E-ISSN: 2663-1067
P-ISSN: 2663-1075
IJHFS 2019; 1(2): 44-47
Received: 21-05-2019
Accepted: 25-06-2019

Pratiksha Wagle
Student, M.Sc. Agriculture, Agriculture and Forestry University, Rampur, Nepal

Ritesh Kumar Jha
Student, M.Sc. Agriculture, Agriculture and Forestry University, Rampur, Nepal

Krishna Prasad Thapaliya
Assistant Professor Center for Development Studies and Rural Sociology, Agriculture and Forestry University, Rampur, Nepal

Corresponding Author:
Ritesh Kumar Jha
Student, M.Sc. Agriculture, Agriculture and Forestry University, Rampur, Nepal

Effect of chemical and hormonal treatments on breaking the dormancy of edy mayal (*Malus baccata*) seeds at Jumla, Nepal

Pratiksha Wagle, Ritesh Kumar Jha and Krishna Prasad Thapaliya

Abstract
The experiments were led to study the effect of pretreatments (concentrated sulphuric acid and gibberellic acid) on germination behavior of Malus baccata by analyzing three parameters i.e. GP: germination percentage, MGT: mean germination time (days) and GRI: germination rate index for various time interval in petridishes. Pre-sowing treatment included immersion in sulphuric acid (98%) for 10, 20 and 30 minutes and immersion in gibberellic acid of 1000, 1250 and 1500 ppm for 24 hrs. The sowing in distilled water (Control) had no positive effect on the germination induction. Generally, the seed pretreatments with sulphuric acid (98%) were very useful to improve germination. Time of immersion significantly ($P < 0.001$) affected GP, MGT and GRI. Treating Malus baccata seeds with sulphuric acid (98%) for 10, 20 and 30 minutes significantly broke the seed dormancy and promoted the germination of the seeds compared to control seeds (treated with distilled water) and gibberellic acid. However, increasing duration had a negative effect on seed germination, reducing the final germination percentage from 93.33% at 10 minutes of immersion to 85.33% at 30 minutes. Priming with gibberellins enhanced the germination but did not completely overcome the dormancy of intact Malus baccata seeds.

Keywords: Pre-treatment, dormancy, germination, sulphuric acid, scarification

1. Introduction
Nepal has diverse ecological and edaphic conditions that favor natural existence of many wild fruit species. Some of these species, but more commonly available in Jumla and Mustang districts of western Nepal are crab apple (Ghobligen, Edimayel and Usen), wild peach, wild apricot, wild cherry, wild walnut, and wild pear (DFRS, 2015) [4]. Edy Mayal, deciduous specimens that flower beautifully in the spring but produce only small, bitter apples of less than 2 inches in diameter, are prime rootstock candidates for other apple varieties. In Jumla, Edy Mayal (*Malus baccata*) is collected from the wild or only cultivated to a limited extent.

Crab apple (Edy Mayal) is a deciduous tree with a rounded canopy of spreading branches, ultimately reaching 20 to 50 feet in height valuable for landscaping, animal habitat and food. The trees produce beautiful, fragrant blossoms in the spring and abundant colourful fruit in late summer and early fall. The small fruit may be harvested or left on the trees as food for birds and other wildlife (Karki, Rizal, Manandhar, Atreya, & Gotame, 2017) [8]. Some native species of crab apple yield very small, intensely sour fruit with poor flavour, although many cultivars have been developed that produce flavourful, pleasantly tart and very useful culinary “crabs" (Delden, Dinstel, & Cascio, 2011) [3].

The main problem encountered in Edy Mayal is high level of dormancy and poor seed germination caused by the water-impermeable seed coat. Seed dormancy hinders the completion of germination of an intact viable seed under favorable conditions. Due to water impermeable seed coat of Edy Mayal species, seeds do not germinate promptly when subjected to conditions normally regarded as suitable for germination. This makes it impossible to obtain uniform germination in the nursery and at times it may take Edy Mayal seeds up to 3 months to germinate.

