



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(2): 256-260
Received: 19-01-2017
Accepted: 20-02-2017

Vinod Kumar Kairon
Department of Tree
Improvement and Genetic
Resources, College of Forestry
Dr. Y.S Parmar University of
Horticulture and Forestry,
Nauni-Solan 173230,
Himachal Pradesh, India

HP Sankhyan
Department of Tree
Improvement and Genetic
Resources, College of Forestry
Dr. Y.S Parmar University of
Horticulture and Forestry,
Nauni-Solan 173230,
Himachal Pradesh, India

Sanjeev Thakur
Department of Tree
Improvement and Genetic
Resources, College of Forestry
Dr. Y.S Parmar University of
Horticulture and Forestry,
Nauni-Solan 173230,
Himachal Pradesh, India

Correspondence

Vinod Kumar Kairon
Department of Tree
Improvement and Genetic
Resources, College of Forestry
Dr. Y.S Parmar University of
Horticulture and Forestry,
Nauni-Solan 173230,
Himachal Pradesh, India

Morphometric and chemical evaluation of seed oil traits of seabuckthorn (*Hippophae rhamnoides* L.) populations under different major gene pool areas of Spiti Valley of Himachal Pradesh

Vinod Kumar Kairon, HP Sankhyan and Sanjeev Thakur

Abstract

A survey was conducted in cold desert of Spiti Valley, Himachal Pradesh to assess morphometric and chemical seed oil traits diversity of *Hippophae rhamnoides* population. Nine major gene pool areas and three growing conditions within major gene pool areas selected for the study showed wide variation among different morphological, biochemical and leaf proximate compositions traits. On the basis of results, eventually, it may be concluded that GPA-3(Sheigo) was superior than other major gene pool areas for morphometric traits and seed oil chemical traits among different major gene pool areas. Over all GC-3 (crop land) was found superior for all morphometric traits and seed oil chemical traits among different growing conditions among different major gene pool areas. For seed oil evaluation Maximum per cent seed oil (5.23) was recorded in GPA-3 (Sheigo) while minimum plant height (4.25) was recorded in GPA-9 (Hurling) gene pool area. On the other hand, Maximum per cent seed oil (4.91) was recorded for GC-3 (crop land) which shows significant variation among different growing conditions. Maximum acid value(4.77 mg KOH/g), saponification value (231.51 mg KOH/g), and iodine value (151.37 g I₂/ 100 g) was recorded for GPA-3 (Sheigo) major gene pool area, and 4.91 mg KOH/g, 216.61 mg KOH/g and 146.37 g I₂/ 100 g respectively for GC-3 (crop land) growing condition. Based on morphometric traits and seed oil chemical traits observations it was evident that large diversity exists in naturally growing populations of *Hippophae rhamnoides* in the Spiti Valley of Himachal Pradesh, and this morphological variation can be exploited for the further selection of suitable genotypes to accelerate the domestication process of this important species.

Keywords: Seabuckthorn, *Hippophae rhamnoides*, morphometric traits, major gene pool areas, growing conditions, cold deserts and seed oil traits

Introduction

Seabuckthorn (Genus *Hippophae*) is a berry-bearing, hardy bush of the family Elaeagnaceae, naturally distributed in Asia and Europe and also introduced in North and South America. It includes four species viz., *Hippophae rhamnoides*, *Hippophae salicifolia*, *Hippophae tibetana* and *Hippophae neurocarpa* and nine subspecies of *Hippophae rhamnoides* which are reported so far from the World, of which, the most important species is *Hippophae rhamnoides* (Linn). It is a unique and valuable plant currently cultivated in various parts of the world. The natural habitat of Seabuckthorn extends widely in China, Mongolia, Russia, and most parts of North Europe. It can withstand extreme temperatures from -43°C to +40°C and is considered to be drought resistant.

Hippophae, commonly called Seabuckthorn and locally known as Chharma, Sutz or Sarla, occupies an important position as a valuable bio resources in the cold desert of Himachal Pradesh, Jammu and Kashmir, Uttarakhand, Sikkim and Arunachal Pradesh. It possesses outstanding qualities such as nitrogen fixing (60 to 180 kg/ha/yr), as soil binder, reduce top soil erosion by 30 per cent and retains soil moisture up to 80 per cent. A symbiotic michorhizal fungus, *Frankia* has been found from nodules and fixes maximum amount of nitrogen present in the atmosphere. Its capacity to fix nitrogen is twice than that of soybean (Rongsen, 1992) [10]. It has extraordinary capability to grow and survive under harsh environmental conditions, on problem soils, improve physical and chemical properties of soil and invades barren areas as pioneer species. Besides these, it is a rich sources of vitamins C, fodder and its fruits and seeds are source for food, cosmetic and pharmaceutical industries.

