Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 3 (2): 61-68, 2009
ISSN 1819-3455

Reproductive Biology and Breeding System of *Aconitum balfourii* (Benth) Muk: A High Altitude Endangered Medicinal Plant of Garhwal Himalaya, India

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Abstract: *Aconitum balfourii* (Benth) Muk an endangered medicinal herb of high altitude region was studied for reproduction biology. Controlled pollination studies were also conducted on plants grown under hothouse. Observation reveals that ravine and scree wild habitats of alpine region had better flowers and seed production. Furthermore, hot house grown plants had far more superiority over wild populations for flowers and seed production. Protandry type of dichogamy was observed and is viewed as an anti-selfing mechanism. In general, higher pollen germination was achieved comparatively at low concentrations of GA_3, IAA and IBA (1 ppm). Tube elongation was maximum upto 65 μm in IAA 1 ppm and 63 μm in IAA (5 ppm) and sucrose 5%. Dark condition along with violet color inhabits pollen germination whereas it enhances pollen tube elongation. Apomixis as well as autogamous self pollination was not observed in the species. However, fruit set differed significantly between the hand-selfed and hand-crossed treatments. Seed characteristics of open pollinated plants viz., number of seeds and seed yield per pod and plant were significantly at par than hand self pollinated flowers. Self-compatibility in the species may be a derived condition, considering that flowers are insect pollinated. The abundance and efficiency of pollinators may also affect mating patterns. The results of this study on the floral biology and breeding system of *A. balfourii* indicate reproductive potential of the species for cross-pollination, which would limit the production of selfed seeds and as such is likely to maintain sustainable levels of heterozygosity among the various populations.

Key words: Apomixis, control cross, pollen germination, protandry, reproductive phenology

INTRODUCTION

Aconitum balfourii (Benth) Muk locally known as *Meetha vish* of family Ranunculaceae is very important medicinal plant in Indian Traditional System of Medicines (TSM) including Ayurveda as *Vatsnabha*. In fact, it is cited by *Susrutas* (in Ayurveda) as one of the 13 bulb poisons and 4 varieties (species) of *Vatasnabha* among the 55 stable poisons. Information on taxonomy (Stapf, 1905; Naitnani, 1984), chemical composition and active ingredients (Anonymous, 1985; Bahuguna *et al*., 2000) and uses (Thakur *et al*., 1989; Uniyal, 1998) of the species is available. As per the National Medicinal Plant Board (NMPB), Government of India website, annual demand of the species during

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2001-02 was 322.3 tons which went up to 3426.8 tons during 2004-05 with an annual growth rate of 30%. Due to great market demand, over and illegal exploitation from wild is going on and the species is identified as an endangered (Nautiyal *et al*., 2002) in Garhwal Himalaya and vulnerable (Vu) in entire Uttarakhand by CAMP (2003). Therefore, to ensure sustainable utilization as well as conservation of the species, cultivation is recommended (Nautiyal and Nautiyal, 2004; Nautiyal *et al.*, 2005).

Information on pollination biology not only required for comprehensive understanding of the efficiency of breeding system of a species and its evolutionary success but also for effective optimization of yield, conservation and rational genetic improvement (Shivanna and Mohan Ram, 1993). Pollination success in plants is determined by the timing of flower opening, anther dehiscence and stigma receptivity. These depend on the environment/species interaction, pollen vector accessibility and recognition events in the stigma and style. The timing of these events generally differs between species and cultivars, but their synchronization governs fruit set, yield and quality (Nautiyal *et al*., 2009). A detailed understanding of the relationship between these flowering events in *A. balfourii* is needed given that breeding program efficiencies and production of new varieties/hybrids relies on manipulation of processes such as pollination and the pollen-pistil interaction. Pollen germination studies are essential for the estimation of quality of the pollen required for controlled pollination. Artificial germination of the pollen grains is a surest test of pollen fertility, which is important for the understanding of any breeding programme. These aspects of reproductive biology in *A. balfourii* remain unclear so far, only the pollen structure is studied by Sharma (1985). Keeping in view all the above facts in mind, this study was aimed at understanding the phenology and breeding system of *A. balfourii* so that further genetic improvement can be done to meet the demand of TSM and pharameutical industries.

