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Pollination mechanisms in *Hippophae rhamnoides* L. (Elaeagnaceae) and its implication in orchard management of seabuckthorn in Kargil (Ladakh)

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ABSTRACT

Hippophae rhamnoides L. commonly known as sea-buckthorn belongs to the family Elaeagnaceae is a spiny deciduous multipurpose shrub. It is predominantly dioecious bearing male and female flowers on separate individuals and some rare cases of hermaphrodites and polygamomonoecious are also known. Pollination studies play important role in the cultivation techniques for improvement of crops. Such studies are useful in designing and managing an orchard. In the light of this, the present study has been undertaken and highlighted some interesting aspect of the pollination mechanism in this highly economically important plant which can be readily be utilized for its orchard management.

Keywords : pollination, hanging slide experiment; dioecy.

Hippophae rhamnoides L**.** known as sea-buckthorn, a member of the family Elaeagnaceae is native to the cold-temperate regions of Europe and Asia (Li and Schroeder 1996). Sea-buckthorn because of its numerous multipurpose uses is in great demand in several industries including medicine and food. Plants also play important role in ecological services such as stabilizing sand dunes, reduces soil erosion, enrich the fertility of the soil etc. (Xu *et al.* 2001, Zeb 2004, Dwivedi *et al.* 2006, Utioh *et al.* 2007, Gupta 2012). It used in the food, cosmetics industries, in traditional medicine, as animal fodder, in horticulture, and for ecological purposes and because of its multi-purpose economic importance is also known as “million dollar plant” (Ali and Kaul 2015). At present sea-buckthorn is cultivated in many parts of the world (Li and Schroeder 1999). Cultivation techniques however, vary from region to region depending upon its climatic and topographical features (Lizumi and Ramankutty 2015). In Ladakh the plants grows wild in nature along the river banks and mountain sides. In order to harness its full potential, it is imperative to cultivate the plant by locals. Generally the farmers avoid its cultivation because of the fact that they lack proper knowledge about is cultivation and management. Present study aims to help these farmers to cultivate this nature’s gift in their orchards.

MATERIAL AND METHODS

Studies were conducted on two populations of Kargil viz; Barutsogs and Khomenibagh of Kargil District (UT Ladakh). The two are separated by a distance of 4 km; the plants were subjected to;

Hanging Slide Experiment: In hanging slide experiments, slides smeared with Mayer 2 s albumin (egg albumin and glycerine in 1:1 ratio with a pinch of sodium salicylate) were hanged with the help of thread at different distances from the male plant (5m, 10m, 20m, 30m) on the female plant. Plants were selected in such a way that no male plant falls in the vicinity except for the plants (2 to many thickets of male plants) from where distance was calculated. Similar slides were also hanged at three different levels of the female plants randomly such as upper (5-7 feet), middle (3-5 feet) and lower (less than 3 feet). Twenty four hour later the slides were collected and scanned for pollen load under binocular microscope (Olympus CH20i) and the quantity record. (In total, 10 slides were hanged per plant and 10 plants were utilized per population)

Pollen/Ovule ratio: Pollen ovule ratio per flower was calculated by dividing the total number of pollen grains in a male flower to total number of ovules in a female flower. The ratio was also calculated at the levels of inflorescence and plants. Average number of flowers per inflorescence were estimated and multiplied by the average number of pollen or ovule per flower. The two counts so obtained were used to estimate P/O per inflorescence. This procedure was repeated at the level of an individual plant.

Bagging experiments: Since it was not possible to bag an individual female flower or inflorescence because of their minute size, 6 inches long twigs bearing pre-anthesis floral buds were randomly selected, flowers number counted and the whole twig was enclosed in butter paper bags. Care was taken to bag only those flowers, which were unpollinated and unfertilized. Thorns were clipped to ensure effective bagging and prevent damage to the bags. After 90 days, the bags were removed and number of fruits formed counted.

RESULTS AND DISCUSSION

Since the plants are dioecious, this makes cross pollination imperative. Both male and female flowers are small, inconspicuous and the latter is without any specialized pollinator attracting organs like showy sepals or petals and nectar.

