Thapliyal M *et al.* (2021)
Notulae Scientia Biologicae
Volume 13, Issue 4, Article number 11044
DOI:10.15835/nsb13411044
Research Article

Seed germination response of Indian wild pear (*Pyrus pashia)* to gibberellic acid treatment and cold storage

Manisha THAPLIYAL*, Namitha N. KALIYATHAN, Kanupriya RATHORE

Forest Research Institute, Forest Tree Seed Laboratory, Dehradun 248006, India;
*manishathapliyal98@gmail.com (*corresponding author); namithafri@gmail.com; kanupriyarathore16@gmail.com*

Abstract

Knowledge of seed germination behaviour of different seed sources of tree species is useful in selecting the most responsive and adaptive ones for propagation and germplasm conservation. The wild Himalayan pear (*Pyrus pashia* Buch-Ham ex D. Don) produces highly nutritious edible fruits that are consumed by local communities. The populations of the species are threatened due to exploitation and lack of adequate conservation programmes. The study was conducted to examine the germination response of *P. pashia* seeds from two sources (S1-Champawat and S2-Pithoragarh) in Uttarakhand state of India, to different GA_3 treatment and also to assess the viability and longevity of the seeds in cold storage (5 °C) for three years. In both sources germination percent (GP) increased significantly under GA_3 treatment and speed of germination was also enhanced (reduction in mean germination time). In Source 1, GP increased under all GA_3 application, highest being 94% under GA_3 500 ppm treatment. In Source 2, GP of seeds doubled under 100 ppm GA_3 treatment while its higher concentrations did not improve the germination. However, the differences observed in germination between the seed sources could be due to differences in the dormancy levels and/or sensitivity to dormancy breaking elements across their geographical range. Thus, exogenous application of GA_3 is suggested for enhancing the germination in seeds of *P. pashia*. Seeds responded to cold storage by increased germination with duration, i.e. highest after three years in storage, indicating that the seeds got the required chilling treatment for overcoming dormancy.

Keywords: conservation; GA_3 treatment; physiological dormancy; seed source; storage; wild Himalayan pear

Introduction

Pyrus pashia commonly known as Indian Pear, wild Himalayan Pear, belongs to the family Rosaceae. It is a moderate-sized deciduous tree with branches dark-brown in colour and often spiny. Leaves are simple, glabrous and ovate-lanceolate in shape (Kanjilal, 1928). Fruit of *P. pashia* is Pome, brown in colour, pulpy and contains approximately five seeds per fruit. Leaves of *P. pashia* are used as fodder, wood is utilised as fuel and for making small agricultural implements. Fruits are edible, highly nutritious and are included in the diet by

Received: 10 Aug 2021. Received in revised form: 02 Nov 2021. Accepted: 05 Nov 2021. Published online: 11 Nov 2021.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

local people. They provide multiple ecosystem services as a source of nutrition, pollination, genetic variation for breeding programs, and even of valuable income for people living around them (Powell *et al.*, 2013).

The wild populations of *P. pashia* are distributed in India, Nepal, Bhutan, Afghanistan, Pakistan, Thailand, China, and Indochina at an altitude of 750-2500 m. In India, the species are found in Himalayan region and north-eastern regions (Troup, 1975). *Pyrus* species are threatened with about 85% pear varieties lost in 19th century (Sindelar, 2002). Furthermore, due to various insect pests, pathogens and abiotic stresses, valuable and highly resistant pear germplasm has been lost (Fowler and Mooney, 1990). Therefore, more efforts to conserve the genetic resources of these species must be made. The genetic diversity can be best preserved by systematic collection for establishing field germplasm banks and long-term storage of seeds in Seedbanks.

Pyrus species are widely cultivated in many parts of the world but physiological dormancy associated with seed causes irregular germination in the species. Dormancy plays a major role in regulating germination in many tree species. Dormancy release is regulated by a combination of environmental and endogenous signal with both synergistic and competing effects. The balance of abscisic acid (ABA); gibberellin (GA) levels and sensitivity is a major, but not the sole, regulator of dormancy status (Finkelstein *et al.,* 2008). Pear seeds belong to Rosaceae family usually show dormancy at maturity and require 2-3 months at 0-7 °C temperature in a moist, well-aerated medium to break dormancy (Hartmann *et al.,* 1990), which is similar to the requirements for apple seeds (Lewak, 2011). Gibberellic acid (GA) activates the production of α-amylase, proteolytic enzymes, nucleases, etc., leading to loss of seed dormancy and promotion of seed germination (Dominguez *et al.,* 2004; Mrva *et al.,* 2006). Studies evaluating the exogenous application of gibberellic acid have shown to relieve certain types of dormancy, including physiological dormancy, thermo-dormancy and photo dormancy, and to replace partially or fully the necessary cold treatments in different plants (Hartmann *et al.*, 1997).