MATERIALS AND METHODS

Four wild populations of *A. balfourii* i.e., Tungnath, Dayara, Kilpur and Panwali-Kantha, in alpine region of Garhwal Himalaya, India were selected and the reproductive phenology viz., time of anthesis, anther dehiscence, stigma receptivity, pollination and fruit and seed setting were recorded daily on ten randomly selected plants from August to October 2005. In order to estimate flower production, the total number of flowers per plant were counted manually in all the selected plants (N = 10) of each populations. The same procedure was carried out to quantify production of pods. Seeds per pod were estimated on ten selected pods per plant (N = 10 plants). These observations were aimed to identify the populations as best seed source for future conservation and crop improvement study.

Reproductive biology of species was studied at Alpine Field Station of High Altitude Plant Physiology Research Centre (HAPPRC), located at Tungnath (3550 m.a.s.l.), Uttarakhand, India. The area lies between 30°14' N Latitudes and 79°13' E Longitudes of Western Himalaya. Observations on flowering time and duration, time of anthesis, anther dehiscence, stigma receptivity, pollination, fruit and seed setting, were recorded daily from August to October 2005. Receptivity of stigma was analyzed with H_2O_2 method (Dafni, 1992) However, controlled pollination studies were conducted on plants those had been brought into cultivation in the hothouse as a protective measure since hail storm and frosting is common feature of alpine environment during this period.

Pollen counts were made on 5 anthers from different flowers. The anthers were obtained from closed flower just prior to anthesis, placed in the small vial containing 1 mL of glycerin 1%, smacked and pollen grains were suspended. From this concentrate, five 10 μL droplets were removed and pollen grains were counted under the microscope. Production of pollen grains per flower was estimated by multiplying number of pollen grains per anther by the number of anthers per flower. A factorial experimental design (Tuinstra and Wedel, 2000), was used to evaluate the effects of sucrose, IAA, IBA

and GA_3 (1, 5 and 10 ppm), thiourea and KNO_3 on pollen germination. Sucrose, boric acid and calcium nitrate have been shown to be key substrates for pollen germination in other alpine species (Raina *et al*., 2003; Nautiyal *et al*., 2009). Sucrose was initially tested at 1, 5 and 20%; IAA, IBA and GA_3, thiourea and KNO_3 were tested at 1, 5 and 10 ppm. The experiment was blocked in time with five replications in a randomized complete block design. Pollen was collected from undehisced anthers. Bulk of the pollen was distributed onto germination media in cavity slides and placed at room temperature (15°C) for 52 h. Germination was quantified as the percentage of germinated pollen grains per 100 evaluated. Pollen grains were considered germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra and Wedel, 2000). Pollen germination at different light conditions, viz., dark, blue, green, violet and red, was also evaluated on optimal germination medium (5% sucrose) to test the effect of light on pollen germination.

The assessment of breeding system involved randomly selected five plants in each treatment. Following treatments were performed: (1) natural pollination-flowers were not manipulated, (2) autogamous self pollination-buds were bagged throughout their flowering period, (3) hand self pollination - bagged flowers were hand pollinated with their own pollen, (4) open cross pollination-anthers were emasculated and stigmas left for open pollination, (5) hand cross pollination-emasculated bagged flowers were pollinated with pollen from another plant. Emasculation were done with methods of Verma *et al*. (1979) approximately 16 h before anthesis and (6) apomixes-anthers and stigma of buds were clipped. Fruit setting among hand-selfed and hand cross treatments and among open pollinated and hand-cross treatments were also compared. An indirect measure of self-incompatibility was obtained by dividing the average fruit set after self pollination by the average fruit set after cross-pollination (Lloyd and Schoen, 1992). The value of one indicates complete self-compatibility.

