

# SEED GERMINATION CHARACTERISTICS OF RATANJOT, ARNEBIA EUCHROMA (ROYLE) I.M. JOHNSTON, A THREATENED MEDICINAL PLANT SPECIES FROM COLD DESERT OF SPITI (HIMACHAL PRADESH, INDIA): IMPROVEMENT BY GA<sub>3</sub>, SODIUM NITROPRUSSIDE AND PROLINE

# ANUPRIYA SHARMA<sup>1</sup> AND SHANTI S. SHARMA<sup>1,2</sup>

<sup>1</sup>Department of Biosciences, Himachal Pradesh University, Shimla 171005, India <sup>2</sup>Department of Botany, School of Life Sciences, Sikkim University, Gangtok 737102, India Email: shantissharma@hotmail.com Date of online publication: 30th June 2020 DOI:10.5958/2455-7218.2020.00020.0

Arnebia euchroma (Royle) I. M. Johnston, commonly known as Ratanjot, is a threatened, medicinally important plant species. It is used to cure an array of ailments including cough, lung problems, hair fall, dandruff and menorrhagia etc. The roots are also used to colour food preparations by the inhabitants of Lahaul and Spiti. We have characterized the seed germination aspects of *A. euchroma* from the cold desert region of Spiti (Himachal Pradesh, India) in W. Himalaya with an emphasis on achieving improved seed germination performance through pre-treatment of seeds with GA<sub>3</sub> and certain physico-chemical effectors. The freshly harvested seeds exhibited poor germination (30%). Of the tested effectors, GA<sub>3</sub> was most effective in promoting the seed germination. Besides, sodium nitroprusside (SNP) and proline proved nearly as effective as GA<sub>3</sub>; these effectors have not been commonly employed for germination improvement in high altitude medicinal plants. KNO<sub>3</sub> was marginally promotory but acid scarification of seeds had virtually no effect. Analyses of certain biochemical parameters such as free proline contents, total phenolic contents, MDA contents and  $\alpha$ -amylase activity provided insight into the mechanism of stimulation of seed germination by the stated effectors. The findings have implications for the conservation and cultivation of *A. euchroma*.

Keywords: Arnebia euchroma, GA<sub>3</sub>, Proline, Seed germination, Sodium nitroprusside

The Indian Himalayan region (IHR) is a part of Himalayan centre of biodiversity, one of the mega hot spots of biological diversity (Myers et al. 2000). It harbours numerous endemic, threatened and rare plants which the local inhabitants use for different purposes including medicine, food, fodder, fuel, timber, making agricultural tools and religious ceremonies (Samant et al. 1998). Spiti, a well-known cold desert, an integral part of North Western Himalaya, is home to many medicinal plants used in local Amchi as well as in other traditional healing systems. Although the vegetation in entire Spiti valley is sparse owing to dry, arid and harsh climate, it is varied and diverse. The unique tiny populations of medicinal plants in the region are prone to overexploitation due to ever increasing local and market demands. Indeed, a significant depletion of the medicinal plant populations in natural habitats has occurred due to unscientific collection and habitat destruction

(Kala 2000). The prevalent extreme climatic conditions significantly restrict the seed germination and propagation of these plants. Apparently, the conservation and cultivation issues of threatened and rare medicinal plants need to be addressed on priority basis.

Although many of these plants also propagate by vegetative means, propagation through seeds comprises by far the most convenient and cost effective means for the purpose. The seeds of many medicinal plant species possess dormancy and exhibit erratic germination (Baskin and Baskin 1988, Sharma et al. 2006). These features hold significance in an ecological and plant survival context but they become a disadvantage for seed-based cultivation and propagation of the concerned species. Therefore, a clearer understanding of the seed germination behaviour is critically important for developing the species-specific conservation and cultivation strategies. Three species of genus Arnebia are reported from

