhibitory chemicals in the seed coat are less likely to be responsible for germination irregularities. The condition of the parent plant may influence the viability of the seed, and differences among parent populations may account for differences in germination among seed lots. All experiments in this study were conducted under the seed germinator up to 30 days. Examination of the conditions in which different seed lots are produced coupled with an investigation into the responses of different lots to the treatments used in this study would provide further information.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

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5. REFERENCES

[1] Troup R.S. 1921. The Silviculture of Indian Trees, 3: 830-833

[2] Hegde, R., Suryaprakash, S., Achoth, L. and Bawa, K.S. 1996. Extraction of Non-Timber Forest Products in the Forests of Biligiri Rangan Hills, India, 1: Contribution to Rural Income, Economic Botany, 50: 243-51.

[3] Kalla, J.C. (1988). Forestry and Economic Development. Indian Journal of Agricultural Economics, 43: 237.

[4] Sekar, C., Vinay, R.S. and Ramasamy, C. 1996. Role of Minor Forest Products in Tribal Economy of India: A Case Study. J. of Tropical Forest Science, 8: 280-88.

[5] Shiva, M.P. and Mathur, R.B. 1995. Management of Minor Forest Produce for Sustainability. New Delhi: Oxford and IBH.

[6] Kirtiker, K.R and Basu, B.D. 1993. *Phyllanthus emblica* In: Indian medicinal plants, Ind. Edu. LalitMohan Basu Pub Calcutta. pp:220.

[7] Dassanayake, M. D. and Fosberg, F. R. 1988. A revised hand book of the Flora of Ceylon.pp: 219-220. New Delhi: Model Press Pvt. Ltd.

[8] Mawalagedera, S.M.U.P., Perera, G.A.D. and Sooriyapathirana, S.D.S.S. 2014. Prolonged seed dormancy in *Phyllanthus emblica* L. can be overturned by seed scarification and gibberellins pretreatment. Open Journal of Forestry, 4(1): 38-41.

[9] Pushpakumara, D. K. N. G., and Heenkenda, H. M. S. 2007. Nelli (Amla) *Phyllanthus emblic*a L. In: D. K. N. G. Pushpakumara, H. P. M. Gunasena, and V. P. Singh (Eds.), Underutilized fruit trees in Sri Lanka. World Agroforestry Centre .pp: 180-221. New Delhi: South Asia Office.

[10] Govinda, N. D. 2006. Propagation studies in Aonla (*Phyllanthus emblica* L.), pp: 5-39.

[11] Goyal, D. and Bhadauria, S. 2008. *In-vitro* shoot proliferation in *Emblica officinalis* var. Balwant from nodal explants. Indian Journal of Biotechnology, 7: 394-397.

[12] Valbuena, L and Vera, M. L. 2002. The effects of thermal scarification and seed storage on germination of four heath land species. Plant Ecology,161: 137-144.

[13] ISTA (1999). International rules for seed testing. Seed science and Technology, pp: 21:288

[14] Czabator, F. J. 1962. Germination value: An index combining speed and completeness of pine seed germination. Forest Science 8: 386 – 395.

[15] Abdual-baki, A.A. and Anderson, J.D. 1973. Relationship between decarboxilation of glutamic acid and vigour in soybean seed, Crop Science, 13, 222–226.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

[16] Sahoo, U.K. 2007. Effect of seed pretreatments on germination and seedling vigour of four different species of *Albizzia*. Seed Research, 35(1): 124-128.

[17] Sahoo, U.K., Upadhyaya, K. and Lalrempuia 2007. Effect of pretreatment and temperature on the germination behavior of seeds of Parkia roxburghiiG.Don. Forests, Trees and Livelihoods, 17(4):345-350.

[18] Amusa, T.O. 2011. Effect of pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers). Journal of Horticulture and Forestry, 3(4): 96-103.

[19] Gray, D. and Thomas, T.H. 1982. Seed germination and seedling emergence as influenced by the position of development of the seed on, and chemical applications to, the parent plant. In: A.A. Khan (ed.). The Physiology and Biochemistry of Seed Development, Dormancy and Germination.Elsevier Biomedical Press, New York. Pp: 81-110.

