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Floral architecture, breeding system, seed biology and chromosomal studies in endangered Himalayan *Angelica glauca* Edgew. (Apiaceae)

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Abstract. Endangered *Angelica glauca* an important medicinal plant of temperate Himalaya is valued for its roots which are used to treat several diseases besides food flavouring. Reproductive biology studies conducted in this species for the first time have revealed i). presence of umbels of different orders with only bisexual flowers ii). occurrence of sterile seeds (without embryo) apart from fertile ones iii). seed set in only early blooming umbels (primary and lateral-I) iv). 2n=22 chromosomes besides presence of chromosomes in a group at metaphase and anaphase-I and cytomixis in some pollen mother cells and v). extreme protoandry and cross pollination behavior (upto 95%) of the species. These observations have implications for developing any conservation plan for the species.

Keywords. Endangered, umbel order, Apiaceae, cross pollination, low seed set, embryo less seeds.

INTRODUCTION

Apiaceae an angiosperm family consists of 300-455 genera and 3000-3750 species worldwide (Pimenov and Leonov 2004) and many of these species are highly valued for being economically and medicinally important (Butola and Badola 2006; Sher et al. 2011). The genus *Angelica* is one of the very important genera belonging to family Apiaceae and is represented by about 110-115 species worldwide and almost 87 species in Asia (Pimenov and Leonov 2004). This genus is represented by mainly three species viz. *A. glauca* Edgew., *A. archangelica* L. and *A. nubigena* Clarke in Himalayan region. *A. nubigena* is poorly known species found in Sikkim (Pimenov and Kljuykov 2003) and other species i.e. *A. cyclocarpa* (C.Norman) M. Hiroe and *A. oreadum* Diels have also been reported from Indian Himalaya, Pakistan and Afghanistan (Pimenov and Kljuykov 2003).

Angelica glauca Edgew. (Family: Apiaceae; English name: Himalayan Angelica; Local name: chora, chokhara & gandrayan) is an endangered perennial temperate medicinal and aromatic herb distributed in moist and shady regions of Himalaya at an altitude of 2000-3800m amsl (IUCN 1993; Samant et al. 1998; Chauhan 1999; Butola and Budola 2004; Samant et al.2009) in Afganistan, Pakistan and India (Jammu and Kashmir, Himachal Pradesh and Uttrakhand) (Bisht et al. 2003; Butola and Budola 2004; Saeed and Sabir 2008; Butola and Vashistha 2013).Valued for roots which are used to treat dismenhorrea, metorrhagia, amenhorrea, polycystic ovary syndrome, rheumatism, infantile atrophe (Bisht et al. 2003; Butola and Samant 2006; Butola and Budola 2008; Butola and Vashistha 2013; Goswami et al. 2012), also acts as stimulant, cholagogue, cardio-active, carminative, sudorfic and expectorant (CSIR 1985; Singh and Rawat 2000; Bisht et al. 2003; Butola and Samant 2006). Besides this, roots also yield essential oil used to flavour liquor and food items (Nautiyal and Nautiyal 2004; Butola and Samant 2006). Due to remote distribution of *A. glauca* in inaccessible areas of Himalaya coupled with small size of flower because of being an Apiaceae member, very less work has been carried out on its reproductive biology. However, breeding behavior and reproductive biology studies are crucial for understanding plant pollinator interaction, reproductive bottlenecks as well as for developing conservation plan. Therefore reproductive biology has been studied in details for the first time in this species under the present investigation.

Material and methods

Studies were carried out at Shillaru (2130m amsl, 30°45'00.48"N, 76°59'12.22"E; District Shimla, Himachal Pradesh, India); at Shilly (1550m amsl; 30°54'30"N, 77° 07'30"E; District Solan, Himachal Pradesh, India) and Medicinal plants laboratory of department of Forest Products, College of Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-173230 (Himachal Pradesh, India) during years 2013-2016. Population Shillaru had almost 100 plants whereas in Shilly population 50 plants were present.

The vegetative and floral studies were conducted as per standard literature (Lawrence 1951; Weberling 1989; Kaufman et al. 1989). Pollen-ovule ratio was studied as per Cruden (1977) and pollen viability was calculated on the basis of one percent acetocarmine staining test.

Young floral buds, fixed in absolute alcohol, glacial acetic acid and chloroform in the ratio of 1:1:1(v:v:v) for 24 hours, washed and then stored in 70% alcohol at low temperature, were used for meiotic studies. One percent acetocarmine stain was used for chromosomal staining by usual squash method. For open pollination plants with unopened healthy umbels were tagged and left as such, whereas for assessing autogamy umbels were enclosed (Figure 2a) at pre flowering stage. Increase in ovary size and its transformation into fruit was taken as the basis of fruit set. Seed viability was tested by Topographical Tetrazolium Test (TTZ) test and germination by petri plate method.

Petri-plate germination test was performed at 23 ± 2°C in growth chamber and radicle protuberances were taken as sign of germination. Topographical Tetrazolium Test (TTZ) (0.1% pH 6.0) for 48 hours after extracting seeds was conducted by soaking seeds in water, then excised to expose embryo followed by immersing in TTZ solution (0.1% pH 6.0) under dark conditions for 48 hours. Darkly red stained embryos were taken as viable.