RESULTS

Flowering and seed production potential of wild populations, cultivated in an alpine garden and hothouse is presented in Table 1. It appears that populations dominated by tree canopy had minimum flowers as well as seeds per fruits, followed by scrub dominated populations. However, ravine and scree habitats of alpine comparatively had better flowers and seed production. Cultivation further improved flowers as well as seed production while hothouse grown plants showed far more superiority over wild populations. Flowering is asynchronous and lasts for 43 days (7 August-20 September). Flowers arranged in straight racemose and the flowers at base bloom first which remain up to 10-15 days and gradually decreased up to 5 days in terminal flowers (Table 2). Floral display has been summarized in Table 3. Flowers are many, tomentellous and bluish in color. Pedicels erect or lower

Table 1: Flowering potential of *A. balfourii* in different wild locations, alpine garden and hothouse condition at Tungnath during 2006

Populations	Flowers plant^{-1}	Fruit weight (mg)	No. of seeds fruit^{-1}	Seed weight (mg 10 seeds^{-1})	Seed size (mm)	
					Lenght	Widht
Tungnath (3200-3600 m) alpine	20.15±5.15	32.85±4.15	8.20±1.50	18.85±2.50	3.6±0.3	2.1±0.1
(ravine and scree slopes) timberline	25.20±4.15	33.50±6.20	8.50±0.50	18.95±1.25	3.8±0.2	1.9±0.1
(under scrubs)						
Kilpur (3000-3500 m) alpine	21.25±6.15	33.00±4.20	8.50±1.50	19.25±1.80	3.5±0.2	2.0±0.2
(under scrubs)timberline (ravine)	28.15±5.00	34.00±5.25	11.50±1.50	20.85±2.25	3.9±0.9	2.2±0.1
Dayara (2800-3400 m) alpine	30.15±10.15	28.00±3.25	8.25±0.50	17.65±1.25	3.6±0.2	1.8±0.3
(ravine) timberline under canopy	15.10±5.15	30.15±6.15	9.15±1.33	18.14±1.25	3.7±0.3	1.9±0.3
Panwali (2800 m) timberline	12.15±3.15	25.10±4.33	8.15±1.33	16.15±2.25	3.5±0.6	1.7±0.2
(under canopy)						
Tungnath (Alpine garden	35.15±6.15	35.15±6.45	9.15±1.20	21.15±2.51	3.5±0.2	1.8±0.2
(Polyhouse)	65.15±20.15	38.45±3.15	12.50±1.50	25.15±2.50	4.0±0.2	2.4±0.2

Table 2: Flowering phenology of *A. balfourii* (out of 50 plants observed)

No. of flowering plants	Flowering period	Total No. of days of flowering
2	7 Aug.-15 Aug., 2006	15
5	15 Aug.-20 Aug., 2006	12
5	21 Aug.-25 Aug., 2006	10
15	26 Aug.-31 Aug., 2006	12
15	1 Sept.-7 Sept., 2006	14
5	8 Sept.-15 Septe., 2006	8
3	16 Sept.-20 Sept.,206	5

Table 3: Floral biology of *A. balfourii*

Flowering period	Aug.-Sept.
Inflorescence type	Straight, racemose
Flower type	Hermaphrodite, Hypogynous
Color	Tomentellous, bluish
Nectar	Present, glabrous,12-13 mm long
Odour	Absent
Anthesis	7-10 am
Time of anther dehiscence	7.30-11.00 am
Mode of anther dehiscence	Longitudinal
Stigma receptivity	3-6 days
Type of dichogamy	Protandry
No of anther/flowers	20
No. of pollen grains/anther	45000±1500
No. of pollen grains/flower	90000
Pollen viability	-
Pollen shape	3-zonicolpate, prolate with size 30×20 μm
Stigma type	Capitate, pentacarpellary, apocarpous
Ovule type	Trilocular with axile placenta
Days taken for capsule maturity	20-35
Seed	Obpyramidal, 3-3.5 mm long, dark brown
Seed/plant	3500-5500

ascending, bracteoles if any, dentate and small. Sepals blue, pubescent, uppermost helmet shaped, semi orbicular in profile, slightly convex in front and shortly beaked. The anthesis was highly temperature dependable and observed between 7:00 to10:00 am. The temperature in hot house conditions in between 08:00-10:00, 10:00-12:00 and 12:00-14:00 h of the day averaged at 10.7±0.5, 22.2±0.9 and 18.1±0.9°C, respectively. Whereas, the mean daily minimum and maximum temperature in alpine condition was recorded as 6.5±0.3 and 20.7±0.7°C, respectively. At low temperature and during night, corolla remains closed, therefore, are temperature sensitive. This process continues till fertilization when stigma lobes become dry and shedding of corolla start. Nectar is glabrous and odorless. The number of flowers per plant in natural populations varied from 12-35, whereas the plants grown under hot house conditions produce massive flowers and the number goes up to 65 flowers plant^{-1}.