Malus baccata propagation is usually through seeds. All the *Malus baccata* species collected in Jumla have a problem of seed dormancy thus limiting its cultivation. Seed dormancy caused a major difficulty in Edy Mayal cultivation. Seeds having exogenous dormancy will not germinate until subjected to stratification

(Ahmadloo, Kouchakesaraei, Goodarji, & Salehi, 2017) [1]. To release the *Malus baccata* seeds from dormancy, cold stratification is done in sand for 2-3 months to reach germination percentage of 65-70%. The present study was carried out to investigate the effect of some recommended seed presowing treatments like sulphuric acid and gibberellic acid on the germination parameters of intact Edy Mayal seeds.

The study was conducted with the general objective to study the effect of chemical and hormonal treatments on breaking the dormancy of Edy Mayal (*Malus baccata*) seeds and specific objecives to investigate the effect of sulphuric acid and gibberellic acid on the germination capacity, to shorten the germination period and to increase the germination percentage of Edy Mayal seeds.

2. Materials and methods

The experiment was conducted under laboatory conditions at Karnali Technical School (KTS), Chandannath-4, Jumla located about 3 Km north east of Khalanga (headquarter of Jumla district). The school is located at longitude 81°10'45"E and Latitude 29°18'30.3"N with an altitude of 2514 MASL.

2.1 Experimental Design

The experiment was conducted in completely randomized design with three replicates. The treatments were control (distilled water), sulphuric acid 98% (10, 20 and 30 min) and gibberellic acid (1000, 1250 and 1500 ppm). The sieving and the flotation were used to remove empty, broken and damaged seeds. Only the seed that sank and settled at the bottom of the beaker were then spread on filter paper to dry and subjected to respective treatments. The seeds from respective treatments were then arranged in the petridishes (20 seeds per petri-dish) per replicate papered with two layers of moist Whatman filter paper and placed under favourable conditions for germination. Filter paper was moistened with distilled water every 48 hrs.

2.2 Pre-Sowing Treatments

For gibberellic acid treatment, the different concentration were prepared by weighing the different amount of gibberellin powder on digital weighing balance. For 1000 ppm, 0.1gm of gibberellin powder was weighed in digital balance and then it was taken in a beaker. N/10 NaOH was added drop-wise and stirred until the GA_3 was dissolved. The P^H of the solutionwas checked using P^H meter and was made neutral by adding N/10 HCL drop by drop. When the P^H was neutral, the solution was made 100 ml by adding distilled water that is 1000 ppm solution of GA_3. For 1250 ppm, 0.125 gm and for 1500 ppm, 0.15 gm gibberllin was used.

For sulphuric acid (98%) treatment, heat resistant non-corrosive beakers (100ml) were used for the seed immersion. Sulphuric acid (98%) was poured slowly from the side of the beaker. The three seed lots were left in beaker for different time interval i.e. 10, 20 and 30 min after which the seeds were thoroughly washed and rinsed to remove all the acid.

In the sulphuric acid (98%) treatment, seeds were divided into three 100 ml heat resistant non-corrosive beakers and sulphuric acid (H_2SO_4) was poured slowly on the side of the beaker to a level where all seeds were covered (50 ml). The three seed lots were left in beaker for different time interval i.e. 10, 20 and 30 min, after which the seeds were removed and the acid drained off into another beaker. The seeds were thoroughly washed and rinsed to remove all the acid.

2.3 Germination Parameter

Number of seedlings emerging daily were counted from days of planting the seeds till the time germination was completed or ceased.

Kinetics of germination: To estimate better the physiological meaning of the germination, the number of germinated seeds was counted on the 20^{th} 25^{th}, 30^{th}, 35^{th} and 40^{th} day.

Germination Rate Index (GRI): It reflects the speed of germination or emergence. GRI is computed by using the following formula:

$$GRI = \Sigma \frac{\text{No. of Germinated Seeds}}{\text{No. of days}} \quad \text{i.e.}$$

$$GRI = (G_1/1) + (G_2/2) + \ldots\ldots\ldots\ldots + (G_x/x)$$

Where, G is the germination day 1, 2, and x represents the corresponding day of germination.