Statistical analysis was made as per CRD factorial (seed germination and viability testing under laboratory conditions), RBD factorial (seed set % in field) as well as T-test (pollination studies). Statistical analysis was conducted as per Gomez and Gomez (1984). Ocular and stage micrometer (ERMA, Tokyo, Japan) were used for micro-measurements and microscopic examination was made using Olympus trinocular research microscope (Model - CH20iBIMF, New Delhi, India).

RESULTS

Morphology and floral architecture

The qualitative and quantitative features are tabulated (Table 1). Plants of this species are erect perennial herbs, inflorescence is compound umbel with umbels of different order i.e. primary, lateral-I, lateral-II and lateral -III umbels based on whether they are borne on main stem or lateral branches (Figure1a, b). Percentage of plants with different umbel order varied in two studied populations (Table 2) and quantitative features of umbels are presented in Table 3. All the morphological features of stem, roots, leaves, inflorescence, flower, fruit and seeds were similar to earlier reports except for the presence of variation in seed size and presence of seeds without embryo which are is being reported for the first time in *A. glauca* (Figure 2b, c).

Phenology

Sprouting starts in spring season (last week of April month onwards) and continues up to June month (first week). Floral buds start appearing during July first week

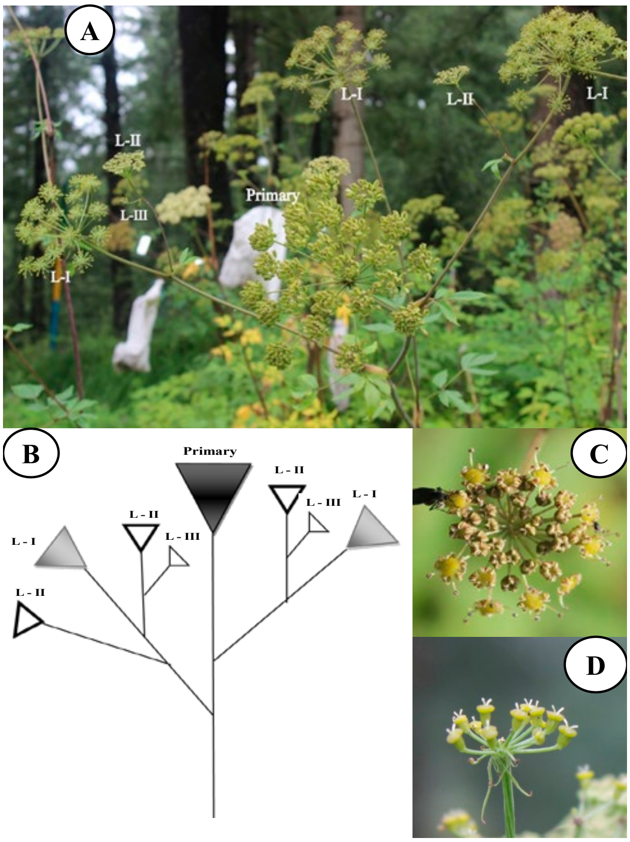

Fig. 1. *A. glauca* a. Arrangement of umbel orders *primary, lateral-I (L-I), lateral –II (L-II), lateral –III (L-III)*; b. Schematic representation of a; c. Anther dehiscence stage; d. Protruding style at receptive stage.

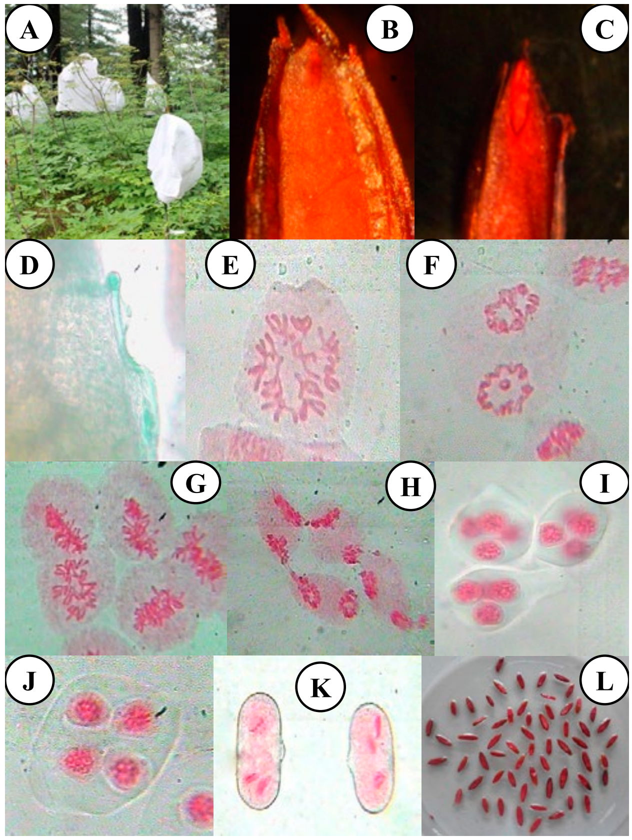

Fig. 2. *A. glauca* a. Bagging for autogamy; b & c. Seed without and with embryo respectively after TTZ staining; d. Pollen germination on receptive stigma; e. Metaphase-I (n=11, 2n=22); f. Ring formation at poles at anaphase-I; g. Clumping of chromosomes at Metaphase-I; h. Pollen mother cells showing cytomixis i. Decussate and tetrahedral tetrads; j. Isobilateral tetrads; k. Trinucleate pollen grains; l. Seed viability through TTZ test.