Anthers dehisced longitudinally between 7.30 to 11.00 am; strongly depend on higher level of temperature. Anthers numbered 20 per flower and the pollen grains per anther varied between 43500-46500, which means an average of 90000 pollen grains per flower (Table 3). Pollen remains viable only up to 3 days after dehiscence. The anthers densely surround stigma up to 3 days before anthesis and moves towards corolla and attain maturity thereafter dehisce to discharge of pollen. Small gap thus separate stigma and anther which provide passage of insect (mainly bumble bee) for pollination. Colour pattern of corolla attract insect vectors to effect pollination. Anther dehiscence was not synchronous rather they dehisced at different time for 5-8 days. The stigmatic lobes at the time of anther dehiscence remain in adpressed conditions. After the completion of anther dehiscence, stigmatic lobes start opening till 3-6 days, which was the stage of stigma receptivity for pollen germination (Table 4). Table 5 and 6 summarize the data on effect of different growth hormones, nitrogenous compounds (thiourea, KNO_3) and different light colors on pollen germination and tube

Table 4: Different sequences in development of pollen and stigma receptivity in *A. balfourii*

Stages of anthesis	Pollen development	Stigma development
6-3 DBA	All anthers in group at the centre of corolla tube	Stigmatic lobes below anther level
3-1 DBA	anthers move towards corolla and 2-4 anthers reach above stigma and pollen dehiscence started, flower opens (anthesis takes place)	Style and ovary start increasing in size
1-5 DAA	Pollen dehiscences continued	Stigmatic region almost in level with anther and lobes start opening
6-8 DAA	Dehiscence complete, pollen lost viability, few non-viable pollen attached in anther lobe	Lobes continue to open, receptive to pollen germination and divide into 5 lobes
9-10 DAA	Anthers shrinked and start withering	Lose receptivity and start drying

Table 5: Effect of growth hormones and other medium on pollen germination and tube elongation

Treatments	Pollen germination (%)	Pollen tube elongation (μm)
Sucrose (%)		
1	47.66±2.51	60.33±2.51
5	55.00±2.64	63.33±1.52
20	55.33±1.51	59.66±2.08
	F = 48*, p = 0.002	F = 72*, p = 0.0
IAA (ppm)		
1	42.66±2.51	64.66±2.08
5	40.33±2.08	63.33±1.15
10	31.33±3.21	56.66±5.85
	F = 57*, p = 0.001	F = 244.9*, p = 0.0
IBA (ppm)		
1	40.66±2.08	60.33±2.08
5	41.00±1.73	62.00±2.00
10	39.33±1.52	57.33±1.52
	F = 174*, p= 0.001	F = 344.2*, p = 0.0
GA_3 (ppm)		
1	44.66±1.52	57.00±2.64
5	39.66±1.52	51.66±2.08
10	37±4.35	50.66±5.13
	F = 104.27*, p = 0.005	F = 214.2*, p = 0.0
Kno_3 (ppm)		
1	37.66±2.51	47.00±2.00
5	32.33±1.52	48.33±2.8
10	24.66±2.08	41.33±2.3
	F = 32*, p = 0.0	F = 142.0*, p = 0.0
Thiourea (ppm)		
1	20.67±2.08	43.00±1.00
5	15.67±2.08	46.66±2.52
10	14.33±2.51	42.00±4.38
	F = 12.72*, p = 0.02	F = 169.2*, p = 0.0

*Significant

Table 6: Effect of light on pollen germination and tube elongation

Light color	Pollen germination (%)	Tube elongation (μm)
Dark	21.00±3.60	60.33±1.52
Red	37.00±2.64	62.00±2.64
Green	31.33±3.21	55.00±3.00
Blue	28.00±3.00	56.00±2.00
Violet	23.66±1.52	50.00±5.29
	F = 14.37*, p = 0.0	F = 51.10*, p = 0.0