Seed viability can be extended by cold or dry storage at seed moisture content below 5% (Huang *et al.,* 2003). Cold storage reduces or prevents the effects of ageing was indicated by the slower rate of damage caused by low temperature (Spano *et al.*, 2006). The aim of our study was to examine the germination response of *P. pashia* seeds from two sources (Champawat and Pithoragarh) of Uttarakhand state in India, to different GA_3 treatment (soaking in 100, 300, 500 and 700 ppm solutions for 24 hours) and also to assess the viability and longevity of the seeds stored at moisture content of 8% at 5 °C.

Materials and Methods

Seed material

Mature fruits of *Pyrus pashia* were collected in October 2017 from natural stands at two locations namely Champawat Forest Range (Source 1 or S1) and Pithoragarh Forest Range (Source 2 or S2) in Uttarakhand state of India. Geographical coordinates of the two sources of the species are presented in Table 1. Fruits were brought to Forest Tree Seed Laboratory of the Institute at Dehradun in permeable bags. The species produces pulpy fruits with seeds consisting of hard endocarp. Fruits were macerated, de-pulped and washed under running tap water to extract the seeds. Extracted seeds were shade- dried in ambient conditions for 3-4 days.

Table 1. Geographical locations and altitudes of the two seed sources of *Pyrus pashia*

Site of seed collection	Co-ordinates	Altitude (m asl)
Source1 Champawat	29^{0}32'50.2"N 80^{0}11'07.1"E	1937
Source 2 Pithoragarh	29^{0}58'15.3"N 80^{0}39'16.3"E	2540

Moisture content of the seeds

The moisture content of the seed was determined by low constant oven dry method at 103 ±1 °C for 17 hrs (ISTA, 2010) in 4 replicates of 10 seeds each. Moisture content was determined on the fresh weight basis (%) using the formula:

$$MC = \frac{(Fw - Dw)}{Fw} \times 100$$

where MC is the moisture content, Fw is the initial fresh weight of the replicate and Dw is the dry weight.

Germination test

Germination test was conducted on fresh seeds from both seed sources. The experiment was designed in completely randomised block in four replications of 25 seeds each, which were placed on moist Whatman filter paper in sterile Petri dishes (15 cm dia.) and kept in a seed germinator (NSW New Delhi, India) at 25 °C temperature (ISTA, 2010) with 8 hours photoperiod. A seed was considered to have germinated when the radical was 1 cm long. The observations on seed germination were taken every day (except weekends) up to 28 days from the date of sowing (ISTA, 2010). Germination parameters such as germination percent; Mean Germination Time (Orchard, 1977) and Germination value (Djavanshir and Pourbeik, 1976) of the species were measured as per the standard methods. Germination percent of seed was expressed as:

Germination percentage (G) = total number of seed germinated at end of germination test/total number of seeds placed for germination test ×100.

MGT - a parameter used for monitoring the speed of germination was calculated as:

Mean germination time (MGT) = ΣFx/ΣF; where F is the number of seeds germinated on day x.

Germination value was expressed as ∑DGS/N x (Final cumulative Germination Percent/10); where DGS is daily germination speed which is calculated by dividing cumulative germination percent by the number of days since beginning the test, N is number of counts and 10 is constant through germination test.

Gibberellic acid treatment

For observing the effect of gibberellic acid pre-treatment on the germination and its speed, seeds from both sources were pre-treated with GA_3 at different concentrations; seeds were soaked in 100 ppm, 300 ppm, 500 ppm and 700 ppm GA_3 solution for 24 hours before placing for germination experiment. For each test separate seed samples were used. Untreated seeds served as control.

Seed storage

Fresh seed of *P. pashia* from both sources after quality evaluation (seed germination test) were desiccated to 8% moisture content from initial 11% using silica gel in 1:1 ratio. Desiccated seeds were thereafter stored at 5 ± 1 °C temperature in air-tight plastic boxes in a seed storage chamber and their viability was evaluated through germination test every six months, up to a period of three years (results only presented for yearly germination) to evaluate the germination pattern and longevity of seeds under storage conditions.