Flowering in both male and female plant initiates in the second week of April. Flowers are borne on inflorescences on both lateral branches as well as on the main axis. However, the frequency being higher on the lateral branches (Fig. 1a). The inflorescences are densely clothed with trichomes which harbor both vegetative and reproductive structures. Male Inflorescence is a raceme, oval to conical in outline varying in colour from brown to dark brown in different plants of a population. Mature inflorescence exhibits plant-to-plant variation in size from 4 to 20 mm in length across populations. These bear a striking morphological resemblance with the cones of gymnosperms (Fig. 1d). Number of flowers varies between 4 and 18 per inflorescence. Each flower has four anthers with their individual filaments attached basally. Morphometry of individual flower parts is given in Table 1.

On the other hand, female inflorescence is a small raceme of 3 – 12 flowers borne in the axil of a leaf bud (Fig. 1b). Arranged in relatively loose whorls or spirals they form a diffuse inflorescence that measures 4-12 mm in length, and 2-5mm in width (Table 1). Female flower is minute varying in size between 4 and 10mm, non-showy without any attractants. Each comprises of two tepals fused at the base and free only at the tips. The two enclose the ovary (Fig. 1c) and together with bract have been collectively referred to as perianth.

Since the plant is dioecious, making cross pollination indispensable in the species under investigation. To measure the extent of pollen flow through wind, hanging slide experiments was conducted on two above mentioned populations. Ample amount of pollen grains on the slides hanged at different distances from male plant confirms the aforementioned statement (Fig. 2- A, B). Pollen count on the slides hanged at 5m was, on an average, 186 and 593 pollen grains/cm^2 for Khomeni Bagh and Barutsogs respectively (Table 2). At 10 meter distance, the mean number of pollen grains reaches 415/cm^2 (Khomeni Bagh) and 1058/cm^2 (Barutsogs). Thereafter, a gradual decline in the pollen count from 15 m to 30 m was recorded. Similarly, slide hanged at the upper, middle and lower parts of female plants also trapped pollen with the maximum pollen scored for the middle part of the plant 268 and 723 pollens respective to Khomeni bagh and Barutsogs. The flower structure indicates that the plants are anemophilous. Its anemophily nature is further justified by the presence of very high pollen ovule ratio ($3.48x10^4$) and the results of hanging slide experiments. The Pollen count on the slides reaches the peak (415 and 1058/cm^2) at 10 meter. Thereafter, the count gradually declines and falls to the minimum of around 124 and 151 pollen grains/cm^2 respective to the two aforementioned populations in the same order at 30

Table 1- Morphometric details of male and female inflorescences and flowers of *H. rhamnoides* L.

Sr. No.	Sex	Characters	x ± S.E (Range)	
			Length (mm)	Width (mm)
1	Male(n=50)	Inflorescence	8.2±1.2 (4-20)	5.2±0.6 (3.6-9.0)*
		Bract	3.8±0.4 (2-7)	2.6±0.1 (2.0-3.5)
		Flower	2.1±0.2 (2-4)	2.5±0.1 (2.0.-3.5)
		Tepal	2.8±0.2 (2-4)	2.4±0.1 (2-3.1)/2.6±0.2 (1.5-3.5)
		Anther	1.8±0.04 (1.5-2.0)	0.8±0.2 (0.5-1.0)
2	Female(n=50)	Inflorescence	6.0±0.3 (4-12)	3±0.1 (2-5)
		Bract	5.2±1.0 (3-10)	3.6±0.2 (2-5)
		Flower	6.5±0.5 (4-10)	2.1±0.1 (1.5-3.0)
		Stigma	3.5±0.2 (2.0-6.0)	1.3±0.2 (0.5-2.5)
		Style	0.18±0.02 (0.1-0.3)	0.33±0.06 (0.3-0.5)
		Tepal	4.6±0.3 (2.0-6.0)	1.9±0.1 (1.0-3.0)
		Ovary	0.76±0.02 (0.7-0.9)	0.58±0.02 (0.5-0.7)

*Mean ± S.E, range in parenthesis

m distance from male plant. In another but similar experiment, slides hanged at different levels of the upper, middle and lower parts of female plants, also trapped pollen evidencing the pollen receipt via wind by female flowers. In bagging experiments, 8 bags were used to cover approx. 505 flowers, of which 9 transformed into fruits at a percentage of 3.99%.

Another set of experiment was conducted to confirm that fruit set is a product of pollination and not by any other means, bagging experiment was conducted, for which twelve bags were utilized. Of the twelve, 8 bags remained intact which is quite obvious since these populations are not protected. Nevertheless, among these 8 bags covering approx. 505 flowers, 9 had transformed into fruits, making the total to approximately 4% of the total flowers bagged (Table 3).