*Significant

elongation. The results revealed that pollen germination was higher in 5 and 20% sucrose with significant variation. Among growth hormones treatments, the maximum germination was observed in GA_3, 1 ppm. In general, higher germination was achieved comparatively at low concentrations of growth hormones. The low concentrations of the nitrogenous medium (KNO_3 and thiourea) also proved as better germination medium but success rate of germination was lower than the growth hormones and sucrose. Tube elongation was maximum upto 65 μm in IAA 1 ppm. Analysis of data

Table 7: Effect of different pollination methods on fruit/seed set in *A. balfourii* species

Treatments	Fruit set (%)	Pod weight (mg)	No. of seeds pod^{-1}	Seed weight pod^{-1} (mg)*	No of seeds plant^{-1}	Seed weight plant^{-1} (mg)
Natural pollination	82.15	34.15±4.15	15.15±6.15	47.15 (2.34)	4000±1500	9360
Autogamous self-pollination	0.00	0	0	0	0	0
Hand self pollination	30.00	24.70±3.13	7.15±1.25	14.65 (2.05)	1000±450	2050
Open cross pollination	75.50	20.15±4.50	7.50±2.50	14.25±2.50	2500±550	4120
Hand-cross pollination	85.00	24.15±4.30	8.15±2.13	17.12 (2.12)	1500.50±550	3180
Apomixes	0.00	0	0	0	0	0

(single factor ANOVA) on pollen germination and tube elongation revealed significant improvement due to different concentrations and treatments. These concentrations represent the requirements for optimal pollen germination and pollen tube elongation. Even at optimal media composition, only 55% pollen germination was observed.

The effect of different light colors on pollen germination and tube elongation was also observed (Table 6) as the alpine region experiences high intensity of solar radiation (Korner, 1999). Observations revealed that dark condition and violet light inhibited pollen germination whereas, dark condition enhanced tube elongation. The maximum pollen germination and tube elongation was observed in red light. Furthermore, variation in pollen germination ($F = 14.37$; $p = 0.0$) and pollen tube elongation ($F = 51.30$; $p = 0.0$) was found highly significant using single factor ANOVA.

The results from controlled pollination are summarized in Table 7. Flowers used to test for apomixes, did not set fruit. Fruit set differed significantly between the hand-selfed and hand-crossed treatments with an ISI value of 0.37 (Lloyd and Schoen, 1992). Seed characteristics viz., number of seeds and seed yield per pod and plant were significantly at par than hand self pollinated flowers.

DISCUSSION

Observations reveal that shade is detrimental factor for flowering and seed production in wild habitats. Furthermore, domestication of the species in the hot house showed optimal flowering and seed production. These results support earlier observations (Nautiyal and Purohit, 2000) on the species. Likewise, variation in the time of anther dehiscence and stigma receptivity indicate protandry forms of dichogamy in individual flower to present the potential for cross-pollination. Protandry in particular, is viewed as an anti-selfing mechanism because it provides opportunities for the receipt of outcross pollen before self-pollen is shed and is more common in self-compatible than self-incompatible taxa (Lloyd and Webb, 1986; Bertin, 1993).

It appears that different growth hormones and nitrogenous compounds influenced pollen germination and tube elongation differently (Setia *et al*., 1985). Chhabra and Malik (1978) interpreted that quiescent pollen contains RNAs and proteins needed for the tube emergence. IAA pretreatments stimulate the synthesis of new RNAs and thereby increase proteins needed for tube growth. Mascarenhas and Mermelstein (1981) also emphasized the need of newly synthesized protein for tube growth. Over the years, an array of plant growth hormones and other chemicals have been empirically added to the culture medium to promote pollen germination and tube growth and the positive effect of some of these substances have led to speculation about their biochemical functions. Further, pollen germination and tube elongation are two independent processes governed by separate set of conditions (Malik, 1985). Maximum germination and tube elongation in red colour suggest the involvement of phytochromes, as red colour synthesize phytochrome protein and its biological manifestation (Sharma and Malik, 1978; Katiyar, 1989).