Statistical analysis

Statistical analysis of cumulative germination data was performed with SPSS Statistics for Windows, version 16.0' (SPSS Inc., Chicago, Ill., USA). Statistical analysis was performed using GLM multivariate ANOVA for testing the significance of treatment (4 levels for GA_3 and 3 for storage duration), population (two levels) and their interactions on GP, MGT and GV. All tests were analysed at a significance level of α = 0.025.

Results

Germination response of P. pashia seeds to GA_3 treatment

Untreated seeds (control) exhibited a GP of 62 % in S1and 15 % GP in S2. This difference could be due to the degree of dormancy related to different seed sources. In S1, MGT was recorded as 12.37 days and in S2 MGT was recorded as 15.45 days (Figure 1) indicating significantly lower germination and slow speed of germination in seeds of Source 2. GV was observed as 12.02 in S1 and much lower (0.46) in S2 (Figure 2).

GA_3 treatment of seeds significantly increased the germination percent against that in untreated seeds. Highest GP (94 %) was observed in the seeds pretreated with GA_3 500 ppm followed by the ones treated with 300 ppm (93 %), 700 ppm (92 %) and 100 ppm (86 %). Seeds pretreated with GA_3 at 100 ppm resulted in highest GP (31 %) in Source 2 followed by 700 ppm (29 %), 300 ppm (25 %) and 500 ppm (23 %) (Figure 1). Mean germination time (MGT) which indicates the speed of germination ranged from 5.4 to 6.6 days in S1 and 7.4 to 10.6 days in S2. Germination was fastest (lowest MGT) in the seeds treated with 700 ppm GA_3 in S1 and 500 ppm GA_3 in S2. Highest MGT (slow speed of germination) was observed in the seeds treated with 100 ppm GA_3 in both the sources (Figure 1) which means that seeds took longer to complete the germination. GV ranged from 42.6 to 60.4 in S1 and 2.4 to 4.8 in S2, which was significantly lower than that of seeds of S1 as the germination of seeds was significantly lower impacting the overall value of germination. Highest GV was recorded in seeds treated with 700 ppm GA_3 in both sources (Figure 2).

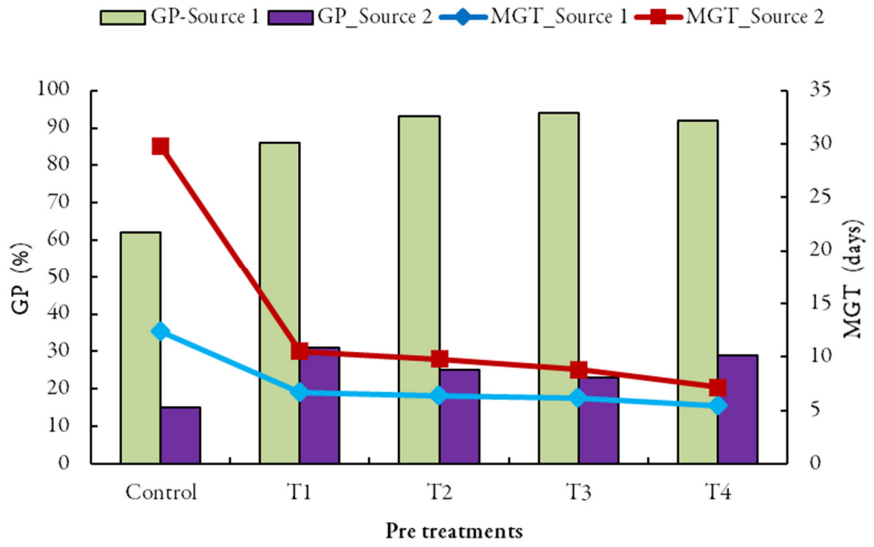

Figure 1. Germination Percent (GP) and Mean Germination Time (MGT) of *Pyrus pashia* seeds from Source 1 (Champawat) and Source 2 (Pithoragarh) after pre-soaking seeds in T1-100 ppm, T2-300 ppm, T3-500 ppm and T4-700 ppm GA_3 solution