Leaves are alternate, narrow and lanceolate, with a silver-gray colour. The male bud consists of four to six apetalous flowers, which produce wind-distributed pollen whereas, the female bud usually consists of one single apetalous flower with one ovary and one ovule.

The female plants produce berry-like fruit 6–9 mm in diameter, soft, juicy and rich in oils. The ripe berries are drupe-like and orange/red in colour, consisting of a single seed surrounded by a soft, fleshy outer tissue. Seeds are dark brown, glossy, ovoid to elliptical in shape and 2.8–4.2 mm in size (Rousi, 1971; Bartish *et al.*, 2002) [11, 5].

The most important pharmacological activities attributed to Seabuckthorn oil or pulp oil include: anti-inflammatory, antimicrobial, pain relief and the promotion of tissue regeneration. Seabuckthorn oil is also recommended as a treatment for oral mucositis, rectal mucositis, vaginal mucositis, cervical erosion, radiation damage, heat burns, scalds, duodenal ulcers, gastric ulcers, chilblains, skin ulcers caused by malnutrition, poorly healing wounds and other skin damage. More than ten different drugs have been developed from Seabuckthorn in Asia and Europe and are available in different forms, such as liquids, powders, plasters, films, pastes, pills, ointments, suppositories, and aerosols.

Seabuckthorn oil extract is beneficial for skin and facial care, used to restore, improve and nourish healthy skin. One of the most well recognized properties of Seabuckthorn oil is nourishing revitalizing and restorative action on the skin and mucous. It is known to improve gum condition, combat gum bleeding and eczema. High content of fat soluble vitamins (A and E) and nutrients (EFAs, phytosterol) make Seabuckthorn indispensable as restorative, anti ageing and revitalizing agent for skin care.

In the view of great economic and ecological potential of the species, a number of studies have been conducted in different regions to assess the morphological and biochemical variations in natural growing populations of seabuckthorn in Himachal Pradesh and Ladakh region of Jammu and Kashmir (Khosla *et al.*, 1994; Singh 1994; Singh and Dogra, 1995; Singh and Singh, 2004; Sankhyan *et al.*, 2004 and Yadav *et al.*, 2006) [8, 13, 14, 15, 12, 17]. However, no studies have been conducted and reported so far to assess the variation of different growing conditions of Seabuckthorn populations under different major gene pool areas of Spiti valley of Himachal Pradesh in particular and other regions of cold deserts of India, in general. The present study, therefore, was carried out for morphometric and seed oil chemical evaluation of Seabuckthorn populations, under different major gene pool areas in Spiti valley in different growing conditions of Himachal Pradesh for further selection and breeding works and to obtain further improved genetic gain.

Materials and Methods

The present study is an outcome of the survey of Seabuckthorn populations under different growing conditions, in Spiti valley, a cold desert of Himachal Pradesh. Plants were selected from Nine sites (i.e. Major gene pool areas) of Spiti Valley. At each site, three growing conditions (viz. Pure stand, Mixed stand and Crop land) were selected during the end of growing season. Five plants were selected from each growing condition in each gene pool area selected. The selected plants were used for recording observation on morphological and seed oil characteristics. Details of sampling sites are presented in Table - 1 and 2. Only female plants showing most promising morphological features were randomly selected during September – October, 2014 to 2016, at the time of fruiting for present study.

Table 1: Selected experimental sites (Major gene pool areas) of Seabuckthorn populations in Spiti valley of Himachal Pradesh.

Serial No	Major gene pool areas and Code	Latitude (north)	Longitude (east)	Altitude (m)
1	Rangreek (GPA1)	32°15.179'	078°00.242'	3826
2	Kaza (GPA2)	32°13.596'	078°03.815'	3601
3	Sheigo (GPA3)	32°10.583'	078°06.242'	3538
4	Schilling (GPA4)	32°08.648'	078°13.195'	3437
5	Poh (GPA5)	32°07.083'	078°19.186'	3386
6	Tabo (GPA6)	32°05.710'	078°22.166'	3342
7	Lari (GPA7)	32°04.379'	078°25.463'	3309
8	Mane (GPA8)	32°02.006'	078°14.192'	3453
9	Hurling (GPA9)	32°03.787'	078°33.048'	3149

Table 2: Different growing conditions of Seabuckthorn populations in Spiti valley of Himachal Pradesh.

