Long-term storage-dependent changes in seed physiological aspects of *Hyoscyamus* niger from a cold desert region of Trans-Himalaya

Shashi Sharma, Rajender Kumar Sharma

Department of Biosciences, Himachal Pradesh University, Shimla, India

Abstract

Henbane (*Hyoscyamus niger* L.), an economically important medicinal plant, has an endangered status in Himachal Pradesh (India) that needs appropriate conservation interventions. We have examined seed physiological aspects of *H. niger* from Lahaul (Himachal Pradesh, India), a cold desert region. The freshly harvested seeds exhibited complete dormancy. Gibberellic acid (GA_3) and chilling treatment strongly promoted seed germination which was accompanied by increased α-amylase activity. KNO_3, NaN_3 and sodium nitroprusside (SNP), an NO donor, also promoted germination. During storage, the seeds retained high viability even after a storage of 72 months under ambient conditions. However, they remained dormant during the entire storage period. The responsiveness of seeds to GA_3 and chilling treatment gradually declined with progression of storage period. Concomitantly, the triphenyl tetrazolium chloride (TTC) reduction ability of seeds was lowered. The seed responsiveness to KNO_3, NaN_3 and SNP during storage increased until one year and decreased thereafter. With the progression of the storage period, seeds exhibited elevated lipid peroxidation and reduced catalase activity implying a role of oxidative stress in observed changes. The involvement of phenolics in seed dormancy of *H. niger* was not evident. The findings are of significance for conservation and cultivation of *H. niger* through seeds in the arid mountain region.

Introduction

Hyoscyamus niger L. (*Solanaceae*), commonly known as henbane, is an important multipurpose medicinal plant. It is used as a narcotic, anodyne, sedative, antiseptic and mydriatic, besides being used in nervous conditions, asthma, whooping cough, muscular pain and toothache.[1-3]

Medicinal properties of henbane are principally due to diverse alkaloids (such as, hyoscyamin, hyoscine, scopolamine, belladonine, hyoscypikrin and atropine) present in most parts of the plant. The plant also contains certain non-alkaloidal components, e.g. grossamide and cannabisins that have been reported to exhibit moderate cytotoxicity in cultured human prostate cancer cells.[4] The plant grows well in regions with a semi-arid and cold climate; high temperature and humidity are detrimental to plant growth and alkaloid contents. Furthermore, the plants from higher altitude regions, as in the present study are notably richer in alkaloids than their lower altitude counterparts.[1]

The Indian Himalayan Region (IHR) harbors many highly valuable medicinal plants. *H. niger* populations are distributed in several areas of Himachal Pradesh in the IHR, particularly Kinnaur and Lahaul-Spiti. This species has an endangered status in Himachal Pradesh owing to unsystematic exploitation and habitat loss.[5] The mode of regeneration in *H. niger* is through seeds. Despite a large number of seeds produced, only a few seedlings are able to establish due to poor seed germination.[6] Obviously, an understanding of seed physiological aspects of *H. niger* could be expected to contribute to the efforts of seed-based *in situ* conservation as well as *ex situ* cultivation. In a previous study, we reported the stimulation of seed germination by GA_3 and KNO_3 in a *H. niger* population from Lahaul, a cold desert region in Himachal Pradesh.[6] The region remains devoid of any significant precipitation, because the main Himalayan ranges form a massive barrier which prevents most of the monsoon rain from reaching the area and creates a vast rain-shadow zone.[7] The scanty rainfall, low humidity, high altitude and cold climatic conditions of the region seem to be conducive to the cultivation and conservation of *H. niger*. We report here the long-term storage-dependent changes in seed physiological parameters of such a population.

Materials and Methods

Seed source

Seeds of *H. niger* were collected during August-September 2003 from the cold desert area of Lahaul (latitude 32° 8' and 32° 59' N, longitude 76° 49' and 77° 47' E and altitude 2,950 m above mean sea level), Himachal Pradesh, India. The seeds were collected manually, air-dried under shade and were stored in polyethylene jars at room temperature for subsequent studies (up to 72 months).