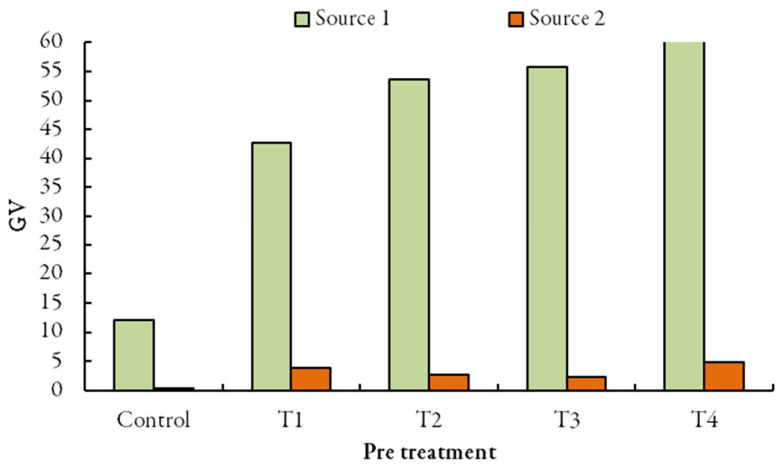

Figure 2. Germination value (GV) of *Pyrus pashia* seeds from Source 1 (Champawat) and Source 2 (Pithoragarh) in pre-soaked seeds in T1-100 ppm, T2-300 ppm, T3-500 ppm and T4-700 ppm GA_3 solution

Germination response of seed to cold storage condition

Study assessed the seed germination behaviour of stored seed for three years. In S1, germination percent of seed was 55 % after one year of storage, which increased to 64.5 % after second year and further to 82.5 % in third year while in case of seeds of other source (S2), GP was 12.25 %, 17.33 % after first and second year respectively, that doubled to 34.75 % after three years of storage. In both sources, highest GP was observed in third year of storage as compared to first year (Figure 3) which clearly indicated that the seeds required cold treatment which they got and responded favourably by increased GP. In S1, lowest and highest MGT was 9.4 and 12.8 days. Lowest MGT in seed was recorded in the second year of storage that exhibited faster speed of germination in the seeds. In S2, lowest MGT of 1.4 days was recorded in the third year and highest was 8.9 days after the first year of storage (Figure 3). Highest GV was recorded in seeds stored for three years in both sources (Figure 4) as germination was also highest in them.

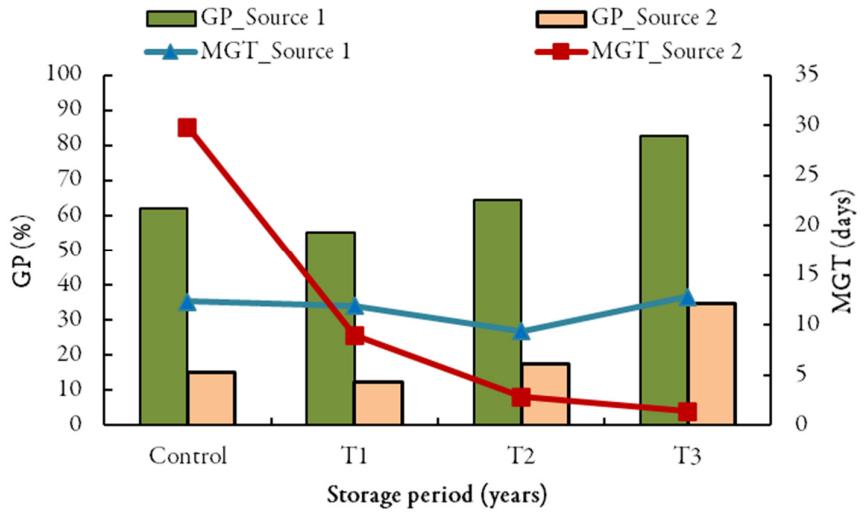

Figure 3. Germination Percent (GP) and Mean Germination Time (MGT) of *Pyrus pashia* seeds from Source 1 (Champawat) and Source 2 (Pithoragarh) in cold storage (5 °C) after T1- 1 year storage, T2- 2 years storage and T3- 3 years storage duration