Mean Germination Time (MGT): It represents the meantime, a seed lot requires to initiate and end seed germination. MGT is calculated by the formula:

$$MGT \text{ (days)} = \frac{\Sigma n.D}{\Sigma n}$$

Where:

n is no. of seeds newly germinated at time D, D is days from the beginning of the germination test, and Σn is final germination

Final Germination Percentage: Germination % is calculated as;

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Number of tested seeds}} \times 100\%$$

(*Source*: Kader, 2005) [7]

2.4 Data Analysis

All data were systematically arranged on the basis of various observation parameters. Statistical software Gen stat 64-bit Release 18.2 and MS Excel were used for data analysis. DMRT was used for mean separation at 5% level of significance.

3. Results

The data related to kinetics of germination of *Malus baccata* seeds as influenced by gibberellic acid and sulphuric acid is presented in Table 1.

Table 1: Effect of various concentrations of Gibberellic acid and sulphuric acid on kinetics of germination of *Malus baccata* seeds

Treatments	Germination percentage at different days of treatment (%)				
Gibberellic acid (GA_3)	20 DAT	25 DAT	30 DAT	35 DAT	40 DAT
1000 ppm	6.67 ^{c}	45.00 ^{b}	70.00 ^{b}	83.33 ^{a}	85.55 ^{a}
1250 ppm	5.00^{c}	46.67 ^{b}	78.33 ^{ab}	85.00 ^{a}	85.00 ^{a}
1500 ppm	11.67 ^{c}	50.00 ^{b}	80.00 ^{ab}	90.00 ^{a}	91.67 ^{a}
Conc. H2SO4					
10 min	30.00^{b}	61.67 ^{ab}	85.00 ^{a}	93.33 ^{a}	93.33 ^{a}
20 min	38.33^{ab}	71.67 ^{a}	85.00 ^{a}	90.00 ^{a}	90.00 ^{a}
30 min	50.00^{a}	76.67 ^{a}	85.00 ^{a}	86.67 ^{a}	86.67 ^{a}
Control	3.33^{c}	21.67 ^{c}	33.33 ^{c}	45.00 ^{b}	53.33 ^{b}
CV%	43.1	18.0	8.4	6.5	6.5
LSD	15.64***	16.77***	10.81***	9.36***	9.55***
SEM	5.16	5.53	3.56	3.09	3.15
Grand mean	20.7	53.3	73.8	81.9	83.6

Note: Means with the same letter do not differ significantly at p=0.05 at DMRT, CV: Coefficient of variation, LSD: Least significant differences, SEM: Standard error of mean, ** denotes significant at 0.1% level

Significant variation was found for germination at 20 DAT. In case of kinetics of germination, significant differences were seen among different treatments in 20th, 25th, 30th, 35th and 40th day after treatment. It was found that the highest germination of seed was given by immersion in sulphuric acid (98%) for 30 min (50.00%) in initial days of germination. However extension of the soaking time showed unfavorable results on germination. Acid treatment exhibits higher kinetics compared to other treatments.

The data related to the germination rate index, mean germination time and germination % of *Malus baccata* as influenced by seven treatments are shown in table.

Table 2: Effect of various concentrations of GA_3 and conc. H_2SO_4 on germination rate index, mean germination time and germination percentage of *Malus baccata* seeds

Treatments	GRI	MGT	Germination %
Gibberellic acid (GA_3)			
1000 ppm	0.6691 ^{d}	26.08 ^{b}	85.00 ^{bc}
1250 ppm	0.6912 ^{cd}	25.03 ^{b}	85.00 ^{bc}
1500 ppm	0.7610 ^{bc}	24.96 ^{b}	91.67 ^{ab}
Conc. H2SO4			
10 min	0.8426 ^{ab}	23.32 ^{ab}	93.33 ^{a}
20 min	0.8558 ^{a}	22.02 ^{a}	91.00 ^{abc}
30 min	0.8399 ^{ab}	20.69 ^{a}	85.33 ^{c}
Control	0.4652 ^{e}	31.64 ^{c}	68.33 ^{d}
CV%	6.8	5.9	4.6
LSD	0.0871***	2.573***	6.889***
SEM	0.0287	0.848	2.271
Grand Mean	0.732	24.82	85.24

Note: Means with the same letter do not differ significantly at p=0.05 at DMRT, CV: Coefficient of variation, LSD: Least significant differences, SEM: Standard error of mean, *** denotes significant at 0.1% level