DISCUSSION

Plants of *H. rhamnoides* ssp. *turkestanica* are predominantly dioecious (Ali 2013) with male and female sexes separated in time and space (Ali 2013). Two populations viz; Barutsogs and Khomeni Bagh were investigated and subjected to detailed analysis of their pollination mechanism. In wind-pollinated plants, siring success is likely to be determined by lottery-model competition (Charlesworth and Charlesworth 1981, Lloyd 1984) resulting in stigma either receiving many or negligible pollen grains. Willson (1983) advocates that there is “no practical limit to the amount of pollen that wind can carry and wind does not cause pollen to be lost”. However, velocity and direction of wind is important in deciding the distance travelled by and quantum of gene flow via pollen (Willson and Burley 1983, Niklas 1985). Ladakh (Kargil), during the flowering season, is essentially dry and

Table 2. Results of hanging slide experiments in two sea-buckthorn populations

S. No	Population	Distance (m) from male plants					Position with respect to the height of female plant (ft)		
		5	10	15	20	30	Upper5-7	Middle3-5	Lower1-3
1	Khomeni Bagh	186.85±7.03 (149-248)*	415.20±89.19 (230-552)	265.36±9.60 (225-356)	144.91±6 (114-196)	124.91±3.63 (106-158)	205.46±2.52 (191-232)	268.85±4.57 (228-291)	228.93±10 (172.91-320.3)
2	Barutsogs	593.34±17.02 (462-708)	1058±43.4 (813-1348.75)	511.31±61.89 (330-793)	170.42±3.92 (150-200)	151.33±9.49 (88.41-219.5)	636.33±32.3 (479-942)	723±52.37 (442-1183)	212.08±12.86 (144-321)

*Mean±S.E; Range in parenthesis

Table 3. Results of bagging experiment in different populations of seabuckthorn

S. No.	Population	Approximate number of female flowers bagged	No. of fruit produced	% total fruit set	F value
1	Barutsogs	77	0	3.66	25.9
		53	2		
		29	0		
		51	6		
2	KhomeniBagh	67	0	0.33	159.8
		78	0		
		62	1		
		88	0		
	Total	505	9	3.99	

[$F_{(1,6)}$ p>0.01]

wind velocity is generally moderate to very high which produce directional movements. Given that sea-buckthorn carpel has predominantly a single ovule and that, a single pollen tube ultimately reaches ovules, afore mentioned conditions are cumulative and provide ambience in optimum pollen load deposition. This is further bolstered by the results of the hanging slide experiments.

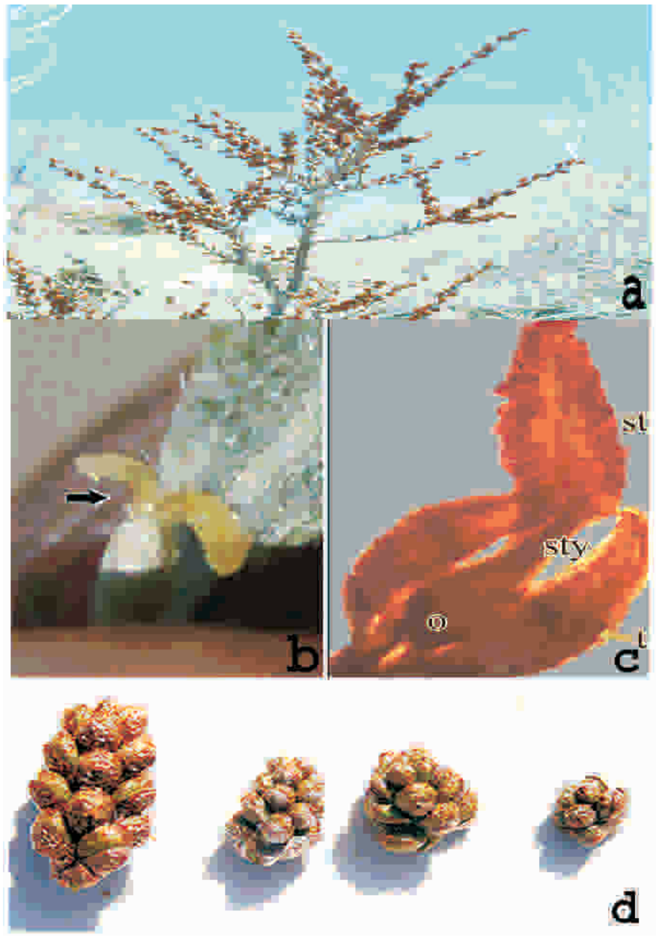
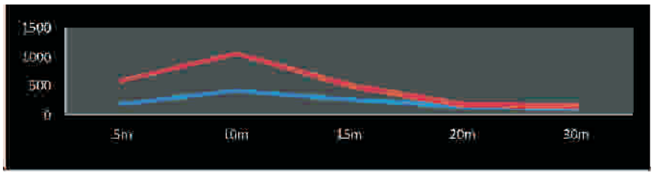
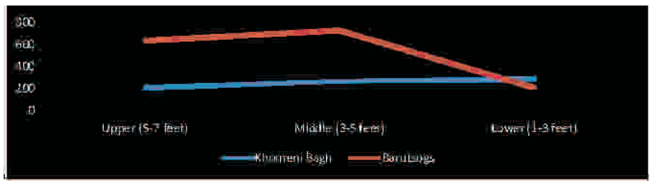