Spiti (Aswal and Mehrotra 1994). Of these, *A. euchroma* (syn. *Macrotomia perennis*, Boiss) is commercially very important. It is known as Dimok or Khamed in the Spiti valley and as Ratanjot in trade. It is distributed in dry areas of alpine Western Himalaya, Western Tibet and Nepal, usually between 3300-4500 m asl. (Polunin and Stainton, 1984). It is found growing on dry, sandy slopes near the villages of Demul (4500 m asl) and Kibber (4200 m asl). *A. euchroma* is a threatened plant species that has been categorised as critically endangered (Singh *et al.* 2007) and later as endangered on the basis of IUCN criteria (Barik *et al.* 2018)

*A. euchroma* is a perennial herb with multiple erect stems (30-40 cm in height) covered with trichomes and emerging from axils of basal leaves. The insect pollinated hermaphroditic purplish-white flowers appear during June to August while the seeds mature from July to September. The roots yield a purple dye used as spice in food preparations. The powdered roots are used to cure cough, lung problems and menorrhagia. Root powder mixed with mustard or apricot oil is used to impart red colour to hair and also to prevent dandruff and hair fall.

Root extracts of A. euchroma yield a shikonin derivative which has significant antimicrobial, anti-inflammation and anti-tumour activities (Ghorbani 2005). The plant also exhibits a potent anti-HIV activity (Kashiwada et al. 1995). In view of the immense medicinal value and threatened status (Singh et al. 2007, 2012, Barik et al. 2018), there is an urgent need to develop realistic strategies for conservation of A. euchroma. As such, there is a dearth of information concerning the seed germination behaviour of A. euchroma and underlying metabolic basis thereof. The present study aimed at determining the seed germination characteristics of A. euchroma from Spiti with an emphasis on achieving improved germination through physico-chemical and hormonal pre-treatments and getting insight into the underlying metabolic mechanism(s).

The findings are expected to have implications for cultivation as well as conservation of the Spiti population of *A. euchroma*.

## **MATERIALS AND METHODS**

**Study Area:** The present work concerns a population of *Arnebia euchroma* from Spiti, a sub-division of Lahaul and Spiti district of Himachal Pradesh (H.P.). It is bordered by Ladakh on the North, Lahaul on the West, Kinnaur on the South and Tibet on the East. With an average elevation of 4000 m above sea level, Spiti is a typical cold desert of middle and great Himalayan ranges. It predominantly remains cool and dry throughout the year except the period of May to September when it receives very little rain. The temperatures typically range from sub-zero during winters to quite high during summers.

**Seed source:** The ripened fruits/mature seeds of *Arnebia euchroma* were collected from Kibber village (4200 m asl) of Spiti subdivision. The manually separated seeds from the fruits were air dried for at least a fortnight at room temperature and stored in airtight polyethylene containers for further studies.

Seed germination assays: The seeds were surface sterilized with 0.1% HgCl<sub>2</sub> followed by thorough washing with water. After soaking in distilled water for 24 h, they were transferred to Petri plates lined with 2 layers of filter paper made wet with distilled water. The Petri plates were placed in a seed germinator/incubator at  $25 \pm 2^{\circ}$  C under continuous illumination (PAR: 40  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) provided by fluorescent white light. The seed germination was recorded at periodic intervals until the final germination count. Emergence of 2 mm of radicle was considered as seed germination (ISTA, 1999). The experiments were performed in triplicate with 30 seeds each (10x3). In addition to germination percentage, following parameters were calculated:

A. Emergence index (EI) =  $\frac{dn}{n}$  Where, dn is day in which emergence ends and n is day when emergence starts

B. Germination Speed =  $\frac{Percentage \ germination}{Day \ of \ completion \ of \ germination}$ 

C. Mean Germination Time (MGT)= $\Sigma(nxd)/N$ 

Where, n= the number of seeds which germinated after each period in days (*d*) and *N* = the total number of seeds germinated at the end of experiment (Hartman and Kester, 1989).