to mid of August month. Primary umbel floral buds appear first followed by buds of lateral-I, lateral-II and lateral-III umbels. Peak flowering occurs during August and is asynchronous even among plants occurring at same niche. Within a plant also, phenological events are asynchronous among primary, lateral-I and lateral-II umbels. Fruit formation commence during the last week of August completing (full maturity) by September last week. Fruit shedding occurs with beginning of October month onwards and physical as well as physiological changes leading to the prennation commence with the beginning of autumn season. This inactive phase lasts upto next spring season.

Breeding system studies

Floral biology

Anther dehiscence (Figure 1c) asynchronously starts with the opening of floral buds through longitudinal slits and continues for 2-3 days. Stigma become receptive (observed by *in vivo* artificial pollination and resultant pollen germination) when all the anthers of a flower are shed and style reached its maximum length after protruding out of stylopodium (Figure 1d). At stigma receptive stage, stylopodium is with shiny surface. Period of stigma receptivity depended upon umbel order.

Elongated, trinucleate (at shedding stage) and bicolpate pollen show 76% to 100% (average 92.64 ± 1.71%) stainability and their number/flower vary from 17400 to 40700 (average 26128.75± 1323.41). With two ovules/flower, pollen-ovule ratio ranged from 8700 to 20350 (average 13064.37± 661.71). Number of seeds produced ranged from 334 to 1024 (average 670.27 ± 65.24) in primary umbel and 112 to 344 (average 216.64 ± 18.88) in lateral-I umbel whereas, no seed set in lateral-II and lateral-III umbels was observed as these umbels dry before fruit formation.

Table 1. Qualitative and quantitative morphological features of *A. glauca*.

Plant part	Qualitative	Quantitative
Habit and habitat	Erect perennial temperate and alpine herb.	
Stem	Erect, cylindrical, hollow inside, smooth and swollen at nodes. Covered with white powder and variously colored (entire shoot purple; purple upto middle and green at top or opposite; to green with purple patches).	**L:** 172.67 ± 6.36 cm
Roots	Perennial consisting of tuberous roots, pale yellow to yellowish brown, surface smooth or wrinkled and occasionally tap root splits into two near collar region.	**Tap L:** 18.90 ± 1.66 cm **B:** 13.56 ± 0.97 mm **Secondary L:**12.53 ± 1.23 cm **B:** 1.40 ± 0.36 mm
Leaves	Large, petiolated, tripinnate, alternate, with very long rachis. Petiole base sheathing. Leaflets: lance-ovate to ovate, tip narrowly-acute to acute, base cuneate, margin irregularly toothed and reticulate venation. Adaxial surface of leaflets dark green and smooth. Abaxial surface grayish white and smooth.	**Cauline leaves/ plant:** 5-12
Inflorescence	Compound umbel with umbels of different orders.	**Umbels/plant:** 2 –9
Involucre bracts	6-10 in number, linear and green colored.	**L:** 2.73 ± 0.27 cm
Bracts	4-11 in number, linear and green colored.	**L:** 1.72 ± 0.17 cm
Flower	Bisexual, pedicillate, epigynous, actinomorphic and pentamerous.	**Spread:** 3.39 ± 0.08 mm
Pedicel	Green colored, length decrease from peripheral towards the centre.	
Calyx	Absent or obsolete.	
Corolla	Petals five, free, valvate, obovate with inward curved tip, green in bud stage and whiter on maturity.	**L:** 2.08 ± 0.03 mm **B:** 1.52 ± 0.07 mm
Androecium	Stamens five, green colored, bilobed, dorsifixed, exerted, alternate to petals, dehisce by longitudinal slits and filaments green colored. Anthers remain bend inwards in bud stage and spread outwards at maturity.	**Filament L:** 3.15 ± 0.09 mm **Anther lobe L:** 0.97 ± 0.02 mm **Anther lobe B:** 0.77 ± 0.01 mm
Gynoecium	Ovary inferior, bicarpillary syncarpous, bilocular bearing single solitary ovule in each locule and placentation apical. Style bifid, erect, white coloured and attain full development after anther dehiscence. Stylar base swollen to form stylopodium.	**Ovary L:** 1.30 ± 0.07 mm **B:** 1.72 ± 0.06 mm **Style L:**1.67 ± 0.06 mm **Ovule size:** 0.65 ± 0.04 × 0.29 ± 0.01 mm
Fruit	Fruit mericarp, green colored, oblong, smooth, flat, pale white to brown, on maturity divides longitudinally into two halves joined with the help of carpophores bearing a single seed in each half.	**L:** 1.66 ± 0.05 cm
Seed	Flat, pale whitish to brown, with five ridges, two lateral ridges form oblong membranous wings that surrounds the seed, wing color pale white or brown.	**Small seed L:** 0.59 ± 0.03 cm **Medium seed L:** 0.94 ± 0.02 cm **Large seed L:** 1.34 ± 0.04 cm
Floral formula	⚥ , ⊕, $K_{\text{0 or obsolete}}$, C_5, A_5, $G_{(2)}$	

L: length; B: breadth.