Seed viability

Seeds were subjected to 2,3,5-triphenyl tetrazolium chloride (TTC) test for viability evaluation shortly after collection and at regular intervals during the subsequent storage period. The seeds were surface-sterilized and imbibed in distilled water for 24 h at 25±1°C. Thereafter, the seeds were cut at one end to facilitate the diffusion of TTC, incubated with 0.1% TTC solution in the dark for 24 h and examined for staining intensity. Seeds with completely stained embryo were considered viable. The TTC reduction by seed tissue was quantified by measuring the formazan formed. For this, the seeds, at the end of incubation with TTC, were homogenized with 5 ml MetOH and centrifuged at 10,000 g for 10 min; absorbance was recorded at 485 nm. TTC reduction was expressed as A_{485}/50 embryos.

Seed germination assays

Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min, washed thoroughly under tap water and soaked in distilled water for 24 h at 25±1°C. Thereafter, they were transferred to Petri dishes lined with three layers of moist filter paper in a seed germinator at 25±1°C under continuous illumination (PAR: 40 μmol $m^{-2}s^{-1}$). Seeds were considered germinated upon the emergence of 2-5 mm radicle and scored periodically for germination. The mean germination time (MGT) was calculated as fol-

Correspondence: Shashi Sharma, Department of Biosciences, Himachal Pradesh University, Shimla 171 005, India.
E-mail: shashi_sh22@yahoo.co.in

Key words: catalase, lipid peroxidation, medicinal plant, seed dormancy, sodium nitroprusside

Acknowledgments: the award of Senior Research Fellowship and Research Associateship by CSIR, New Delhi (9/237(74)2002-EMR-1, 9/237(124)/2008-EMR-1) to Rajender K. Sharma is thankfully acknowledged.

Contributions: SS developed the concept and designed experiments; RKS collected the plant material, carried out seed viability, germination tests and measured enzyme activities (α-amylase and catalase) and certain metabolites (MDA and phenolic contents). The analysis and interpretation of data was carried out by both the authors.

Conflict of interest: the authors report no conflicts of interest.

Received for publication: 12 April 2010.
Revision received: 26 May 2010.
Accepted for publication: 27 September 2010.

International Journal of Plant Biology 2010; 1:e17
doi:10.4081/pb.2010.e17

lows:[8] MGT=Σ (nd)/N, where n=number of seeds germinated after each incubation period in days, d and N=total number of seeds emerged at the end of the test.

Application of physico-chemical and GA_3 treatments for dormancy removal/germination improvement

Before subjecting to germination conditions, the seeds were treated with the following effectors: i) cold-stratification. The surface-sterilized seeds soaked in distilled water for 24 h were kept at 4°C for one month; ii) acid scarification. Seeds were treated with concentrated H_2SO_4 for 1 min; this duration was decided on the basis of preliminary experiments in which various periods of time were considered. Thereafter, the seeds were washed thoroughly under tap water and allowed to imbibe in distilled water for 24 h and then transferred to germination conditions; iii) chemical treatments. The surface-sterilized seeds were soaked in aqueous solution of 0.2% potassium nitrate (KNO_3), 1 mM sodium nitroprusside (SNP), 0.1 mM sodium azide (NaN_3), respectively for 24 h, before subjecting to germination conditions; iv) GA_3 treatment. The surface-sterilized seeds were soaked in aqueous solutions of gibberellic acid (0.1 and 1.0 mM) for 24 h followed by germination on moist substratum; v) acid-scarification + GA_3 (1 mM). The acid-scarified seeds were imbibed in aqueous solution of GA_3 for 24 h and transferred to germination conditions.

α-amylase activity

α-amylase activity was assayed following the method described by Filner and Varner.[9] Seeds were homogenized with chilled Tris-HCl buffer (pH 7.2) and centrifuged at 10,000 g for 10 min at 4°C. The supernatant served as crude enzyme extract. Enzyme 1 ml was added to 1.0 mL substrate (0.15 % starch, 0.2 mM $CaCl_2$ in Tris-HCl buffer, pH 7.2) and incubated for 10 min at 25°C. The reaction was quenched by adding 3 mL IKI reagent (0.6% iodine in 6% KI; 1 mL of this diluted to 50 mL with 0.05 N HCl) and absorbance recorded at 620 nm. The amount of starch degraded was determined with the help of a calibration curve prepared with starch.

Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) contents according to the method of Dhindsa *et al.*[10] In brief, the seeds were homogenized with 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 g for 10 min. One ml supernatant was reacted with 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) and incubated at 95°C for 30 min. The reaction was terminated by cooling the reaction mixture and centrifuged. The absorbance was read at 532 nm and corrected by subtracting the absorbance at 600 nm. The MDA contents were measured using the extinction coefficient at 155 mM^{-1} cm^{-1}.