Physico-chemical and GA, pre-treatment of seeds: The surface sterilized seeds of A. euchroma were pre-treated with  $GA_3(0.1 \text{ and } 1$ mM), sodium nitroprusside (SNP, a nitric oxide donor: 1 and 10 mM), proline (1 and 10 mM) and KNO<sub>3</sub> (2 mM), by keeping them submerged in test solutions for 24 h. Seeds simultaneously imbibed with water constituted the control. For acid scarification, the seeds were treated with 98% H<sub>2</sub>SO<sub>4</sub> for 1 min. after which they were washed thoroughly under tap water and soaked in distilled water for 24 h. Following the pre-treatments, the seeds were placed in Petri plates lined with two layers of filter paper moistened with distilled water and incubated in a seed germinator under the above stated conditions. Different germination

parameters as stated above were measured.

Measurement of biochemical parameters: The seeds pre-treated with different effectors for 24 h were subjected to determination of  $\alpha$  amylase activity and contents of certain metabolites.

 $\alpha$  -amylase was assayed following the method of Filner and Varner (1967). In brief, the seeds were homogenised with Tris-HCl (pH 7.2) buffer and the homogenate centrifuged. The supernatant served as the enzyme extract. One ml substrate (0.15% starch) was incubated with 1 ml enzyme extract for 10 min at 25°C after which 3 ml IKI reagent was added and absorbance read at 620 nm. The amount of starch degraded was calculated with the help of a calibration curve and enzyme activity expressed as µg starch degraded min<sup>-1</sup> mg<sup>-1</sup> protein. Protein contents in the extract were measured using Bradford reagent (Bradford, 1976).

Free proline contents were determined following the method of Bates *et al.* (1973). The seeds were homogenised with 3% sulfosalicylic acid and the obtained homogenate was centrifuged. One ml supernatant was reacted with 1 ml freshly prepared ninhydrin reagent and 1 ml glacial acetic acid for 1 h at 95°C. Thereafter, the

Seed Treatments	% Germination on 15 <sup>th</sup> day	Emergence index	Germination speed	Mean Germination Time, MGT (days)
Control	30±10.0	1.29	3.33	8
KNO <sub>3</sub> 2 mM	40±10.0	2.2	3.64	8
SNP 1 mM	70±5.77	2.25	5.83	7
SNP 10 mM	60±10.0	2.2	5.45	8
GA 0.1 mM	66.67±3.33	3.33	6.67	6
GA 1 mM	86.67±3.33	4.67	6.19	6
Proline 1 mM	60±15.3	2.75	5.45	8
Proline 10 mM	46.67±8.82	2.8	3.33	9
H <sub>2</sub> SO <sub>4</sub> 1 min.	$30 \pm 2.16$	2.8	2.14	10

**Table 1**. Effect of different seed pre-treatments on seed germination (%), emergence index, germination speed and mean germination time (MGT) in seeds of *A. euchroma*. Data are arithmetic means  $\pm$  SE., n=3.



Figure 1. Arnebia euchroma growing in natural habitat in Spiti valley (altitude: 4200 masl)



**Figure 2.** Time course of seed germination of *Arnebia euchroma* as affected by pre-treatment of GA<sub>3</sub> and proline (A); SNP, KNO<sub>3</sub> and acid scarification (AS) (B). Data are arithmetic means of three replicates each comprising 30 seeds  $\pm$  SE. Different letters represent significant differences among different pre-treatments (ANOVA; Tukey's test, p<0.05).



**Figure 3.** Effect of GA<sub>3</sub> and certain chemical pre-treatments (24 h) on  $\alpha$ -amylase activity in the seeds of *Arnebia euchroma*. Data are arithmetic means ± SE, n=3. Different letters represent significant differences among different pre-treatments (ANOVA; Tukey's test, p<0.05).



**Figure 4.** Effect of GA<sub>3</sub> and certain chemical pre-treatments (24 h) on total phenol (A), free proline (B) and malondialdehyde (C) contents of *Arnebia euchroma* seeds. Data are arithmetic means  $\pm$  SE, n=3. Different letters represent significant differences among different pre-treatments (ANOVA; Tukey's test, p<0.05).

reaction mixture was cooled, chromophore extracted with 2 ml toluene and absorbance read at 520 nm. The proline contents were determined with the help of a calibration curve.