The obtained results highlight the effects of various treatments which has a very important role in seed germination. The results on Table 2 showed that control (sowing in distilled water) had no positive effect on the induction of germination activity. The data indicated that sulphuric acid treatments exerted a significant effect on GRI, MGT, and germination % of seeds as shown above in Table 2. Acid treatment exhibited highest GRI and lowest MGT. The positive responses of seeds to the pre-sowing scarification treatments (sulphuric acid) suggest that the hard testa is responsible for the low percentage germination of untreated seeds by preventing imbibitions of water. Concentrated sulphuric acid scarifies the *Malus baccata* seeds and promoted water imbibitions by the seed, hence increases seed germination. However, the germination percentage decreased from 95.33 to 85.33 with the increase in time of exposure to sulphuric acid because of the corrosive nature of sulphuric which acid damages the embryos of the seeds.

4. Discussion

Concentrated sulphuric acid scarifies the *Malus baccata* seeds and promoted water imbibitions by the seed, hence increases seed germination. The findings of the experiment were in line with the (Emongor, Mathowa, & Kabelo, 2004) [5] who reported that concentrated sulphuric acid overcame the seed dormancy in *Corchorus tridens* but exposing the seeds for more than 10 min to 98% sulphuric acid, decreased significantly the germination capacity because sulphuric acid being corrosive might have damaged the embryos of some seeds.

Acid treatment exhibited highest germination and lowest MGT. The results were in agreement with the earlier findings of (Asl, Sharivivash, & Rahbari, 2011) [2] who reported that the more rapidly the seed coat is ruptured the faster the rate of germination. Sulphuric acid is thought to disrupt the seed coat and expose the lumens of the macro sclereids cells, permitting imbibitions of water which trigger germination but prolonged immersion may be injurious to the seeds as the acid may rupture vital parts of the embryo.

5. Conclusion

The results of the present study showed that treating *Malus baccata* seeds with concentrated sulphuric acid (98%) for 10, 20 and 30 minutes significantly broke the seed dormancy and promoted the germination of the seeds compared to gibberellic acid and control. Treating *Malus baccata* seeds with sulphuric acid for more than 10 minutes significantly decreased germination capacity. Priming with gibberellins enhanced the germination but did not completely overcome the dormancy of seeds.

6. Acknowledgement

The authors would like to express deepest acknowledgement, gratitude and appreciation to Prime Minister Agriculture Modernization Project, Agriculture and Forestry University, Karnali Technical School and all concerned respondents for their support during research work.

7. References

1. Ahmadloo F, Kouchakesaraei MT, Goodarji GR, Salehi A. Effects of gibberellic acid and storage temperature on the germination of hawthorn seeeds. Journal of forest science, 2017, 417-424.
2. Asl MB, Sharivivash R, Rahbari A. Effect of Different Treatments on Seed Germination of Honey Locust (*Gleditschia triacanthos*). East Azarbaijan: Canadian Center of Science and Education, 2011.
3. Delden KV, Dinstel RR, Cascio J. *Crabapples.* Alaska: University of Alaska Fairbanks, 2011.
4. DFRS. High mountains and high himal forests of Nepal. Kathmandu: Government of Nepal, 2015.
5. Emongor VE, Mathowa T, Kabelo S. The Effect of Hot water, Sulphuric Acid, Nitric Acid, Gibberellic acid and Ethephon on the Germination of Corchorus (*Corchorus tridens*) seed. Journal of Agronomy, 2004, 196-200.
6. Gilman EF, Watson DG. *Malus baccata,* Siberian Crab Apple, 1994.
7. Kader MA. A Comparison of Seed Germination Calculation Formulae and The Associated Interpretation Resulting data. Journal & Proceedings of the Royal Society of New South Wales, 2005, 65-75.
8. Karki S, Rizal G, Manandhar R, Atreya PN, Gotame TP. Minor Fruits in Nepal; Utilization and conservation Efforts. *Conservation and Utilization of* Agricultural Plant Genetic Resources in Nepal. Kathmandu, 3, Kathmandu, 2017.
9. Kheloufi A, Mansouri LM, Boukhatem FZ. Application and use of sulphuric acid pretreatment to improve seed germination of three acacia species. REFORESTA, 2017, 1-10.