Catalase assay

Catalase activity was assayed polarographically by measuring the H_2O_2 dependent oxygen evolution at room temperature with an oxygen electrode unit (Hansatech, UK). In brief, the seeds were homogenized with chilled Na-phosphate buffer (pH 7.4) and centrifuged at 10,000 g for 5 min at 4°C. The supernatant served as crude enzyme. Assay (1 mL) including 880 μL phosphate buffer (0.1 M) pH 7.4 and 100 μL of 0.1 M H_2O_2. The reaction was initiated by addition of 20 μL enzyme extract. The amount of O_2 evolved/min was recorded with the help of a chart recorder. The catalase activity was calculated through electrode calibration and slopes obtained on chart paper.

Protein contents

Protein contents were estimated according to the method of Lowry *et al.*[11]

Total phenolic contents

Total phenolic contents were determined according to the method of Goldstein and Swain.[12] The seeds were homogenized in 0.3 N HCl in methanol, and centrifuged at 10,000 g for 10 min. Supernatant was collected and pellet was again extracted in 0.3 N HCl in methanol and centrifuged. The supernatants were pooled and evaporated on a hot water bath. The residue obtained was dissolved in distilled water and final volume made to 5 mL. To this, 0.5 mL of Folin-phenol reagent was added and shaken vigorously. After 3 min, 1 ml 35% sodium carbonate solution was added, shaken and allowed to stand for 1 h. Absorbance was recorded at 630 nm and the amount of total phenols was determined with the help of calibration curve prepared with gallic acid.

Statistical analysis

All experiments were carried out in triplicate. Data are presented as arithmetic means and standard deviation. The statistical significance was tested using Student's t-test.

Results

Seed viability

Freshly harvested seeds exhibited 100% viability. During storage, the viability status was maintained for at least 24 months. Thereafter, only a slight decline in viability was observed. After 72-month storage, a 12% decrease in seed viability was observed (Figure 1). Besides, the qualitative assessment of seed viability, the TTC reduction ability of seeds was also measured. This remained high for up to 24 months followed by a subsequent gradual decline (Figure 1).

Seed dormancy/germination

Freshly harvested seeds exhibited dormancy as was apparent from a lack of germination in control. Of the various pre-treatments tested for their effectiveness to overcome dormancy/improve germination, GA_3 was found to be most effective. The GA_3 effect intensified with an increase in concentration. Thus, GA_3 0.1 and 1 mM induced 33 and 100% germination, respectively, within 10 d. Cold-stratification of seeds for 30 d was also effective causing 84% germination. GA_3 and chilling not only stimulated the seed germination but also effectively reduced the time required for germination as is evident from lower MGT values (Figure 2). Other seed pre-treatments tested, namely, potassium nitrate (KNO_3), sodium nitroprusside (SNP) and sodium azide (NaN_3), were effective in dormancy removal, only to a lesser extent than GA_3 and cold-stratification. Acid (H_2SO_4) scarification of seeds was altogether ineffective in altering the dormancy/ germination status (Figure 2).

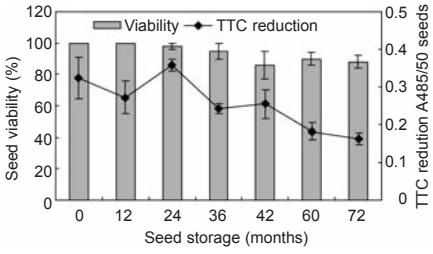

Figure 1. Storage-dependent changes in seed viability of *H. niger*. Data are average of 3 measurements each ± s.d.

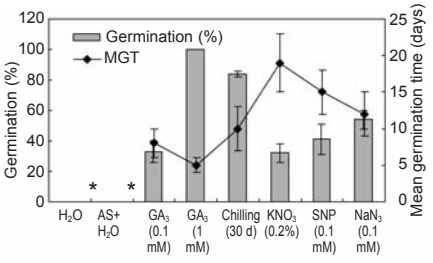

Figure 2. Effect of different seed pre-treatments on germination and mean germination time of freshly harvested seeds of *H. niger*. Data are average of 3 replicates each ± s.d. AS: acid scarification; *no germination.