Lipid peroxidation was estimated in terms of malondialdehyde (MDA) contents following the method described by Dhindsa *et al.* (1981). The seeds were homogenised with 0.1% trichloroacetic acid and the homogenate was centrifuged. The supernatant was reacted with 0.5% thiobarbituric acid in 20% trichloroacetic acid at 95°C for 1 h. After

cooling, the absorbance was read at 532 nm. Measurement corrections for unspecific turbidity were made by subtracting the absorbance at 600 nm. The MDA contents were determined using the extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ .

Total phenol contents were determined according to Goldstein and Swain (1963). The seeds were homogenised with 0.3 N HCl in methanol and the homogenate was centrifuged. The supernatant was evaporated and the residue dissolved in water which was reacted with 1N Folin-Ciocalteau reagent and 35% sodium bicarbonate. After 1 h, absorbance was recorded at 630 nm. The phenol contents were determined using a calibration curve prepared with gallic acid.

Statistical analysis: All experiments were carried out in triplicate and data are presented as arithmetic means  $\pm$  SE. The data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test for significant differences between individual means.

#### RESULTS

#### Seed germination in response to various pretreatments

The germination assays were performed on freshly harvested seeds (within two months after harvest) of A. euchroma; a plant growing in natural habitat is shown in figure 1. In control, seed germination started after 7 d of incubation. A maximum of 30% germination was evident on 15 d. With the aim of achieving improved seed germination performance, the seeds were subjected to various physicochemical and GA<sub>3</sub> pre-treatments. Among all the treatments applied, GA<sub>3</sub> (1 mM) was most effective (Fig. 2A). With this, germination started after 2 d of incubation that increased gradually to 87% after 15 d. The lower concentration (0.1 mM) was also promotory and caused 67% germination. Like GA<sub>3</sub> SNP(1 mM) pre-treatment induced early seed germination and strongly promoted the same causing 70% germination after 15 d (Fig. 2B). The higher SNP concentration (10 mM) was not as effective. Proline (1 mM) pre-treatment also promoted the seed germination substantially leading to 60% after 15 d; effect of 10 mM proline was lower in magnitude (47%) (Fig. 2A). In contrast to GA<sub>3</sub> SNP and proline, KNO<sub>3</sub> (2 mM) promoted the seed germination only marginally (Fig. 2B). Acid scarification in fact somewhat suppressed the

seed germination particularly during initial period although the final count was comparable to that in control (Fig. 2B). Other germination characteristics namely, emergence index and germination speed were consistent with the seed germination data. Thus, their values were highest in case of pretreatment with GA<sub>3</sub> followed by that with SNP, proline, KNO<sub>3</sub> and acid scarification (Table 1).

Biochemical changes due to various seed pre-treatments: To get insight into the possible mechanism(s) underlying the germination enhancement induced by different effectors, certain biochemical parameters namely,  $\alpha$ -amylase activity, free proline contents, lipid peroxidation levels (MDA contents) and phenol contents were measured in the seeds after pre-teatment of 24 h. The  $\alpha$ amylase activity in the seeds was strongly promoted by 1 mM GA<sub>3</sub> (2.62-fold) and SNP 1 mM (1.73-fold). In contrast, proline and KNO<sub>3</sub> had no effect and AS marginally suppressed the activity (Fig. 3). Seed phenolic contents declined due to  $GA_3$  (1 mM), AS and proline (1 mM) treatment by 34, 28 and 12%, respectively. However, SNP (1 mM) and KNO<sub>3</sub> (2 mM) did not alter the same (Fig. 4A). Due to pre-treatment with exogenous proline (1 mM), the endogenous seed proline contents increased 2.35-fold. They increased by 18 and 17% due to SNP (1 mM) and KNO<sub>3</sub> (2 mM), respectively but remained unchanged under GA<sub>3</sub> (1 mM) treatment (Fig. 4B). The seed MDA contents tended to be lower than the control levels due to KNO<sub>3</sub> GA<sub>3</sub>, proline and AS; lowest levels were observed in KNO<sub>3</sub>treated seeds. SNP had no effect (Fig. 4C).

