Incidence of apomixis in sea buckthorn (*Hippophae rhamnoides* L.), a dioecious taxon of immense economic importance

Hippophae rhamnoides L., commonly called sea buckthorn, is a core dicot of the family Elaeagnaceae placed in the order Rosales[1,2]. The plant is a treasure trove of bioactive substances with a potential to sustain more than one kind of industry. Countries with its natural populations are eyeing it as a new millennium food, as well as health and beauty resource. Valued for all its parts, the species is prized particularly for its fruit pulp and seed oil, which among others are a rich source of vitamins and omega-3 fatty acids[3–5]. In Jammu and Kashmir (J&K), India, the species grows luxuriantly in Ladakh along riversides of the Indus, Suru, Shyok and Zanskar. *H. rhamnoides* L. is strictly dioecious in majority of the populations surveyed[6,7]. Therefore, effective sexual reproduction and seed formation require co-cultivation of male and female plants with the former acting only as pollen donors. As such, a proportion of the population is always non-fruit-bearing.

Mass cultivation of cultivars or varieties of both the sexes is important to mitigate the demand from the growing industries. However, the objectives for obtaining male and female cultivars differ, because, qualitatively more criteria are to be met in the female sex track[8]. Because of its dioecious nature, the species is outcrossed and generates lot of variability[6]. Promising lines tend to segregate in each generation and fixation of heterozygosity through suckers though possible poses numerous difficulties. Apomixis, that is the formation of non-sexual seed in plants, is an effective tool to circumvent these drawbacks. A phenomenon of great promise, it not only fixes heterozygosity and paves the way for mass cultivation of superior genotypes, but also makes the need of male parents minimal. Found to be closely associated with perenniality, hybridity and polyploidy, to name only a few conditions, apomixis in a sexually reproducing diploid species is considered rare[9–13]. To find it in a dioecious, diploid sexual is all the more interesting and intriguing, and something similar has been recorded in *H. rhamnoides* growing at Ladakh.

As a part of our endeavour to work out the breeding system of this taxon, plants belonging to five populations, two from Leh (Thiksay and Shey about 4 km apart) and three from Kargil (Khomeni Bagh, Barutsogs and Kanoor; Khomeni Bagh is 4 km away from Barutsogs which is 22 km away from Kanoor along the National Highway) were randomly selected. Although a couple of treatments were given to plants for the purpose, only that of bagging, i.e. pollen exclusion is presented here and a comparison drawn with fruit formation that takes place on its own in nature. Taken as control, the latter is henceforth referred to as open or natural pollination[6]. The treatment of pollen exclusion or bagging was undertaken with the sole purpose of knowing whether fruits are set and seeds are formed in the absence of any kind of pollen/pollination stimulus.

Although dioecy eased the treatments by doing away with emasculation, the architectural design of the inflorescences and minute size of an individual flower did not permit bagging at these levels[6,14]. Therefore twigs of female plants with inflorescences bearing mature pre-anthesis floral buds were randomly selected and enclosed in butter-paper bags. These bags were tailored to accommodate all the inflorescences of individual branches. Care was taken to include only pre-anthesis flower buds, because they are not pollinated. The total number of flowers bagged was estimated by counting the number of flowers per inflorescence (average of three inflorescences per branch) and subsequently multiplying it by the number of inflorescences on that branch. Thorns were clipped to ensure effective bagging. The bagged twigs were harvested and brought to the work station after about 90 days. Bags were removed and number of fruits recorded.

A total of 25 bags, each enclosing 3 twigs of a single plant, were used to cover approximately 1500 flowers. Of these, 19 with 1066 flowers remained intact. Since all the populations chosen for the study are wild and unprotected, damage to some bags could not be avoided despite regular visits and monitoring. Interestingly, of the 1066 flowers, 33 transformed into fruits. Fruit set of 3.09% in the absence of pollen deposition indicates that these have formed on account of non-pseudogamous apomixis operating in the species. This fruit set was interestingly recorded for all the five populations spread over two districts of

Table 1. Results of bagging experiment in different populations of *Hippophae rhamnoides* spread over three years

Year	Population	Number of flowers bagged per plant	Number of fruits produced in bagged flowers per plant	Fruit set (%)
2010	Thiksay	67	0	–
		75	5	6.66
		89	12	13.48
		46	0	–
2010	Shey	68	0	–
		54	0	–
		32	4	12.5
		21	1	4.76
2011	Barutsogs	77	0	–
		53	2	3.77
		29	0	–
		51	6	11.76
2012	Khomeni Bagh	67	0	–
		78	0	–
		62	1	1.61
		88	0	–
2012	Kanoor	47	2	4.25
		38	0	–
		24	0	–
	Total	1066	33	3.09

Table 2. Results of fruit formation in tagged flowers on open or natural pollination across the five populations