Storage-dependent changes in seed dormancy/germination

During 72-month storage seeds generally remained dormant and showed no germination. During storage, the seed responsiveness to GA_3 (1 mM particularly) and cold-stratification remained unchanged for up to 24 months (Figure 3). Beyond this period, the seed responsiveness to these treatments declined slowly with the progression of storage period. After 72-month storage, 30 and 36% decline in seed response to GA_3 and cold-stratification was observed. Such a decline was not evident when seeds had been acid-scarified prior to the application of GA_3 (Figure 3).

The storage-dependent alteration in the responsiveness of seeds to other pre-treatments, namely, KNO_3, SNP and NaN_3 in causing dormancy removal/seed germination improvement appeared to be biphasic. After storage of up to 12 months, the seed response to the above effectors increased by 200, 50 and 38%, respectively, as compared to the similarly treated freshly harvested seeds. In contrast, when storage exceeded beyond 12 months, the responsiveness to all above effectors decreased gradually; after 42 months, the effect was strongly diminished (Figure 3). Despite the changes in responsiveness of seeds to various pre-treatments during storage, the MGT was altered only slightly (*data not shown*).

α-amylase activity

The α-amylase activity in seeds increased until 24 months of storage and declined thereafter. GA_3 pre-treatment of freshly harvested seeds induced a 2-fold increase in α-amylase activity. This GA_3-dependent enhancement, however, diminished with the progression of storage period. Chilling treatment also significantly increased the α-amylase activity but to a lesser extent as compared to GA_3. KNO_3 and SNP treatment did not have any significant effect on α-amylase activity (Figure 4).

Lipid peroxidation and catalase activity

During storage, the lipid peroxidation status (MDA contents) of seeds increased, particularly beyond 36 months; leading to a 54% increase after 72 months. Seeds pre-treated with GA_3, KNO_3 and SNP showed higher MDA contents. For example, 20, 21 and 40% higher MDA contents were observed due to these treatments, respectively, in freshly harvested seeds. Moist-chilling did not significantly alter the MDA contents of freshly harvested or differentially stored seeds (Figure 5A).

The freshly harvested seeds exhibited a higher level of CAT activity as compared to stored seeds. The CAT activity declined with the progression of the storage period; a 52% decline in CAT activity was evident after 72 months. The activity was promoted by GA_3 but suppressed by KNO_3 pre-treatments in seeds stored up to 36 months. Moist-chilling did not considerably alter the CAT activity (Figure 5B).

Total phenolic contents

Maximum total phenolic contents were found in freshly harvested seeds. During storage, the total phenolic contents of the seeds declined significantly, particularly beyond 24 months storage. After 72 months storage a 37% decline in seed phenolic content was observed. Chilling treatment also reduced the phenolic contents of the seeds. Various other seed pre-treatments did not considerably alter the quantity of total phenolic content of the seeds (Table 1).

Discussion

H. niger, an economically important, multi-purpose medicinal plant, has an endangered status in Himachal Pradesh, India.[5] Therefore, it requires an appropriate intervention for conservation. In addition, its *in situ* cultivation might reduce the pressure on wild populations as well as contribute to the economy of inhabitants. The mode of regeneration in the *H. niger* is through seeds. However, the seeds exhibit dormancy,[6] a situation unfavorable for seed based regeneration/multiplication. Obviously, a clearer understanding of seed physiological aspects including the storage-dependent alterations thereof would be immensely valuable. The high degree of viability in freshly harvested seeds was generally maintained even after a 72-month storage of

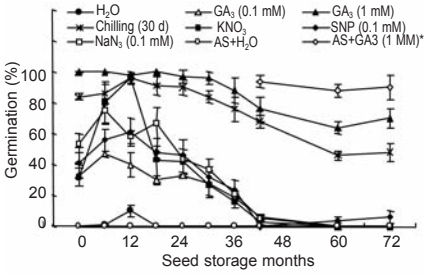

Figure 3. Storage-dependent changes in seed germination and responsiveness to different pre-treatments in *H. niger*. Data are average of 3 replicates each ± s.d. ASacid scarification; *tested 42 months storage onwards.

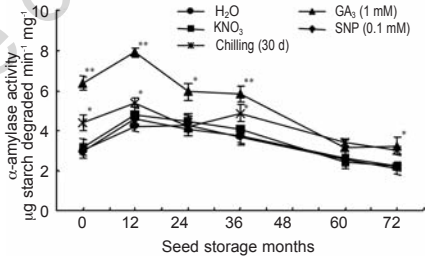

Figure 4. Effect of different seed pre-treatments on α-amylase activity of differentially stored seeds of *H. niger*. Data are average of 3 measurements each ± s.d. Different from respective controls based on 't' statistic: * (P≤0.05); ** (P≤0.01).