Chromosomal studies

In most of the pollen mother cells, the bivalent upto metaphase stage appeared to be clumped together without clear separation (Figure 2g), however, some cells with separate 11 bivalents were also observed (Figure 2e). Anaphase-I was interesting as chromosomes at each pole were present in groups forming ring like structure and the number of chromosome in each group at each pole was 11 (Figure 2f). In 7-8% pollen mother cells, cytomixis (Figure 2h) was observed, however other abnormalities like laggards, bridges, etc. were absent. Pollen grains were trinucleate at pollen shedding stage (Figure 2k).

Breeding system

Floral visitors like bees, flies, beetles, butterflies and ants were observed visiting its flowers. Open pollination resulted in 670.27 ± 65.24 seeds/primary umbel and under autogamous conditions, only 21.82 ± 15.36 seeds/primary umbel were set (Table 4). Based on the average number of flowers per primary umbels (591 ± 38.96) with two ovules per flower, 56.71 ± 5.52% seed set under open pollination conditions and 2.63 ± 1.82% under autogamous conditions was observed. Out of is 56.71 ± 5.52% seed set under open conditions, after microscopic examinations, 27.49 ± 2.67% of such seeds was with embryo with the rest (29.22 ± 2.84%) being with-

Table 2. Percentage of plants with different umbel order in *A. glauca*:

Population	Primary	Primary + Lateral- I	Primary + Lateral -I + Lateral -II	Primary + Lateral -I + Lateral -II+ Lateral- III
Shillaru	100%	24%	72%	4%
Shilly	100%	60%	40%	0 %

Table 3. Quantitative features of umbels of different order in *A. glauca*:

Umbel order	Characters			
	Primary	Lateral-I	Lateral-II	Lateral-III
Number per plant	1	2-4	0-5	0-1
Diameter	15.56 ± 0.52 cm × 15.6 ± 0.53 cm	11.59 ± 0.80 × 11.53 ± 0.81 cm	3.93 ± 0.21 cm × 3.93 ± 0.21 cm	It was observed to be simple umbel, with upto 10 flowers, very weak and dried later on before blooming.
Umbelet number	18.50 ± 0.86	18.05 ± 0.85	15.08 ± 1.22	
Number of flowers	591 ± 38.96	539.35 ± 16.92	247.47 ± 31.09	
Diameter of peripheral umbelet	3.05 ± 0.07 cm × 3.05 ± 0.07 cm	2.32 cm ± 0.13 × 2.28 ± 0.14 cm	0.75 ± 0.08 cm × 0.75 ± 0.08 cm	
Diameter of central umbelet	2.43 ± 0.06 cm × 2.42 ± 0.06 cm	1.61 ± 0.16 cm × 1.61 ± 0.16 cm	0.43 ± 0.03 cm × 0.43 ± 0.03 cm	
Number of flowers in peripheral umbelets	33.65 ± 0.91	30.95 ± 1.01	16.05 ± 1.15	
Number of flowers in central umbelets	24.90 ± 0.91	20.8 ±0.89	10.9 ± 1.03 cm	
Length of peripheral rays	8.2 ± 0.45 cm	5 ± 0.29 cm	1.17 ± 0.80 cm	
Length of central rays	5.2 ± 0.35 cm	3.01 ± 0.26 cm	0.64 ± 0.05 cm	
Length of flower stalk in peripheral flowers of peripheral umbelets	1.09 ± 0.07 cm	0.85 ± 0.04 cm	0.25 ± 0.02 cm	
Length of flower stalk in central flowers of peripheral umbelets	0.48 ± 0.04 cm	0.32 ± 0.03 cm	0.16 ± 0.02 cm	
Length of flower stalk in peripheral flowers of central umbelets	0.73 ± 0.04 cm	0.54 ± 0.03 cm	0.1 cm	
Length of flower stalk in central flowers of central umbelets	0.36± 0.03 cm	0.19± 0.02 cm	Central flowers were underdeveloped	

Table 4. Impact of different pollination methods on seed set and viability in primary umbel of *A. glauca*:

Pollination Conditions	Observations – Primary umbel						
	Average number of seeds** per umbel*	Total seed** set* %	Seed set % (with embryo)*	Seed set % (without embryo)*	Seed viability %***		100 Seed# weight grams*
					With embryo	Without embryo	
Open Pollination	670.27 ± 65.24	56.71 ± 5.52	27.49 ± 2.67	29.22 ± 2.84	100	0.00	1.20 ± 0.44 g
Self Pollination	21.82 ± 15.36	2.63 ± 1.82	1.27 ± 0.88	1.36 ± 0.93	100	0.00	0.85 ± 0.06 g
T calculated value	9.22	8.87	8.87	8.87			4.19

* Statistically significant.
**On the basis of number of ovules involved in study. Refers to all these structures that appeared to be like seed (with or without embryo).
*** Refers to seeds with embryo only. Seeds without embryo did not show any positive viability due to absence of embryo.
# Refers to all these structures that appeared to be like seed (with or without embryo).