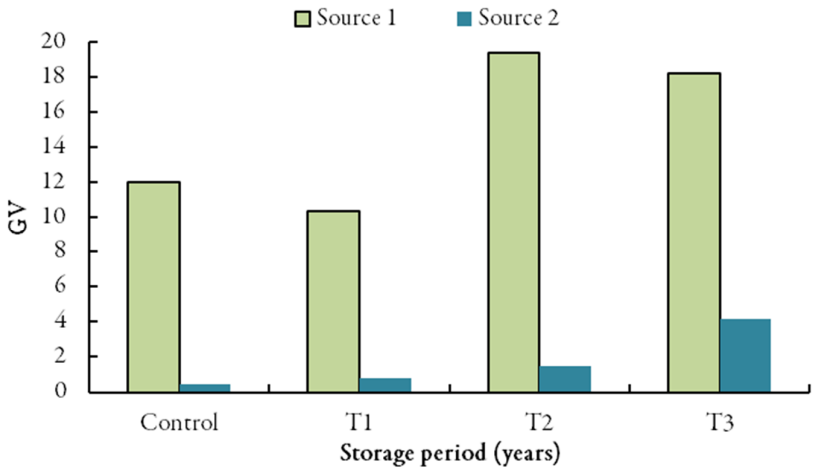

Figure 4. Germination Value (GV) of *Pyrus pashia* seeds from Source 1 (Champawat) and Source 2 (Pithoragarh) in cold storage (5 °C) after T1- 1 year storage, T2- 2 years storage and T3- 3 years storage duration

The effect of GA_3 treatment (various concentrations) on germination percent and mean germination time of seeds of *P. pashia* was significantly different ($p < 0.025$), highest germination occurred in seeds pre-soaked in 500ppm GA_3 solution in S1 and those in 100 ppm in S2. Similarly, the effect of population on germination percent and mean germination time of seed was significantly different ($p < 0.025$) but the effect of interaction between treatment and population on germination percent and mean germination time of seed was not significantly different ($p > 0.025$) (Table 2). There was significant effect of treatments, population and interaction between treatment and population on germination value meaning that the germination value which is an index of expression of speed and totality of germination, under different treatments differed in the two populations significantly ($p < 0.025$).

Table 2. Summary of General Linear Model (GLM) multivariate analysis for testing significant effects of seed sources and treatment

Variable	Source of variation	DF	SS	MS	F-value	p-value
GP	TRT	7	7030.6	1004.4	10.268	0.000000
	Population	1	48662.9	48662.9	497.519	0.000000
	TRT*Population	7	1649.2	235.6	2.409	0.033846
	Error	48	4694.9	97.8		
	Total	63	62037.6			
MGT	TRT	7	434.888	62.127	16.997	0.000000
	Population	1	54.199	54.199	14.829	0.000348
	TRT*Population	7	65.042	9.292	2.542	0.026118
	Error	48	175.444	3.655		
	Total	63	729.573			
GV	TRT	7	6885.88	983.70	29.0088	0.000000
	Population	1	15826.24	15826.24	466.7086	0.000000
	TRT*Population	7	5700.22	814.32	24.0139	0.000000
	Error	48	1627.70	33.91		
	Total	63	30040.03			

Treatment includes pre-soaking in 100 ppm; 300 ppm 500 ppm; and 700 ppm GA_3 solution and cold storage for 1, 2 and 3 years on germination percent (GP), mean germination time (MGT) and germination value (GV) of *P. pashia* seeds from Source 1 (Champawat) and Source 2 (Pithoragarh) in Uttarakhand state in India. ($P<0.025$) denote significant difference on seed source and treatment independently and combined effect of both on GP, MGT and GV.

Discussion

Fresh seeds of *P. pashia* from both sources yielded lesser germination (without any pretreatment) as compared to GA_3 treatment. It was under GA_3 treatment, highest GP was exhibited in the seed pre-treated with 500 ppm in S1 while it was in 100 ppm in S2. Exogenous application of GA_3 breaks dormancy and accelerates seed germination in many Rosaceae members. Gibberellins stimulate germination by inducing hydrolytic enzymes that weaken the barrier tissues such as endosperm, seed coat, inducing mobilization of seed storage reserves, and stimulating the expansion of embryo (Finkelstein *et al.*, 2008). Kumar *et al.* (1988) studied germination in seeds of *P. pashia* using 0.5%, 1.2% and 5% ethanol and -3 log M, -5 log M and -7 log M GA_3. Unstratified seeds of *P. pashia* responded with the germination of 60% in -7 log M GA_3 and 90% in 5% ethanol treatment.