The results on controlled pollination suggest predominantly self-incompatibility in *A. balfourii*, as no fruit setting was observed from autogamous self pollination which may be due to the existence of protandry as also observed earlier in two high altitude herbs, *Gentiana kurroo* (Raina *et al*., 2003) and *Aconitum heterophyllum* (Nautiyal *et al*., 2009) although, few fruits developed from selfing. Such

fruits were smaller than the fruits produced by open pollinated and from hand-crossed flowers and most aborted early in development. In general, flowering plants possess a wide array of morphological and physiological mechanisms that influence mating patterns, particularly the degree of self fertilization (Eckert and Barrett, 1994). Temporal separation of male and female function (protandry in this case) is one of the most widespread morphological mechanisms as found in >75% co-sexual angiosperm species (Griffin *et al*., 2000). Further, self-compatibility in the species may be a derived condition, considering that its flowers are insect pollinated (Gituru *et al*., 2002). In addition, the abundance and efficiency of pollinators may affect mating patterns. However, observations on pollinators were not undertaken during the course of this study. Nevertheless, aim of this study was to observe floral and reproductive biology and breeding behaviour in *A. balfourii* so that crop improvement programmes could be undertaken in future for better *in situ* and *ex situ* conservation of genetic variability and cultivation for commercial purposes, as the species, already has already been specified vulnerable to endangered (CR) in the wild (Nautiyal *et al*., 2002; CAMP, 2003).

CONCLUSION

The results of this study on the floral biology and breeding system of *A. balfourii* indicate the species reproductive potential for cross-pollination, which would limit the production of selfed seeds and as such is likely to maintain sustainable levels of heterozygosity among the wild populations. This fact furthermore could be useful in future crop improvement study considering the fact that there already exist a great heterozygosity among the wild populations of this very important medicinal herb of high altitude Himalaya.

ACKNOWLEDGMENTS

Author (BPN) is thankful to researchers Dr. Rajeev K. Vashitha, Gunjan, Poonum, Anurag Dhyani and Karan Singh for their help during observations. The author BPN gratefully acknowledges the SERC division, DST, GOI, New Delhi for partial financial support.

REFERENCES

Anonymous, 1985. The Wealth of India (Raw Material). Vol. 1A, Publication and Information Directorate, CSIR, New Delhi.

Bahuguna, R., M.C. Purohit, M.S.M. Rawat and A.N. Purohit, 2000. Qualitative and quantitative variations in alkaloids of *Aconitum* species from Garhwal Himalaya. J. Plant Biol., 27: 179-183.

Bertin, R.I., 1993. Incidence of monoecy and dichogamy in relation to self-fertilization in angiosperms. Am. J. Bot., 80: 557-560.

CAMP., 2003. Threat assessment and management prioritization of selected medicinal plants of Western Himalayan states, India. Proceedings of the Conservation Assessment of Medicinal Plants Workshop, May 22-26, Shimla, Organized by FRLHT, Banglore, India, pp: 1-1.

Chhabra, N. and C.P. Malik, 1978. The Effect of Nucleic Acid and Protein Synthesis Inhibitors on Iaa-Induced Pollen Tube Elongation in *Pisum Sativum*. In: Advances in Reproductive Physiology, Malik, C.P. (Ed.). Kalyani Publishers, New Delhi, pp: 57-63.

Dafni, A., 1992. Pollination Ecology: A Practical Approach. Oxford University Press, New York.

Eckert, C.G. and S.C.H. Barrett, 1994. Post-pollination mechanism and the maintenance of outcrossing in self-compitable, tristylous, *Decodon verticillatus* (Lythraceae). Heredity, 72: 396-411.

Gituru, W.R., Q. Wang, Y. Wang and Y. Hao Guo, 2002. Pollination ecology, breeding system and conservation of *Caldesia grandis* (Alismataceae), an endangered marsh plant in China. Bot. Bull. Acad. Sin., 43: 231-240.

Griffin, S.R., K. Mavraganis and C.G. Eckert, 2000. Experimental analysis of protogyny in *Aquilegia canadensis* (Ranunculaceae). Am. J. Bot., 87: 1246-1256.

Katiyar, S.R., 1989. Effect of light on pollen germination and pollen tube growth in *Pinus kesiya* Royle ex Gord. Acta Bot. Indica, 17: 133-135.