Table 5. Different seed size classes in A. glauca.

Size class	Size of seeds (cm)	100 seed weight in grams (g)
Small	0.59 ± 0.03 (0.4-0.7)	0.72 ± 0.03 g
Medium	0.94 ± 0.02 (0.8-1.0)	1.02 ± 0.04 g
Large	1.34 ± 0.04 (1.1-1.6)	1.18 ± 0.07 g

out embryo. Similarly out of the 2.63 ± 1.82% seed set under autogamous conditions, 1.27 ± 0.88% seed was with embryo with the rest 1.36 ± 0.93% without embryo respectively. 100 seed test weight under open pollination (1.20 ± 0.44 g) was statistically higher to 0.85 ± 0.06 g under autogamous pollination. TTZ test revealed 100% seed viability (Figure 2l) in seeds with embryo in both open as well as autogamous conditions and on the contrary none of the seed without embryo was found to be viable.

Seed biology

Seed size

Differences in seed size were noticed and were categorized into i). small, ii). medium and iii). large seeds (Table 5).

Seed set percentage in different umbel orders of *A. glauca*

670.27 ± 65.24 seeds were obtained in primary umbel which was statistically higher than 216.64 ± 18.88 obtained in lateral-I umbels thus, seed set percentage was 56.71 ± 5.52% in primary and 20.08 ± 1.75% in lateral-I umbel. Out of these only 27.49 ± 2.67% and 6.53 ± 0.57% with seeds with embryo were present in primary and lateral-I umbel respectively which was statistically significant. 100% viability in seed with embryo was observed irrespective of umbel order by TTZ test. Seed set by primary umbel had statistically significant 100 test seed weight (1.20± 0.44 g) as compared to 0.94 ± 0.28 g in lateral-I umbel (Table 6).

Seeds (with embryo) set percentage as influenced by location, umbel order and position within umbel

In Shillaru population (2130 m amsl, district Shimla, HP, India), statistically non-significant difference in percentage of seed with embryo among primary and lateral-I umbels as well as among peripheral and central regions of these umbel orders was observed (Table 7). 53.80% (maximum) seeds with embryo were observed in peripheral regions of primary umbels and 32.38% (minimum) in central region of lateral-I umbels which was however statistically non significant. On overall basis, 48.47% seeds with embryo were obtained in primary and 32.52% in lateral-I umbel (Table 7).

In Shilly population (1550 m amsl, district Solan, HP, India), maximum (66.18%) seeds with embryo were obtained in central region of primary umbel and minimum (36.68%) in central region of lateral-I umbel which was however statistically non-significant (Table 7). On overall basis maximum (56.56%) seeds with embryo were obtained in primary umbel which was statistically higher to minimum (40.11%) obtained in lateral-I umbels. Maximum (51.43%) seeds with embryo were obtained in central region of umbels and minimum (45.24%) in peripheral regions of umbels which was, however statistically non-significant (Table 7).Amongst the two populations, on overall basis 48.34% (Shilly) and 40.49% (Shillaru) seeds with embryo were obtained which was statistically non-significant (Table 8).

Table 6. Seed set percentage in different umbels of *A. glauca*:

Umbels	Observations						
	Average number of seed per umbel*#	Total seed set*# %	Seed set % (with embryo)*	Seed set % (without embryo)*	Seed viability %**		100 Seed weight*** grams*
					With embryo	Without embryo	
Primary Umbel	670.27 ± 65.24	56.71 ± 5.52	27.49 ± 2.67	29.22 ± 2.84	100	0.00	1.20 ± 0.44 g
Lateral-I Umbel	216.64 ± 18.88	20.08 ± 1.75	6.53 ± 0.57	13.55 ± 1.18	100	0.00	0.94 ± 0.28 g
T calculated value	6.37	6.03	7.30	4.85			4.37

* Statistically significant
\# On the basis of number of ovules involved in study. Refers to all these structures that appeared to be like seed (with or without embryo).
**Refers to seeds with embryo only. Seeds without embryo did not show any positive viability due to absence of embryo.
*** Refers to all these structures that appeared to be like seed (with or without embryo).

Table 7. Percentage of seeds with embryo among different umbel order vis-à-vis umbel part in population in *A. glauca*.