Fogle (1958) reported that soaking seeds of sweet cherry for twenty-four hours in gibberellin at a concentration of 100ppm immediately after collection substitutes for two or three months of the after-ripening period. Pillay *et al.,* (1965) reported that soaking seeds in gibberellin at a concentration of 100 ppm increases the rate of germination and partially replaces the chilling requirements in *Prunus avium* and *Prunus mahaleb*. According to Donoho and Walker (1957) seed treatment with gibberellin could replace the low-temperature requirement to some extent in *Prunus persica*. Treatment of seeds with GA_3 may substitute for cold stratification was also reported for *Prunus persica, Myrica pensylvanica* (Macdonald, 1993), *Arbutus unedo* (Smiris *et al.*, 2006), *Jasione supina* subsp. *supine* (Güleryüz, 2021). In our study also, germination of seeds significantly increased under GA_3 treatment exhibiting the replacement of chilling requirement of seeds for overcoming physiological dormancy which the seeds get during the winters in their natural habitat.

Germination percent of seeds increased from 55 to 82.5 % in accession 1 which was an increase of 27% and from 12.25 to 34.75% i.e. almost three times in S2 from first year to third year. It shows that seeds of *P. pashia* got the required chilling treatment during cold storage that resulted in subsequent increase in the GP and reduction in MGT. According to Baskin and Baskin (1988) some species can come out of dormancy during dry storage but a relative humidity of 30-40% is required for them to do so. Highest germination was observed in the seeds stored for 3 years in both sources. Storage of seeds in the air-dried state at low temperature for after-ripening between 1 °C to 10 °C or 2 °C to 5 °C for periods of some months to several years have been reported to break dormancy for certain forest and fruit trees (Crocker, 1948; Stokes, 1965). Highest germination of *P. pashia* seeds after three years in stored conditions indicate that seeds in cold storage (at 5 °C storage temp, 8% seed moisture content) required several months to reach full ripening, breaking dormancy and accelerate germination. Generally, desiccation plays a crucial role in the after-ripening process, which prepares the seeds for germination. This suggests that one of the functions of the desiccation process is preparation for germination (Wawrzyniak *et al.*, 2020).

Storage conditions and duration significantly affect germination percent has been reported in many species. Huang *et al.* (2003) reported that seed viability can be extended by cold or dry storage at seed moisture content below 5%. Temporary cold storage under high-moisture conditions enhanced germination in *Arbutus unedo* (Ana Vasques *et al.*, 2014). Abdelbasit *et al.* (2012) observed that cold and dry storage increased seed germinability with increasing storage time in *Acacia tortilis* of three provenances.

A comparative study on *P. pashia* seeds from two seed sources revealed a significant difference on GP, MGT of seed under similar pre-treatments ($p < 0.025$) but the effect of interaction between treatments and seed source is not significant in both seed sources under similar storage conditions which indicate the difference in quality of seeds collected from different natural ranges. Provenance /seed source studies in forest trees are very important for determining the quality, identifying the best and highly adaptable provenance and for screening the naturally available genetic variation to utilize the best material for maximum productivity and for further breeding programme (Suri, 1984; Shiv Kumar and Banerjee, 1986). Baskin and Baskin (1998) reported that fresh seed collected from various locations can alter the germination characteristics of seed due to different degree of dormancy. Seed from different accessions reported various depth of dormancy due to genotype-by-

environment interactions in *Arabidopsis* (Donohue *et al.*, 2005; Vidigal, 2016); *Medicago truncatula* (Renzi *et al.*, 2020). In the present study also, the initial germination of seeds from Champawat (S1) was 62% which was more than 4 times that of seeds from Pithoragarh (S2). This could be because of varying dormancy levels in the seeds. Donohue *et al.* (2005) observed that depth of dormancy is determined genetically as well as by the ambient environment during seed formation. Genetic variation for germination can be detected when genotypes are compared in identical environments. This implies that not only the conditions of the germination test must be identical, but also growth conditions during seed development and storage conditions, including the time that the seeds are stored, must be the same (Bentsink and Koornneef, 2008). Germination responses of a seed vary according to geographical and environmental factors viz. altitude, elevation, soil moisture, soil nutrient, temperature, kind and density of plant cover, degree of habitat disturbance of the seed where the seed matures (Ginwal *et al.*, 2005).