Fig. 2a. Male inflorescence in full bloom, 2b. Female flower, 2c. Stigma, style and ovary, 2d. Male inflorescence with variable number of flowers.

Slides hanged at 5, 10 15, 20 and 30m away from male plants had trapped enormous quantity of pollen confirming the mechanism of wind pollination operative in this species. Maximum pollen load was observed at 10 m (415 and 1058/cm^2 of the slide for the respective population). The difference in the pollen flow between the two populations is perhaps because of the topography. The population of Khomeni-bagh is surrounded by hills thereby restricting the wind flow, whereas the population of barutsogs is at the level of suru river and exposed. Unlike the findings of Mangla and Tandon (2014), where they observed the pollen flow restricted with in 15 m range, in present study, pollen flow crossed beyond 15 m to as far as 30 m from the male plants in both the population. At this distance (30m), as expected, the pollen count is comparatively less i.e. 124 and 151/cm^2 for Khomeni bagh and Barutsogs respectively. Natural pollen loads on stigma are very low. In a random sample of 150 (n= 20 plants from different populations across Kargil) stigmas scanned, average pollen loads range between 5 and 30±7.39. This amount shows no increase even 144 hour after anthesis (Ali, 2013) Mangla *et al.* (2013), also support these observations. They reported emergence of 81.88% pollen tubes 24 hour post pollination Further in 90% instances only one and 10% two pollen tube (s) traversed the compitum but invariably only one entered the ventral pore and caused fertilization (Mangla *et al.* 2013).



(A)



(B)

Fig. 2. Line diagram depicting the amount of pollen grains trapped by slides hanged. A – at different distances from male plant. B – at different levels of female plant.

In order to access the pollen flow vertically across the plant, slides were hanged at different levels of the female plants viz, Lower (less than 3 feet), middle (3-5 feet) and upper (5-7 feet), maximum deposition was recorded at middle followed by upper levels. This observation coincides with the parts of female plant where maximum flowers differentiate and require sufficient amount pollen for effective fertilization. In order to ensure that the fruit so formed are the result of fertilization, a total of 12 bags covering 505 flowers among the two populations were bagged and kept undisturbed for more than two months. Of these, 8 bags remained intact and 496 flowers remained as such, however, 9 fruit transformed into fruit with a working percentage of 3.99% thereby confirming the operation of apomixes as already discussed by Mangla *et al.* (2015) and Ali and Kaul (2017).

Now a day, sea-buckthorn is being considered as a potential cash crop and as such present study can be quite useful in its orchard management. Dioecious plant requires plantation of male plants in between the female ones. Mangla and Tendon (2014) on the basis of pollen flow suggested the placement of male plant after every 15m. However this distance can go up to 30m in present study, as the slides hanged at this distance also trapped sufficient amount of pollen grains to effect pollination. Thus, the ratio of female: male increases double i.e. 30:1. This increase in the number of female plant in an orchard will also increase the yield. As far as the optimum height of female plant in an orchard is concerned, 3–7 feet tall plant have been found to fetch maximum pollen grains. This indicates maximum pollination followed by fertilization and ultimately fruit production (harvest).

From the two sets of hanging slide experiments viz. hanged at different distance from male plant and hanged at different levels of female plant, it can be concluded that the former is an indicative of pollen movement only whereas the latter is equivalent to the actual pollen flow since female plants are the recipients of the male gametes. Thus the former is a random phenomenon and likely to lead to over estimation of actual gene flow, while the latter is more effective in predicting the amount of actual pollen grains that reaches the stigma surface.

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