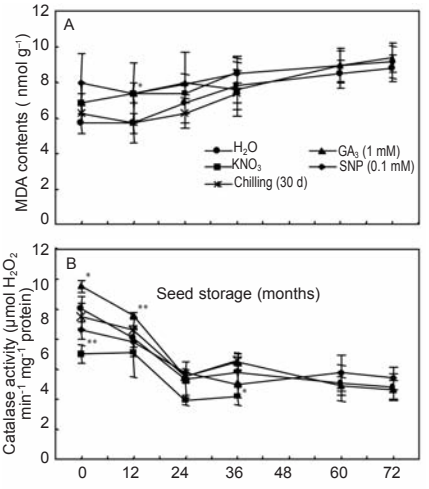

Figure 5. Effect of seed pre-treatments on lipid peroxidation status (A) and catalase activity (B) of differentially stored seeds of *H. niger*. Data are average of 3 measurements each ± s.d. Different from respective controls based on 't' statistic: * (P≤0.05); ** (P≤0.01).

Table 1. Effect of different seed pre-treatments on total phenolic contents of differentially stored seeds of *H. niger*. Data are average of 3 measurements each ± s.d.

Storage period (months)	Total phenolic contents (mg g^{-1} seed)				
	H_2O	GA_3 (1 mM)	KNO_3 (0.2%)	SNP (1 mM)	Chilling (30 d)
0	5.42±0.24	5.47±0.58	5.42±0.38	5.76±0.09*	5.23±0.48*
12	4.90±0.48	4.66±0.67	4.75±0.53	4.66±0.67	4.75±0.29
24	4.13±0.24	4.78±0.15*	4.18±0.19	4.27±0.43	3.79±0.09*
36	3.98±0.72	3.79±0.43	4.68±0.86	4.03±0.43	3.46±0.29
60	3.68±0.28	3.39±0.29	†	3.56±0.16	†
72	3.42±0.02	3.43±0.18	†	3.34±0.26	3.08±0.18*

†not measured; different from respective controls based on 't' statistic: * (P ≤ 0.05); ** (P ≤ 0.01).

seeds under ambient conditions as revealed in TTC reduction assay. This viability status was also reflected in germination observed in seeds treated with GA_3 alone or in combination with acid-scarification. The observed seed viability features under ambient conditions are desirable for seed based plant propagation.

Despite being strongly viable, the freshly harvested seeds exhibited dormancy, which could be alleviated through seed pre-treatment with GA_3; 100% germination was achieved within a week. The seed germination stimulating effect of GA_3 in a *H. niger* population from Turkey was reported by Cirak *et al.*,[13] where about 20% germination was induced. Thus, GA_3 effect seems to be population-specific. The GA_3-induced seed germination accompanied by increased α-amylase activity suggests the role of GA_3 in enhancing the availability of sugars for embryo growth. GA_3 might similarly affect other hydrolytic enzymes too. GAs are likely to influence several other events associated with seed germination. For example, they are known to replace the requirement of after-ripening, light and cold.[14] Furthermore, the GAs have been reported to alleviate mechanical restraints of the testa and endosperm allowing an easy protrusion of the radicle in *Arabidopsis* and tomato.[15,16] The effect of chilling treatment on seed germination and α-amylase activity in dormant seeds of *H. niger* were found to resemble those induced by GA_3 with essentially quantitative differences. Cold stratification is known to inhibit ABA synthesis, promote GA synthesis and/or increase the sensitivity of seeds to GA_3 and induce cell division in the shoot and root meristem of the embryo.[14,17]

KNO_3, NaN_3 and SNP significantly promoted the seed germination of *H. niger*. Nitrite and nitrates have long been known to break dormancy or stimulate seed germination in many plant species.[18] Hihorst[19] found that in seeds of *Sisymbrium officinale*, the nitrate content of the seeds from different habitats was directly proportional to their rate of germination. Nitrate provided during seed development via the maternal plant has been reported to lower dormancy.[20] However, the mechanism(s) involved in seed dormancy removal by nitrite and nitrates are not precisely known. It has been proposed that oxidized forms of nitrogen might promote seed germination by causing a shift in respiratory metabolism to the pentose phosphate pathway or through promotion of respiration by increasing oxidation of NADPH to $NADP^+$, a limiting electron acceptor.[21-23] Such a relationship was, however, found missing in dormant seeds of *Avena fatua*.[24,25] McIntyre *et al*.[26] suggested the involvement of osmotic and nutritional effects of exogenous nitrate on seed germination of *A. fatua*.