Conclusions

In the present study, seed from both sources responded to exogenous GA_3 applications as well as cold storage while higher seed viability and vigour was exhibited by seed collected from S1 in terms of all germination parameters. Seed source plays an important role for the contribution of germination percent; still further research should be extended to large provenance aligned in this direction for a better characterisation of effect of GA_3 and long-term storage on seed germination of Rosaceae members especially species subjected to rapid environmental changes like in *Pyrus pashia* from different provenances/sources.

Authors' Contributions

Conceptualization: MT and NNK, Laboratory work and data curation: KR, Technical Supervision: MT and NNK Data analysis and interpretation: MT, NNK and KR, Manuscript writing and review: MT, NNK and KR. All authors read and approved the final manuscript.

Acknowledgements

The work was supported by the Ministry of Environment, Forest and Climate Change, Government of India (Grant No. 13-17/2012-CAMPA). The authors thank Mr. Raman Nautiyal, Scientist for statistical analysis support to research findings. The authors also thank the anonymous reviewers for critical review of the manuscript for improving its quality.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdelbasit H, Mahgoup S, Eldoma A (2012). Effect of cold and dry storage on seed viability among three provenances of *Acacia tortilis* subspecies *raddiana* and subspecies *spirocarpa*. International Journal of Advanced Biological Research 2:130-137.

Baskin CC, Baskin JM (2004). A classification system of seed dormancy. Seed Science Research 14:1-16. https://doi.org/10.1079/SSR2003150

Baskin CC, Baskin, JM (1998). Seeds: Ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, California, USA. https://doi.org/10.1023/A:1011465920842

Bentsink L, Koornneef M (2008). Seed dormancy and germination. The Arabidopsis Book/American Society of Plant Biologists, pp 6. https://doi.org/10.1199/tab.0119

Crocker W (1948). Growth of plants. Reinhold Publishing Corporation, New York. https://doi.org/10.1002/jps.3030370918

Djavanshir K, Pourbeik H (1976). Germination value: A new formula. Silvae Genetica 25:79-83.

Domínguez F, Moreno J, Cejudo FJ (2004). A gibberellin-induced nuclease is localized in the nucleus of wheat aleurone cells undergoing programmed cell death. The Journal of Biological Chemistry 279:11530-11536. https://doi.org/10.1074/jbc.M308082200

Donoho CW, Walker DR (1957). Effect of gibberellic acid on breaking of rest period of Eleberta peach. Science 126:1178-1179. https://doi.org/10.1126/science.126.3284.1178-a

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005). Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. Evolution 596:740-757. https://doi.org/10.1111/j.0014-3820.2005.tb01750.x

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008). Molecular aspects of seed dormancy. Annual Review of Plant Biology 59:387-415. https://doi.org/10.1146/annurev.arplant.59.032607.092740

Fogle HW (1958). Effects of duration of after ripening, gibberellins and other pretreatments on sweet cherry germination and seedling growth. Proceedings of American Society for Horticultural Science 72:129-133. https://doi.org/10.2307/2440115

Fowler C, Mooney P (1990). Shattering food, politics and the loss of genetic diversity. The University of Arizona Press. Tucson, USA.

Ginwal HS, Phartyal SS, Rawat PS, Srivastava RL (2005). Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* in central India. Silvae Genetica 54:76-80. https://doi.org/10.1515/sg-2005-0012

Güleryüz G, Kırmızı S, Arslan H, Güleryüz E (2021). Breaking of dormancy in the narrow endemic *Jasione supina* Sieber subsp. *supina* (Campanulaceae) with small seeds that do not need light to germinate. Acta Botanica Croatica 80:12-17. https://doi.org/10.37427/botcro-2021-009.

Hartmann HT, Kester DE, Davies FT, Jr., Geneve RL (1997). Plant propagation: Principles and Practices. 6thed. Prentice Hall Upper Saddle River, New Jersey. https://doi.org/10.1016/S0304-4238(97)00085-X

Huang Z, Zhang X, Zheng G, Gutterman Y (2003). Influence of light, temperature, salinity and storage on seed germination of *Haxloxylon annodendron*. Journal of Arid Environments 55:453-464. https://doi.org/10.1016/S0140-1963(02)00294-X

ISTA (2010). International Rules for Seed Testing. Zurichstr.50, CH-8303 Bassersdorf, Switzerland.