#### DISCUSSION

The aim of present study was to characterize the seed germination behaviour of *Arnebia euchroma*, a threatened species, from Spiti (Himachal Pradesh, India) cold desert in W. Himalava. Such information holds significance for its seed-based conservation and cultivation; the latter could potentially ease the pressure on natural populations. The freshly harvested seeds of A. euchroma exhibited poor germination; maximum of only 30% seeds germinated after 15 d of incubation under favourable conditions. Therefore, we explored the possibility of improvement of seed germination performance through the pretreatment of seeds with a couple of uncommon effectors namely, SNP, a donor of nitric oxide (NO) and proline in addition to the common ones including GA<sub>2</sub> acid (H<sub>2</sub>SO<sub>4</sub>) scarification, and KNO<sub>2</sub>. Strongest stimulation of seed germination, in terms of both advancement and germination percentage (87%, 12 d), was evident in case of GA<sub>3</sub> pre-treatment. Such an influence of GA<sub>2</sub> on seed germination has been reported in case of several high altitude populations of medicinal plants e.g. Podophyllum hexandrum, Inula racemosa, Hyoscymus niger, Bunium persicum, Carum carvi, Saussurea costus and Rheum australe (Sharma et al. 2006, 2010, 2014). Improvement in germination performance has also been reported in Arnebia benthamii (Kandari et al. 2008). The role of GA<sub>3</sub> in alleviation of seed dormancy and enhancement of seed germination is well established (Debeaujan and Koornneef 2000, Finch-Savage and Leubner-Metzger 2006; Finkelstein 2008). Dormancy release and germination enhancement involve increase in GA biosynthesis and ABA degradation leading to low ABA/GA ratio (Cadman et al. 2006). GA<sub>2</sub> induces amylase activity in germinating seeds that hydrolyses starch into sugars to sustain the seed germination (Kaneko et al. 2002). Consistent with this, amylase activity in GA<sub>3</sub> pre-treated A. euchroma seeds was strongly enhanced (Fig. 3). GA<sub>3</sub> could replace the requirement of after ripening by increasing its endogenous level (Bewely and Black 1994).

Quite interestingly, SNP, an NO donor and

proline strongly promoted the seed germination of A. euchroma. SNP was demonstrated to break seed dormancy in Arabidopsis thaliana (Bethke et al. 2006). NO promoted seed germination and seedling growth in rice (Oryza sativa L.) under cadmium stress (He et al. 2014). The reversal of NO dependent dormancy removal in Lactuca sativa by cPTIO, an NO scavenger, confirmed the role of endogenous NO in the regulation of the process (Beligni and Lamattina 2000). NO alleviated the dormancy of apple seeds via alteration of ABA and JA biosynthetic or transduction pathways (Andryka-Dude et al. 2019). Recently, the seed germination acceleration by NO in chickpea has been demonstrated to be a consequence of the regulation of respiration and ROS accumulation (Pandey et al. 2019).

ROS production occurs in germinating seeds (Bailly et al. 2004). The MDA contents, however, were not affected by SNP preteatment in A. euchroma seeds. Due to SNP pre-treatment, amylase activity was enhanced that might have contributed to enhanced seed germination in the present study. In germinating wheat seeds, NO stimulated the  $\alpha$  amylase activity (Zhang et al. 2005). SNP promoted imbibition and caused storage reserve degeneration finally leading to stimulated seed germination (Zhang et al. 2007). The stimulation of A. euchroma seed germination by proline could be ascribed to multiple actions of proline. Thus, being an osmoprotectant (Kishor et al. 1995), it would be expected to appropriately contribute to the maintenance of water relations in the seed tissue and seedlings. There is a positive correlation between proline contents and cell elongation. It is quite likely to act as an N source (Claussen 2005). Proline promoted seed germination in wheat seeds by stimulating  $\alpha$ amylase activity (Sultana et al., 2000). Besides, proline could scavenge a variety of free radicals in vitro (Kaul et al. 2008) and in turn is likely to be a component of cellular