Umbel order	Umbel part					
	Shillaru population Seed set %			Shilly population Seed set %		
	Peripheral	Central	Mean	Peripheral	Central	Mean
Primary	53.80 (47.13)	43.13 (39.15)	48.47 (43.14)	46.94 (46.21)	66.18 (58.12)	56.56 (52.16)
Lateral-I	32.65 (34.36)	32.38 (32.37)	32.52 (33.36)	43.55 (41.17)	36.68 (37.12)	40.11 (39.15)
Mean	42.23 (40.74)	37.76 (35.76)		45.24 (43.69)	51.43 (47.62)	
$Cd_{0.05}$						
Umbel order		NS*			12.13	
Within umbel		NS			NS	
Umbel order X within umbel		NS			NS	

Values in parentheses are Arc Sine transformed values.
* Non significant.

Seed size vis-à-vis percentage of seeds with embryo

The large sized seed consisted of 52.12% seeds with embryo at Shillaru as against 58.12% obtained at Shilly, (statistically non significant). Amongst the small seeds, only 37.67% (Shillaru) and 39.15% (Shilly) seeds were with embryo (Table 9). Amongst medium sized seeds 28.66% (Shillaru) and 41.66% (Shilly) were with embryo (table 9). There was observed no statistically significant difference amongst the two locations i.e. Shilly and Shillaru but significant difference in percentage of seed with embryo amongst seeds of different size class was observed at both locations (Table 9) with large seeds having higher proportion of seeds with embryo (55.12%).

Seed germination

Seed size class wise, inter and intra population seed germination was conducted and Shillaru (2130 m amsl, district Shimla, HP, India) population gave maximum (31.00%) germination which was statistically higher (Table 10). Seeds of Kilba (3200 m amsl, 31°31' 18.17" N; 78°11' 49.30"E district Kinnaur, HP, India) population did not germinate at all and in case of Khan Jungle (2300 m amsl, 30°49' 13.40" N; 77°27' 47.82" E, district Sirmour, HP, India) population, large sized seeds gave maximum germination (24.00%) and medium seeds gave minimum germination (6.00%) (Table 10). In case of Jagatsukh (1982 m amsl, 32° 11' 43.20" N; 77° 12' 31.82" E, district Kullu, HP, India) population large seeds gave maximum germination (8.00%) and small seeds did not germinate at all (Table 10). In case of Thandi Dhar (2240 m amsl, 30° 54' 51.42"N; 77° 24' 44.45"E, district Sirmour, HP, India) population, medium seeds gave maximum germination (26.67%) and large seeds gave minimum germination (8.33%) (Table 10). In case of seeds from Rohru Forest Division (2700 m amsl, 31°07'09.49"N; 77°37'35.45"E, district Shimla, HP, India), small seeds gave maximum germination (34.00%) and large seeds minimum (13.33%) (Table 10). In case of Shillaru population, medium seeds gave maximum germination (48.00%) and large seeds gave minimum germination (10.00%) (Table 10). On overall basis, non significant impact of seed size on seed germination was observed (Table 11).

Table 8. Overall percentage of seeds (with embryo) comparison between two population of *A. glauca.*

Populations	Seed with embryo (%)
Shillaru	40.49%
Shilly	48.34%
T calculated value	1.37*

* Non significant.

Table 9. Percentage of seed with embryo amongst different seed size classes in *A. glauca.*

Population	Seed size			
	Small	Medium	Large	Mean
Shillaru	37.67 (34.96)	28.66(31.64)	52.12(46.34)	39.49(37.65)
Shilly	39.15(38.42)	41.66(39.90)	58.12(54.95)	46.31(44.42)
Mean	38.41 (36.69)	35.16(35.77)	55.12 (50.65)	
$Cd_{0.05}$				
Sites		NS*		
Seed size		9.69		
Site X size		NS		

* Non significant.
Values in parentheses are Arc Sine transformed values.

Table 10. Site wise open pollination set seed germination response vis-à-vis seed size in *A. glauca*.

Sites	Category			
	Small	Medium	Large	Mean
Khan Jungle	20.00 (26.54)	6.00 (14.12)	24.00 (29.30)	16.67 (23.32)
Kilba	-	0.00 (0.00)	-	0.00 (0.00)
Jagatsukh	0.00	-	8.00 (16.37)	4.00 (8.18)
Thandidhar	23.33 (28.83)	26.67 (31.06)	8.33(16.72)	19.45 (25.54)
Rohru forest division	34.00(35.64)	-	13.33 (21.37)	23.67 (28.50)
Shillaru	35.00 (36.25)	48.00 (43.83)	10.00(18.27)	31.00 (32.79)
$Cd_{0.05}$				
1. Size categories with in populations		2.12		
2. Between population with number of size categories				
1 and 2		1.84		
1 and 3		1.73		
2 and 3		1.37		
2 and 2		1.50		
3 and 3		1.22		

Values in parentheses are Arc Sine transformed values.

Table 11. Impact of seed size on germination percentage.

Seed size	Germination%
Small	22.47 (25.48)
Medium	20.17 (23.16)
Large	12.73 (20.48)
CD0.05	NS*

* Non significant.