[20] Gutterman, Y. 1982. Phenotypic maternal effect of photoperiod on seed germination, In: A.A. Khan (ed.) The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier Biomedical Press, New York. Pp: 67-79

[21] Asiyire, B.E., Akinro, L.A. and Amoo, S.O. 2008. Seed germination and in vitro propagation of *Piliostigma thonningii*-an important medicinal plant. African Journal of Biotechnology, 8(3): 401-404.

[22] Hossain, M. A., Arefin, M. K., Khan, B. M. and Rahman, M. A. (2005). Effects of Seed Treatments on Germination and Seedling Growth Attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery.Research Journal of Agriculture and Biological Sciences, 1(2): 135-141.

[23] Mayer, A.M. and Poljakoff-Mayber, A. 1963. The germination of seeds. MacMillan, New York, N.Y. pp: 236

[24] Nilkoleave, M.G. 1977. Factors controlling seed dormancy pattern. North Holland Publishing ompany, Amsterdam, pp. 51-74.

[25] Bajpai, P. N. 1970. Studies on flowering and fruit development in aonla (*Emblica officinalis* Gaertn.). Horticultural Advance, VII, 38-67

[26] Sant Ram and Raja Rao, T. 1981.Naturally occurring Auxins and inhibitors and their role in fruit growth of aonla (*Emblica officinalis* Gaertn.). New Phytol, 88,53 – 66,53.

[27] Baskin, C.C. and Baskin, J.M. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, Boston, MA.

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GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

Table 1. Effect of different treatments on the germination of *Emblica officinalis* seeds.

Treatments	Imbibition period (day)	Total germination period (day)	Germination % (GP) (at the end of the test)	Total no. of germination speed (∑DGs)	Total no. of germinated seed (N)	Germination value (GV)= {(∑DGs/N)x (GP/10)}	Germination energy
T1	25.00 (± 1.26)	20.00 (±1.27)	84.00 (± 5.40)	483.66 (±6.86)	210.00	18.88	84.00
T2	6.00 (± 0)	6.00 (± 0)	3.20 (± 0.49)	2.64 (±0.08)	8.00	0.11	3.20
T3	6.00 (±0)	6.00 (±0)	60.00 (± 8.94)	50 (±1.47)	150.00	2.00	60.00
T4	24.00 (±0)	19.00 (±0)	86.00 (± 4.38)	435.73 (± 4.3)	215.00	17.43	86.00
T5	27.00 (±0)	9.00 (±0)	73.60 (± 1.94)	115.65 (±0.97)	184.00	4.63	73.60
T6	0	0	0	0	0	0	0
T7	21.80 (± 0.49)	13.80 (± 0.49)	81.20 (± 5.31)	285.10 (± 4.22)	203.00	11.40	81.20
LSD (P<0.05)	1.72	1.72	16.05	11.70	13.33	2.67	16.05

T_1 = Tap water for 24 hours, T_2 = Thiourea (2%) for 24 hours, T3 = GA_3 500ppm for 12 hours, T_4 = GA_3 500ppm for 24 hours,
T_5 = Stratification at 5^{o}C for 10 days, T6 = Acid scarification for 30 seconds, T7= Control

Table 2. Germination and other growth attributes of *Emblicaofficinalis* under laboratory condition after 7 days treatment

Treatment	Shoot length(cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Biomass (g)	Seedling vigor
T1	3.43(± 0.44)	3.09(± 0.19)	2.79 (± 0.44)	1.04 (± 0.17)	1.75(± 0.28)	547.68(±6.76)
T2	3.37(± 0.21)	2.93(± 0.28)	0.31(± 0.13)	0.01(± 0.05)	0.19(± 0.08)	20.16(±1.20)
T3	1.71(± 0.10)	1.50(± 0.06)	4.37(± 0.78)	1.69(± 0.35)	2.68(± 0.45)	192.6(±0.97)
T4	2.96(±0.98)	1.49(± 0.05)	2.19(± 0.39)	0.79(± 0.14)	1.39(± 0.25)	382.70(±4.32)
T5	2.60(± 0.18)	2.16(± 0.09)	3.97(± 0.83)	1.50(± 0.32)	2.46(± 0.51)	350.34(±4.54)
T6	0	0	0	0	0	0
T7	3.94(± 0.41)	2.62(± 0.24)	5.69(± 1.35)	2.01(± 0.51)	3.68(± 0.84)	532.67(±6.76)
LSD (P<0.05)	0.83	0.74	1.10	1.32	0.35	21.42

Key as in Table 1. Values in parentheses indicates ±SEM, n=5