antioxidant defence network. Through this property, proline might help the redox regulation of germinating seeds in view of the generation of ROS during the process (Bailly et al. 2004). As a consequence of seed pretreatment with exogenous proline, the endogenous contents increased by a factor >2that supports the involvement of proline in stated processes. Proline accumulated in the seeds pre-treated with SNP and GA<sub>3</sub> albeit to a much lower magnitude than that in proline treated ones, pointing to its likely contribution towards germination stimulation. KNO<sub>3</sub> has been reported to have a positive effect on seed germination and seedling growth of many species (Ahmadvand et al. 2012). KNO<sub>3</sub> stimulated germination by enhancing the water uptake in dormant caryopsis of Avena fatua (Hilton 1984). Nitrate induced seed dormancy removal was reported to be accompanied by reduction in ABA levels (Matakiadis et al. 2009; Fredes et al. 2019). In contrast to other pre-treatments, acid scarification did not alter the seed germination of A. euchroma implying the lack of a major role of seed coat hardness in poor germination ability of seeds.

Total phenolic contents of A. euchroma seeds decreased as a consequence of most of the treatments; maximum decrease was evident in GA<sub>3</sub> (1 mM) treated seeds. Phenols are categorized as plant growth regulators and are involved in regulation of seed germination and dormancy (Chien et al. 2004). Mayers and Evenari (1952) observed simple phenols to be the potent germination inhibitors. Thus, the enhanced magnitude of seed germination might partly be due to lowered phenolic levels. In Arnebia benthamii, the seed coat contains the chemical inhibitors which have an inhibitory effect on seed germination (Ganaie et al. 2011). MDA is an indicator of membrane injuries and cellular metabolic deterioration (Fan et al. 2012). In A. euchroma, a cold desert plant species, MDA contents were found to be highest in control showing 30% germination. No effect of acid scarification indicates that the seeds of *A. euchroma* from Spiti (H.P.) possess only physiological dormancy. The parameters such as emergence index, germination speed and MGT support the findings of present study. For example, the lower values of MGT in case of  $GA_3$  pre-treatments signify an acceleration of germination process.

### CONCLUSIONS

In brief, the present study represents the first characterization of seed germination behaviour of threatened, high value *Arnebia euchroma* from the cold desert region of Spiti in W. Himalaya. The findings revealed a strong stimulation of seed germination by SNP and proline, two uncommon effectors, besides that by Ga<sub>3</sub>. Diverse biochemical parameters provide insight into the metabolic basis of the observed stimulation. The observations have implications for conservation and cultivation of the species.

AS thanks the Department of Higher Education, Himachal Pradesh for grant of study leave. The authors are thankful to the inhabitants of the surveyed area for their whole-hearted cooperation and help during field study. Thanks are also due to Dr Vijay Kumar and Mr. Mahesh Kumar for their suggestions about statistical analyses.

# REFERENCES

Ahmadvand G, Soleimani F, Saadatian B and Pouya M 2012 Effect of seed priming with potassium nitrate on germination and emergence traits of two soyabean cultivars under salinity stress conditions. *Amer-Eur J Agr Env Sci* **12** 769-774.

Andryka-Dudek P, Ciacka K, Wisniewska A, Bogatek R and Gniazdowska A 2019 Nitric –oxide induced dormancy removal of apple embryos is linked to alteration in expression of genes encoding ABA and JA biosynthesis or transduction pathways and RNA nitration. *Int. J. Mol. Sci.* **20(5)** 1007.

Aswal B S and Mehrotra B N 1994 Flora of Lahaul-Spiti, a cold desert in North West Himalaya, Bishen Singh Mahendra Pal Singh, Dehradun, India.

Baskin C C and Baskin J M 1998 Seeds: Ecology, biogeography and evolution of dormancy and germination. San Diego, Academic Press.

Bailly C 2004 Active oxygen species and antioxidants in seed biology. *Seed Sci Res* **14** 93-107.