Sodium nitroprusside (SNP), a nitric oxide (NO) donor, promoted seed germination of *H. niger* in the present study. NO has emerged as a major signaling and effector molecule involved in many key physiological processes.[27-30] A few reports implying the involvement of NO in influencing seed dormancy/germination are also available. Thus, the application of NO donors was found to break the dark imposed seed dormancy in lettuce; the effect of NO donors was reversed in the presence of the NO scavenger cPTIO (carboxy 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide).[31] Also, NO-dependent germination of wheat seeds under osmotic stress and stimulation of β-amylase during early stage of germination has been shown.[32,33] Such effects of NO might explain the germination of dormant seeds of *H. niger* induced by SNP.

Quite remarkably, the *H. niger* seeds remained dormant even after 72 months of storage under ambient conditions. However, the responsiveness of seeds to GA_3 and chilling treatment began to decline consistently with the progression of the storage period, showing a correspondence with reduction in seed vigor (TTC reduction ability). In contrast, the storage-dependent alteration in the seed responsiveness to KNO_3, SNP and NaN_3 treatments was biphasic with enhanced effect up to 12 months of storage followed by a gradual decline thereafter. Dry storage or after-ripening is known to widen the temperature range for germination, decrease ABA concentration, increase GA sensitivity, loss of light and nitrate requirement, and also increase the germination velocity.[14] All these factors might eventually lead to enhanced sensitivity of seeds to various effectors. However, a long-term storage is known to decrease the germination ability and sprouting of seeds which is strongly species-specific.[34]

During seed storage, MDA contents increased particularly beyond one year which was accompanied by reduced CAT activity. These observations suggest the involvement, at least in part, of redox metabolic perturbation in the storage-dependent partial loss of seed viability. Sustained production of ROS due to prolonged storage of seeds leads to oxidative stress and related deteriorative events.[35,36] The decline in α-amylase activity with storage, particularly beyond 24 months, might also be ascribed to a certain extent to increased oxidative stress and related protein damage.

Total phenolic contents of seeds, measured as possible dormancy regulators,[20,37,38] decreased gradually during 72-month storage. Despite this, however, no germination was observed; apparently, there was no indication of involvement of phenolics in seed dormancy/germination of *H. niger*.

In conclusion, the seeds of *H. niger* retained high viability even after prolonged storage and exhibited reasonably good germination through certain seed pre-treatments. The findings are of significance for conservation as well as for the possibility of commercial cultivation of *H. niger* through seeds in the arid mountain region.

References

1. Anonymous. Wealth of India, Raw Materials. CSIR, New Delhi; 1959.
2. Chevallier A. The encyclopedia of medicinal plants. Dorling Kindersley Limited, London; 1996.
3. Chauhan NS. Medicinal and aromatic plants of Himachal Pradesh. Indus Publishing Company, New Delhi; 1999.
4 Ma CY, Liu WK, Che CT. Lignanamides and non alkaloidal components of Hyoscyamus niger seeds. J Natural Prod 2002;65:206-9.
5. Ved DK, Kinhal GA, Ravikumar K, et al. CAMP report: conservation assessment and management prioritization for medicinal plants of Jammu and Kashmir, Himachal Pradesh and Uttaranchal. FRLHT, Bangalore; 2003.
6. Sharma RK, Sharma S, Sharma SS. Seed germination behaviour of some medicinal plants of Lahaul and Spiti cold desert (Himachal Pradesh): implications for conservation and cultivation. Curr Sci 2006;90:1113-8.
7. Negi SS. Cold deserts of India. Indus Publishing Company, New Delhi; 1995.
8. Airi S, Rawal RS, Dhar U. Presowing treatment effect on germination of Cornus copitata seeds. Seed Sci Technol 2005;33: 77-86.
9. Filner P, Varner JE. A test for de novo synthesis of enzymes: density labeling with H2O18 of barley α-amylase induced by gibberellic acid. Biochemistry 1967;58:1520-6.
10. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of superoxide dismutase and catalase. J Expt Bot 1981;32:93-101.
11. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
12. Goldstein JL, Swain T. Changes in tannins in ripening fruits. Phytochemistry 1963;2: 371-83.
13. Cirak C, Kevseroglu K, Saglam B. Physical and physiological dormancy in black henbane (Hyoscyamus niger L.) seeds. J Plant Biol 2004;47:391-5.
14. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytol 2006;171:501-23.
15. Koornneef M, Van der Veen JH. Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.) Heynh. Theor App Gen 1980;58:257-63.
16. Groot SPC, Karssen CM. Gibberellins regu-

late seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. Planta 1987; 171:525-31.
17. Chien CT, Kuo-Huang LL, Lin TP. Changes in ultrastructure and abscisic acid level, and response to applied gibberellins in Taxus mairei seeds treated with warm and cold stratification. Ann Bot 1998;81:41-7.
18. Roberts EH, Smith RD. Dormancy and the pentose phosphate pathway. In: Khan AA, editor. The physiology and biochemistry of seed and germination. Elsevier North Holland, Amsterdam; 1977. p. 385-411.
19. Hilhorst HWM. Dose-response analysis of factors involved in germination and secondary dormancy of seeds of Sisymbrium officinale. Plant Physiol 1990;94:1096-102.
20. Finkelstein R, Reeves W, Ariizumi T, et al. Molecular aspects of seed dormancy. Annu Rev Plant Biol 2008;59:387-415.
21. Simmonds JA, Simpson JM. Increased participation of the pentose phosphate pathway in response to after-ripening and gibberellic acid treatment in caryopses of Avena fatua during germination. Can J Bot 1971;49:1833-40.
22. Hendricks SB, Taylorson RB. Promotion of seed germination by nitrate, nitrite, hydroxylamine, and ammonium salts. Plant Physiol 1974;54:304-9.
23. Bewley JD, Black M. Physiology and biochemistry of seeds. Springer-Verlag, Berlin, Heidelberg; 1982.
24. Fuerst EP, Upadhyaya MK, Simpson GM, et al. A study of the relationship between seed dormancy and pentose phosphate pathway activity in Avena fatua L. Can J Bot 1983;61:667-70.
25. Adkins SW, Simpson GM, Naylor JM. The physiological basis of seed dormancy in Avena fatua. IV. Alternative respiration and nitrogenous compounds. Physiol Plant 1984;60:234-8.
26. McIntyre GI, Cessna AJ, Hsiao AI. Seed Dormancy in Avena fatua: Interacting effects of nitrate, water and seed coat injury. Physiol Plant 1996;97:291-302.
27. Delledonne M, Xia Y, Dixon RA, et al. Nitric oxide functions as a signal in plant disease resistance. Nature 1998;394:585-8.
28. Ruan HH, Shen WB, Ye MB, et al. Protective effects of nitric oxide on salt stress-induced oxidative damage to wheat (Triticum aestivun L.) leaves. Chinese Sci Bull 2002;7:677-81.
29. Guo FQ, Mamoru O, Crawford NM. Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science 2003;302:100-3.
30. Neill SJ, Desikan R, Hancock JT. Nitric oxide signalling in plants. New Phytol 2003;159:11-35.
31. Beligni MV, Lamattina L. Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 2000;210:215-21.
32. Zhang H, Shen WB, Xu LL. Effect of nitric oxide on the germination of wheat seed and its reactive oxygen species metabolisms under osmotic stress. Acta Bot Sin 2003;45:901-5.
33. Zhang H, Shen WB, Zhang W, et al. A rapid response of β-amylase to nitric oxide but not gibberellin in wheat seeds during the early stage of germination. Planta 2005;220:708-16.
34. Baskin CC, Baskin JM. Seeds- ecology, biogeography and evolution of dormancy and germination. San Diego, USA: Academic Press; 1998.
35. Bailly C. Active oxygen species and antioxidants in seed biology. Seed Sci Res 2004;14:93-107.
36. Oracz K, Bouteau HEM, Farrant JM, et al. ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant J 2007;50:452-65.
37. Phartyal SS, Thapliyal RC, Nayal JS, et al. Seed dormancy in Himalayan maple (Acer caesium) II: Bioassay of inhibitors. Seed Sci Technol 2003;31:13-20.
38. Chien CT, Kuo-Huang LL, Shen YC, et al. Storage behaviour of Chionanthus retusus seeds and asynchronous development of the radicle and shoot apex during germination in relation to germination inhibitors, including abscisic acid and four phenolic glucosides. Plant Cell Physiol 2004;45:1158-67.