DISCUSSION

Morphology and floral architecture

The traded roots of *A. glauca* are sometimes adulterated by roots of *Pleurospermum angelicoides* (Wall. ex DC) Benth. ex C. B. Clarke and *Angelica archangelica* L., thereby making morphological studies crucial to check the genuiness of the species. Although the studied populations were of genuine *A. glauca* being similar in morphological features reported earlier (Clarke 1885; Kirtikar and Basu 1984; Bisht et al. 2003; Nautiyal and Nautiyal 2004; Vashistha et al. 2006), yet with regard to the sex type present observations have established beyond doubt presence of only bisexual flowers that has been reported earlier by Butola et al. (2010) also however, Bisht et al. (2008) have reported *A. glauca* as andromonoecious (both bisexual and staminate flowers on same individual). Apiaceae members exhibit diverse sexual expression with most of the species being andromonoecious, few bisexual (wild *Foeniculum vulgare* Mill.) and rest either dioecious (*Aciphylla* or *Anisotome*) or gynodioecious (*Gingidia*, *Scandia* and *Lignocarpa* etc.) (Koul et al. 1993; Reuther 2013).

Seed size variation corresponding to test weight is being reported for the first time in this species and such variation was observed irrespective of the umbel order. However, the primary umbel followed by lateral-I umbel only set seed with almost nil seed set by lateral-II and lateral-III umbels. This suggests that only two former types of umbels should be targeted for seed harvest. Seed size variation is also known in the Apiaceae species like *Anethum graveolens* L. and *Pastinaca sativa* L. wherein such variations is correlated with umbel order as well as the portion of flowers within an umbel (Hendrix 1984; Hołubowicz and Morozowska 2011).

Another important seed feature having implications for its reproductive fitness that has been observed is presence of seeds without embryo (thereby sterile) in the species. Low seed germination in *A. glauca* is already known and seeds without embryo probably are the reason. Low seed fertility due to the embryo less seeds may be the reason of its sporadic populations thereby leading to its rarity in nature. This is an important finding and any strategy towards sustainable utilization shall have to factor in this feature. This feature was irrespective of seed produced by different umbel order as well as pollination systems indicating physiological causes. Reduced fertile seed output may have some advantages like allowing enough space for progeny to grow but limit their number. In self incompatible *Stevia rebaudiana* Bertoni, two types of sterile and fertile seeds are produced, however that is due to genetic reasons (Raina et al. 2013).

Phenology

Phenology of *A. glauca* follows the general pattern of temperate perennial herbs that undergo perennation during winter period only to sprout back after snow melting. Flowering commences with the summer season with primary umbels blooming first followed by lateral-I, lateral-II and lateral-III umbels with peak flowering during August month. Seed maturation and shedding commences from last week of August month till October. Flowering is asynchronous among plants occurring in same niche and within plant too, different phenological events were asynchronous even among primary, lateral-I and lateral-II umbels which appears to be an adaptation to limited pollinator services especially insects. Vashistha et al. (2010) have also reported similar phenological events.

Floral biology

Flower of *A. glauca* have been observed to be protandrous with anther dehiscence beginning with the opening of floral buds that continues for 2-3 days. Stigma become receptive after complete anther dehiscence that is also characterized by style extending full beyond stylopodium indicating complete intra floral dichogamy. Shiny stylopodium is also indicator of stigma receptivity. Late maturation of stigma coupled with elongation of style after anther dehiscence facilitate dichogamy in *A. glauca* and appears as an adaptation to avoid autogamy as well as inbreeding depression. Protandry in *A. glauca* has also been reported by Bisht et al. (2008). In *Chaerophyllum bulbosum* L.(Apiaceae), also styles elongates only after pollen is shed and sexual phases are clearly distinguishable indicating extreme '*protandry*' (Reuther and Claßen-Bockhoff 2013). *Foeniculum vulgare* Mill. other member of Apiaceae, has also been found to be strongly '*protandrous*' as pollen are released much before stigma receptivity (Koul et al. 1996). As *A. glauca* thrives in hostile climatic condition, production of trinuclear pollen grains seems to be an adaptive feature for faster germination on stigma leading to reproductive assurance.

Pollen ovule ratio of 13064.37± 661.71, studied for the first time in present studies indicates the species to be an obligate outcrosser as per Cruden (1977). *Foeniculum vulgare*, another member of Apiaceae, is also characterized by high pollen ovule ratio of 12005-14635 (Koul et al. 1996).

Chromosomal studies

The present gametic chromosome count of n=11 is in conformity with the previous diploid count of 2n=22 (Kumar and Singhal 2011) from northwest Himalaya. However, grouping of bivalents at diakinensis and metaphase-I stage into a ring structure has been observed for the first time in this species. In, only few cells could clear 11 bivalents be observed at these stages. Anaphase-I too was characterized by the presence of two rings of 11 chromosomes at each poles. Presence of ring of 11 chromosomes at anaphase-I in *A. glauca* poles appears to be similar to the 'renner' complexes (entire haploid genomes which are inherited as single units) present in genus *Oenothera*, wherein due to reciprocal translocations of chromosome arms, all the 14 chromosomes form two rings of seven chromosome each (Greiner 2008).