Barik S K, Rao B R P, Haridasan K, Adhikari D, Singh PP and Tiwary R 2018 Classifying threatened species of India using IUCN criteria. *Curr Sci* **114** 588-595.

Bates L S, Waldren R P and Teare I D 1973 Rapid determination of free proline for waterstress studies. *Plant Soil* **39(1)**205-207.

Beligni M V and Lamattina L 1999 Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. *Planta* **208** 337-344.

Bethke P C, Libourel IGL and Jones RL 2006 Nitric oxide reduces seed dormancy in *Arabidopsis.JExp Bot* **57** 517-526.

Bewley J D and Black M 1994 Viability, dormancy and environmental control. *Physiology and biochemistry of seeds in relation to germination*. Vol. 2. Springer-Verlag.

Bradford MM 1976 A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72** 248-254.

Cadman C S, Toorop PE, Hilhorst HW and Finch-Savage WE 2006 Gene expression profiles of *Arabidopsis* Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J* **46** 805-822.

Chein C T, Kuo-Huang L L, Shen Y C, Zhang R, Chen S Y, Yang J C and Pharis R P 2004 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 45 1158-1167.

Claussen W 2005 Proline as a measure of stress in tomato plants. Plant Sci 168 241-248.

Debeaujan I, Koornneef M 2000 Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiol 122(2) 415-424.

Dhindsa RS, Plumb-Dhindsa P and Thorpe TA 1981 Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J Exp Bot* **32** 93-101.

Fan W, Zhang M, Zhang H and Zhang P 2012 Improved tolerance to various abiotic stresses in transgenic sweet potato (*Ipomoea batatas*) expressing spinach betain aldehyde dehydrogenase. *PLoS One* **7(5)** e37344.

Filner P and Varner J E 1967 A test for *de novo* synthesis of enzymes: Density labelling with  $H_2O^{18}$  of barley alpha-amylase induced by gibberellic acid. *Biochem* **58(4)** 1520-1526.

Finch-Savage WE, Leubner-Metzger G 2006 Seed dormancy and the control of germination.

*New Phytol* **171(3)** 501-523.

Finkelstein RR, Reeves W, Arizumi T and Steber C 2008 Molecular aspects of seed dormancy. *Ann Rev Plant Biol* **59** 387-415.

Fredes I, Moreno S, Diaz FP and Gutierrez RA 2019 Nitrate signalling and the control of *Arabidopsis* growth and development. *Curr Opin Plant Biol* **47** 112-118.

Ganaie KA, Aslam S and Nawchoo IA 2011 No chilling obligation for germination in seeds of *Arnebia benthamii*: A critically endangered alpine medicinal plant of north-west Himalayas. *Int J. Biodiver Cons* 3(5) 155-159.

Ghorbani A 2005 Studies on pharmaceutical ethanobotany in the region of Turkman Sahra, north of Iran. *J Ethnopharmacol* **102** 58-68.

Goldstein JL and Swain T 1963 Changes in tannins in ripening fruits. *Phytochem* **2(4)** 371-383.

Hartmann HT and Kester DE 1989 Plant Propagation: Principles and Practices. Prentice Hall, New Delhi, India.

He J, Ren Y, Chen X and Chen H 2014 Protective role of nitric oxide on seed germination and seedling growth of rice (*Oryza sativa* L.) under cadmium stress. *Ecotox Environ Saf* **108** 114-119.

Hilton JR 1984 The influence of light and potassium nitrate on the dormancy and seed germination of *Avena sativa* L. seeds and its ecological significance. *New Phytol* **96** 31-34. ISTA 1999 International rules for seed testing. *Seed Sci. Tech.* **27 1-333.** 

Kala C P 2000 Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. *Biol Conser* **93** 371-379.

Kandari L S, Rao K S, Maihkuri R K, and

Chauhan K 2008 Effect of pre sowing, temperature and light on the seed germination of *Arnebia benthamii* (Wall.Ex G.Don). An endangered medicinal plant of central Himalaya, India. *Afr J Plant Sci* **2** (1) 5-11.