Kanjilal UN (1928). Forest flora of the Chakrata, Dehradun and Saharanpur Forest Divisions. Uttar Pradesh, 3rd ed. Govt. of India Press, Delhi.

Kumar A, Nautiyal MC, Prakash S (1988). Enhancement of pear *(Pyrus pashia* L.) seed germination by GA and ethanol. Current Science 57:964-966.

Lewak S (2011). Metabolic control of embryonic dormancy in apple seed: seven decades of research. Acta Physiologiae Plantarum 33:1-24. https://doi.org/10.1007/s11738-010-0524-8

Macdonald B (1993). Practical woody plant propagation for nursery growers. 4th edition. Timber Press, Portland, Oregon.

Mrva K, Wallwork M, Mares DJ (2006). Alpha- amylase and programmed cell death in aleurone of ripening wheat grains. Journal of Experimental Botany 57:877-885. https://doi.org/10.1093/jxb/erj072

Orchard T (1977). Estimating the parameters of plant seedling emergence. Seed Science and Technology 5:61-69.

Pillay DTN, Brase KD, Edgerton LJ (1965). Effects of pretreatments, temperature and duration of after ripening of germination of Mazzard and Mahaleb Cherry seeds. American Society for Horticultural Science 86:102-107.

Powell B, Ickowitz A, McMullin S (2013). The role of forests, trees and wild biodiversity for nutrition-sensitive food systems and landscapes. In: Expert background paper for the International Conference on Nutrition, Food and Agriculture Organization of the United Nations. Rome, Italy.

Renzi JP, Duchoslav M, Brus J, Hradilová I, Pechanec V, Václavek T, ... Smýkal P (2020). Physical dormancy release in *Medicago truncatula* seeds is related to environmental variations. Plants 9:503. https://doi.org/10.3390/plants9040503

Shiv Kumar P, Banerjee AC (1986). Provenance trail of *Acacia nilotica.* Journal of Tree Sciences 5:53-56.
Sindelar J (2002). Toward threatened forest tree species preservation on the example of crab apple (*Malus sylvestris* L.) and wild pear (*Pyrus pyraster* L. [Burgsdorf]). Zprav Lesnic Vyzk 47:199-203.
Smiris P, Pipinis E, Aslanidou M, Mavrokordopoulou O, Milios E, Kouridakis A (2006). Germination study on *Arbutus unedo* L. (Ericaceae) and *Podocytisus caramanicus* Boiss. & Heldr (Fabaceae). Journal of Biological Research 5:85-91.
Spano C, Buselli RF, Castiglione MR, Bottega S, Grilli I (2006). RNases and nucleases in embryos and endosperms from naturally aged wheat seeds stored in different condition. Journal of Plant Physiology 164:487-495. *https://doi.org/10.1016/j.jplph.2006.03.015*
Stokes P (1965). Temperature and seed dormancy. In: Ruhland W (Ed). Encyclopedia of Plant Physiology 15(2):746-803. Springer, Berlin. *https://doi.org/10.1007/978-3-642-50088-6_60*
Suri SK (1984). Analytical study of teak provenance test in North Raipur Division of Madhya Pradesh. The Indian Forester 110:345-363.
Timson J (1965). New method of recording germination data. Nature 207:216-217. *https://doi.org/10.1038/207216a0*
Troup RS (1975). Silviculture of Indian trees (Revised edition). Forest Research Institute Press, Dehradun, India. *https://doi.org/10.5962/bhl.title.24363*
Vasques A, Vallejo VR, Santos MC, Keizer JJ (2014). The role of cold storage and seed source in the germination of three Mediterranean shrub species with contrasting dormancy types. Annals of Forest Science 71(8):863-872. *https://doi.org/10.1007/s13595-014-0395-z*
Vidigal DS, Marques ACSS, Willems LAJ, Buijs G, Méndez-Vigo B, Hilhorst HWM, ... Alonso-Blanco C (2016). Altitudinal and climatic associations of seed dormancy and flowering traits evidence adaptation of annual life cycle timing in *Arabidopsis thaliana*. Plant Cell and Environment 39:1737-1748. *https://doi.org/10.1111/pce.12734*
Wawrzyniak M, Michalak M, Chmielarz P (2020). Effect of different conditions of storage on seed viability and seedling growth of six European wild fruit woody plants. Annals of Forest Science 77:58-77. *https://doi.org/10.1007/s13595-020-00963-z*