As has been discussed earlier, significant proportion of seeds of *A. glauca* were without embryo leading to reduced germination, which may be due to this meiotic anomaly. Further studies on female meiosis, embryo development as well as more extensive studies on male meiosis are required to establish the consequences of meiotic anomaly in *A. glauca*. Although meiotic abnormalities like laggards, bridges, etc. were not observed but in some pollen mother cells, cytomixis was observed in *A. glauca*. Cytomixis often leads to abnormal meiotic behavior, variation in pollen grains size and low pollen viability or sterility e.g. in *Alopecurus arundinaceus* Poir. (Koul 1990), *Polygonum tomentosum* Willd. (Haroun 1995), *Hordeum vulgare* L.(Haroun 1996), *Brassica napus* var. *Oleifera* Delile. and *B. campestris* var. *oleifera* DC (Souza and Pagliarini 1997), *Vicia faba* L. (Haroun et al. 2004), and *Meconopsis aculeate* Royle (Singhal et al. 2008). Despite chromosome arranged in rings as well as cytomixis, pollen stainability did not seem to be impacted much as it ranged from 76% to 100% in different plants of *A. glauca* studied.

Breeding system studies

Significant seed set under open pollination conditions in comparison to autogamous conditions established the species strongly favouring (about 95%) cross pollination. This aspect is being reported for the first time in this species. Interestingly open pollination also resulted in much higher seed (with embryo) set (27.49 ± 2.67%) as compared to autogamous seed (1.27 ± 0.88%), again indicating strong presence of cross pollination.

As it is generally presumed that selfing rates increase with increasing altitudes (Schroter 1926; Bliss 1962; Garcia-Camacho and Totland 2009; Korner and Paulsen 2009) as pollinator abundance and activity become limiting factors due to hostile climatic conditions at higher altitudes (Arroyo et al.1982, 2006; Bingham and Orthner 1998; Medan et al. 2002). Contrary to this view, the species under investigation (*A. glauca*) favours cross pollination and dichogamy seems to play a key role in its cross fertilization. *Eritricium nanum* (Vill.) Schrad.ex Gaudin (Boraginaceae), *Chaetanthera renifolia* (J. Remy) Cabrera (Asteraceae) and *Nardostachys grandiflora* DC (Valerianaceae) other temperate plant species, are also known for higher cross pollination rates at high altitudes (Writh 2010; Diaz et al. 2011; Gautam and Raina 2016). Inflorescence attributes, high pollen ovule ratio and asynchronous opening of flowers in *A. glauca* are also evidence for its cross pollinating nature. However, low seed set in *A. glauca* limits, natural variation essential for genetic improvement.

Seed biology

As has been already discussed only primary and lateral-I umbels set seeds. Among these too primary umbel set significantly more (56.71 ± 5.52%) seed than lateral-I umbel (20.08 ± 1.75%). Higher seed set in primary umbel in *Eryngium alpinum* L. and *Carum carvi* L. (family Apiaceae) is already known (Bouwmeester and Smid 1995; Gaudeul and Bottraud 2004).

Interestingly, despite blooming, lateral-II as well as lateral-III umbels do not set any seed which seems due to their late development or restricted resource allocation and they seem to only for enhancing floral visibility of its plants for pollinator attraction. Of the 56.71± 5.52% and 20.08 ± 1.75% seed set by primary and lateral-I umbels only27.49 ± 2.67% and 6.53 ± 0.57% seed is with embryo respectively indicating higher fertile seed production by primary umbels. This low fertile seed production in *A. glauca* may be the reason for generally low germination rates in this species.

Fruit and seed set percentage is generally low in late blooming flowers than early blooming ones (Zimmerman and Aide 1989), and several reasons like resource competition among the ovaries of an inflorescence (Lee 1988); reduced pollen receipt by later blooming inflorescence (Lee 1988); intrinsic features (Berry and Calvo 1991; Diggle 1995) may be the reasons.

Non significant variations in fertile seed production between peripheral and central flowers of a umbel and also among seed from two ecologically different populations (Shilly and Shillaru) indicates that *A. glauca* as a strategy, limits fertile seed production either for nutrient resource conservation or to ensure better quality fertile seed that can produce a healthy progeny.

Seed germination

Seed germination behavior of any species impacts the genetic variability in any species and lower germination rates deprive such variation. Inter population germination variation was observed amongst the six population viz. Khan Jungle, Kilba, Jagatsukh, Thandidhar, Rohru forest division and Shillaru with Shillaru population excelling others (31.00% seed germination) with no germination in seeds of Kilba.

Apiaceae family members are known for generally low germination rates (Koul et al. 1993) and in *A. glauca* poor and erratic germination with maximum of 8% germination is already reported (Nautiyal et al. 2002; Butola and Budola, 2004; Butola and Samant 2006). Present studies have revealed that low germination in *A. glauca* is not due to dormancy but production of seeds without embryo.

CONCLUSION

Only primary and lateral-I umbels set seeds as other lateral-II and lateral-III only attract pollinators without setting any seed. Production of embryo less seeds (sterile seed) is a major reproductive bottleneck in this species. Seed size variation occurs within same plant as well as within same umbel with large seeds having higher proportion of fertile seeds (with embryo). The species is strongly cross pollinating.

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