Korner, C., 1999. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystem. 1st Edn., Springer-Verlag, Springer-Verlag,.

Lloyd, D.G. and C.J. Webb, 1986. The avoidance of interference between the presentation of pollen and stigma in angiosperms. N. Z. J. Bot., 81: 199-205.

Lloyd, D.G. and D. Schoen, 1992. Self and cross-fertilization in plants. I. Functional dimensions. I. J. Plant Sci., 153: 358-369.

Malik, C.P., 1985. Metabolic Control of Pollen Germination. In: Recent Advances in Pollen Research, Varghese, T.M. (Ed.). Applied Publishers, New Delhi, pp: 25-42.

Mascarenhas, J.P. and J. Mermelstein, 1981. Messanger RNAs: Their utilization and degradation during pollen germination and tube growth. Acta Soc. Bot. Polon., 50: 13-20.

Naitnani, B.D., 1984. Flora of Chamoli. Vol. 1, Botanical Survey of India, Howarh.

Nautiyal, M.C. and A.N. Purohit, 2000. Cultivation of Himalayan aconites under polyhouse conditions. Curr. Sci., 78: 1062-1063.

Nautiyal, B.P., P. Vinay, R. Bahuguna, U.C. Maithani, H. Bisht and M.C. Nautiyal, 2002. Population study of three aconites species in Garhwal for the monitoring of species rarity. Trop. Ecol., 43: 297-303.

Nautiyal, M.C. and B.P. Nautiyal, 2004. Agrotechniques for High Altitude Medicinal and Aromatic Plants. 1st Edn., Mahendra Pal Singh Bishen Pal Singh, Dehradun, ISBN: 81-211-0338-X.

Nautiyal, B.P., V. Parkash, R.S. Chauhan, H. Purohit, R. Vashistha and M.C. Nautiyal, 2005. Cultivation of *Aconitum* species. J. Trop. Med. Plants, 6: 193-201.

Nautiyal, B.P., M.C. Nautiyal, V.P. Khanduri and N. Rawat, 2009. Floral biology of *Aconitum heterophyllum* Wall: A critically endangered alpine medicinal plants of Himalaya, India. Turk. J. Bot., 33: 13-20.

Raina, R., M.C. Behera, R. Chand and Y. Sharma, 2003. Reproductive biology of *Gentiana kurrooa* Royle. Curr. Sci., 85: 667-670.

Setia, N., G.S. Mangat and C.P. Malik, 1985. Effect of Growth Regulators and Antimetabolites on Pollen Germination and Tube Elongation in *Cicer arietinum*. In: Recent Advance in Pollen Research, Varghese, T.M. (Ed.). Allied Publishers, New Delhi, pp: 63-68.

Sharma, R. and C.P. Malik, 1978. Effect of Light on Pollen Germination, Tube Elongation and Some Enzymes in *Lathyrus odoratus*. In: Physiology and Sexual Reproduction in Flowering Plants, Malik, C.P. (Ed.). Kalyani Publishers, New Delhi, pp: 105-111.

Sharma, C.M., 1985. Palynological Studies on Alpine Plants of Garhwal Himalaya. IBH Publication, New Delhi.

Shivanna, K.R. and H.Y. Mohan Ram, 1993. Pollination biology: Contribution to fundamental and applied aspects. Curr. Sci., 65: 226-232.

Stapf, O., 1905. The Aconites of India – A Monographs. Bengal Secretariat Press, Calcutta.

Thakur, R.S., H.S. Puri and A. Husain, 1989. Major Medicinal Plants of India. 1st Edn. Central Institute of Medicinal and Aromatic Plants, Lucknow, India..

Tuinstra, M.R. and J. Wedel, 2000. Estimation of pollen viability in grain sorghum. Crop Sci., 40: 968-970.

Uniyal, M.R., 1998. Agriculturization of Medicinal Plants in India. Vaidhya Nath Ayurvedic Shodh Sansthan, Patana, Bihar.

Verma, M.M., P.S. Paul, J.S. Kumar and S.B. Singh, 1979. Hybridization. In: Plant Breeding Manual, Atwal, A.S. (Ed.). Punjab Agriculture University, Ludhiana, India.