Population	Number of flowers bagged	Number of fruits produced	Fruit set (%)	Overall mean ± S.E. per population (range)
Thiksay	99	49	49.49	45.44 ± 3.2 (26.44–79.66)
	59	47	79.66	
	72	39	54.16	
	70	46	65.71	
	89	52	58.42	
	128	48	37.5	
	103	38	36.89	
	121	32	26.44	
	66	19	28.78	
	116	52	44.82	
	141	39	27.65	
	112	51	45.53	
	88	54	61.36	
	93	48	51.6	
	93	36	38.7	
	138	40	28.98	
	93	34	36.55	
	85	38	44.7	
	99	46	46.46	
Shey	99	57	57.57	59.85 ± 2.32 (45.5–75.9)
	112	65	58.03	
	66	50	75.75	
	82	48	58.53	
	88	52	59.09	
	93	54	58.06	
	102	66	64.7	
	123	56	45.52	
	109	65	59.63	
	83	63	75.9	
	120	62	51.66	
	117	65	55.55	
	108	58	53.7	
	98	63	64.28	
Khomeni Bagh	118	75	63.55	80.51 ± 3.83 (63.55–92.47)
	98	79	80.61	
	114	80	70.17	
	93	86	92.47	
	83	75	90.36	
	101	78	77.22	
	93	83	89.24	
Barutsogs	140	93	66.42	63.64 ± 2.92 (49.65–82.52)
	93	52	55.91	
	81	41	50.61	
	84	46	54.76	
	72	59	81.94	
	93	55	59.13	
	85	67	78.82	
	143	71	49.65	
	71	38	53.52	
	73	49	67.12	
	102	67	65.68	
	105	82	78.09	
	121	93	76.85	
	103	85	82.52	
	132	66	50	
	140	71	50.71	
	93	56	60.21	
Kanoor	119	86	72.26	69.98 ± 2.52 (51.85–86.59)
	93	75	80.64	
	100	82	82	
	93	63	67.74	
	99	71	71.71	
	118	83	70.33	
	122	88	72.13	
	89	61	68.53	
	93	72	77.41	
	97	84	86.59	
	108	56	51.85	
	70	48	68.57	
Total	6897	4148	60.14	

Ladakh region of J&K. Notwithstanding the geographical isolation, greater fruit formation was recorded in plants of Leh district. Overall, population-wise, plants of Thiksay had maximum fruit set varying between 6.66% and 13.48%, followed by those of Shey (4.76–12.5%) and Barutsogs (3.77–11.76%) (Table 1). Fruits to the tune of 4.25% were formed in plants of Kanoor and least (1.61%) in Khomeni Bagh. Results of one-way ANOVA revealed a highly significant difference between the number of flowers bagged and the number that transformed into fruits ($F_{(1,14)} = 39.89$; $P < 0.01$; plants with zero fruit were excluded from calculations). With regard to fruit set per population, no significant difference was found between or within populations on the square root transformed data ($F_{(4,14)} = 0.6054$; $P > 0.01$)[15].

Further, the percentage frequencies of plants bearing such fruits were 25 at Khomeni Bagh, 33.3 at Kanoor and 50 each at Thiksay, Shey and Barutsogs respectively. This indicates that neither all flowers on a plant, nor all plants of a population yield fruits on pollen exclusion. Although fruit set in different populations is highly variable, their formation on pollen exclusion at all places over a three-year study period cannot be dismissed as inconsequential. It points towards the potential of plants to set seed in the absence of pollen.

In the control, flowering twigs (five per plant; number of plants per population is 4–5) belonging to the same as well as different plants of the same populations were tagged at all places to record fruit set on open pollination. Flowers of all these twigs (excluding the tampered ones) transformed into fruits which become visible 6–10 days after pollination. The percentage fruit set per plant varied between 26.44 and 92.47 among the populations; the differences were highly significant ($F_{(4,64)} = 17.05$; $P < 0.01$) (Table 2). Thus, against 1.61–13.48% fruits set in pollen exclusion treatments, formation of fruits under non-experimental conditions was quite high. On an average, the fruit set in control was 60.14% across populations tested, which far exceeded that in bagged inflorescences (3.09%). Population-wise female plants of Khomeni Bagh were efficient fruit producers, setting the highest number amongst the ones studied. However, as of now it is not possible to predict what proportion of this is agamospermous.

This communication, therefore, puts on record the incidence of apomixis in sea buckthorn, which would have been the first report for the species but for the publication by Mangla *et al.*[16] (Field and detailed embryological evidence in favour of the plant being a facultative apomict has recently been published by Mangla *et al.*[16], after we communicated the present study to the journal [I got the link from Rajesh Tandon on 18 August 2015 when the present communication was already under review]. While their work is confined to two populations of the same area, separated by about 4 km, the present work is based on a wider germplasm of distantly placed populations spread across two districts of Ladakh.). Plants revert to apomixis as an escape from seedlessness and sterility or under conditions of severe pollen limitation[12,13]. However, in *H. rhamnoides*, a good fruit set is found on open pollination in almost all the populations studied. Further, barring few anomalies, meiosis through male sex track studied by us is largely normal; pollen produced is 90% viable[6]. All these features make the incidence of apomixes in this stable diploid more interesting and intriguing.

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