Kaneko M, Itoh H, Tanka M U, Ashikari M and Matswoka M 2002 The  $\alpha$ -amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in pithelium. *Plant Physiol* **128(4)** 1264-1270.

Kaul S, Sharma S S, Mehta I K 2008 Free radical scavenging potential of L-proline: evidence from in vitro assays. *Amino Acids* **34** 315-320.

Kashiwada Y, Nishizada M, Yamagishi T, Nonaka GI, Cosentino LM, Snider JV, and Lee KH 1995 Anti-AIDS agents, 18 sodium and potassium salts of caffeic acid tetramers from *Arnebia euchroma* as anti-HIV agents. *J Nat Prod* **58** 392-400.

Kavikishor PB, Hong ZL, Miao GH, Hu CAA and Verma D P S 1995 Overexpression of delta-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol* **108** 1387-1394.

Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou JP, Kamiya Y, Nambara E and Truong HN 2009 The *Arabidopsis* abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. *Plant Physiol* **149(2)** 949-960.

Mayers A M and Evenari M 1952 The relationship between structure of coumarin and its derivatives and their activity as germination inhibitors. *J Exp Bot* **3(2)** 246-252.

Myers N, Muttermeier RA Muttermeier CA, Da Fonseca ABG and Kent J 2000 Biodiversity

hotspots for conservation priorities. *Nature* **40** 853-858.

Pandey S, Kumari A, Shree M, Kumar V, Singh P, Bhardwaj C, Loake, GJ, Parida SK, Masakapalli SK and Gupta KJ 2019 Nitric oxide accelerates germination via the regulation of respiration in chikpea. *J Exp Bot* **70(17)** 4539-4555.

Polunin O and Stainton A 1984 Flowers of the Himalaya. Oxford University Press, New Delhi, India.

Samant S S, Dhar U, Palni L M S 1998 Medicinal Plants of Indian Himalaya: Diversity Distribution Potential Value. Gyanodaya Parkashan, Nainital, Uttaranchal, India 155-158.

Sharma R K, Sharma S and Sharma SS 2006 Seed germination behaviour of some medicinal plants of Lahaul and Spiti cold desert (Himachal Pradesh): Implications for conservation and cultivation. *Curr Sci* **90(8)** 1113-1118.

Sharma R K, Sharma S, Sharma SS 2010 Storage-dependent changes in dormancy and germination of Himalayan Mayapple (Podophyllum hexandrumRoyle) seeds and their response to gibberellic acid. J herbs spices med plants **16 (1)** 69-82.

Sharma R K, Sharma S, Sharma SS 2014 The long-term ambient storage-induced alterations in seed viability, germination and seedling vigour of Saussurea costus, a critically endangered medicinal herb of North West Himalaya. *J Appl Res Med Arom Plants* **1 (3)** 92-97.

Singh K N, Lal B, Singh R D, Todaria N P and Ahuja PS 2007 Species richness, distribution pattern and conservation status of higher plants in the Spiti cold desert of trans-Himalaya, India. *Int J Biodiver Sci Manage* **3(4)** 223-233.

Singh K N, Lal B, Chand G, Todaria N P 2012 Ecological features and conservation of *Arnebia euchroma*, a critically endangered medicinal plant in Western Himalaya. *Int J Conser Sci* **3(3)** 189-198.

Soltani E, Ghaderi-far F, Baskin CC and Baskin JM 2015 Problems with using mean germination time to calculate the rate of seed germination. *Aust J Bot* **63(8)** 631-635.

Sultana N, Ikeda T and Mitsui T 2000 GA<sub>3</sub> and proline promote germination of wheat seeds by stimulating  $\alpha$ -amylase at unfavorable temperatures. *J Plant Prod Sci* **3** 232-237

Zhang H, Shen W, Zhang W, and Lai-Xu L 2005 A rapid response of beta-amylase to nitric oxide but not gibberellin in wheat seeds during the early stage of germination. *Planta* **220(5)** 708-716.

Zhang A Y, Jiang M Y, Zhang J H, Tan M P and Hu X L 2007 Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defence in maize leaves. *New Phytol* **175** 35-60.