Serial No.	Growing Conditions	Code
1	Pure stand	GC1
2	Mixed stand	GC2
3	Crop land	GC3

The observations recorded includes morphometric traits viz., plant height (cm), branch length (cm), leaf density (no. of leaves per 10 cm), 100 fresh fruits weight (g) and 100 seed weight. The data was analysed statistically by using RBD. ANOVA (Analysis of Variance) was used for morphometric traits, as described by. In case of seed oil characteristics percent seed oil, acid value, saponification value and iodine value recorded for each growing condition among different selected major gene pool areas.

Extraction of oil was carried out following the method of Anonymous (1965) [3], in which both seed and kernels will be crushed using pestle and mortar. The oil will be extracted from powdered sample (known weight) in Soxhlet extraction apparatus with petroleum ether (60–80 °C) for six hours without interruption by gently heating it on the heating mantle.

$$\text{Oil content (\%)} = \frac{\text{Weight of oil extracted from the sample}}{\text{Weight of sample (100 g)}} \times 100$$

Acid value

Acid value of seed oil was estimated by titrating a known weight of sample (10g) containing 50 ml neutral solvent (25 ml ether + 25 ml 95% alcohol + 1% phenolphthalein) against 0.1N KOH solution using phenolphthalein as an indicator (Thimmiah, 1999) [16]. The acid value was calculated by using following expression:

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre} \times \text{Normality of KOH} \times 86.1}{\text{Weight of sample (g)}}$$

Saponification value

Saponification value of oil was estimated according to the standard method outlined in AOAC (1995) [4]. The oil sample (5.0 g) was mixed with alcoholic potassium hydroxide and the mixture was refluxed for 30 minutes to completely saponify the sample and then was titrate against 0.5 N HCl using phenolphthalein as indicator. Blank determination was also carried out along with sample. The value represents the average molecular weight of the oil and is useful for comparative study of the oil and is useful for comparative study of the fatty acid chain length in oils.

$$\text{Saponification value} = \frac{28.05 \times (\text{Blank titre} - \text{Sample titre})}{\text{weight of sample taken in grams}} \times 100$$

Iodine value

Iodine value was estimated according to Wijs (carbon tetrachloride-acetic acid solvent) method (AOAC, 1995) [4]. The oil sample (0.26-0.32 g) was mixed with carbon tetrachloride (25 ml) followed by addition of Wijs solution and then store in dark for one hour. After addition of 15 ml of 15 % KI and 100 ml water, the solution will be titrated against 0.1 N sodium thiosulphate using starch solution as an indicator. The iodine value was then calculated by subtracting the sample titre from the blank titre using following expression:

$$\text{Iodine value (g I}_2\text{/ 100 g oil)} = \frac{(\text{Blank titre} - \text{Sample titre}) \times \text{Normality of Na}_2\text{S}_2\text{O}_3 \times 12.69}{\text{Weight of sample (g)}}$$

Results and Discussion

The wide spectrums of variations were observed for different characters studied during the survey (Table – 3-4). There was significant difference among different major gene pool areas

for plant length, branch length, no. of leaves per 10 cm, 100 fruits weight and 100 seed weight. These chemical evaluation of seed oil also showed significant variation for different growing conditions among different major gene pool areas.

Maximum plant height (124.40 cm) was recorded in GPA-3 (Sheigo) followed by GPA-1 (Rangreek, 115.47 cm), while minimum plant height (97.60 cm) was recorded in GPA-9 (Hurling) gene pool area. On the other hand, maximum plant height (116.28 cm) was recorded for GC-1 (pure stand) which shows significant variation among different growing conditions. Variation in branch length under different growing conditions of *Hippophae rhamnoides* in different gene pool areas showed non significant variation. Maximum branch length among different gene pool areas recorded for GPA-4 (Schilling) i.e. 48.33 cm and minimum was observed in GPA-8 (Mane) i.e. 37.67 cm. GC-3 (Crop land) showed maximum (44.69 cm) and the minimum was in GC-2 (Mixed stand) i.e. 42.73 cm for branch length. Maximum (31.40) leaf density was recorded in GPA-4 (Schilling) and minimum (19.80) was recorded in GPA-9 (Hurling). Among different growing conditions, maximum leaf density was recorded in GC-3 (Crop land) i.e. 26.36 and minimum was recorded in GC-2 (Mixed stand) i.e. 23.12. Maximum (18.54 g)/100 fruits weight was recorded in GPA-3 (Sheigo) and minimum (11.36 g) was recorded in GPA-9 (Hurling). Among different growing conditions, maximum (16.50 g) 100 fresh fruit weight was recorded in GC-3 (Crop land) and minimum (12.79 g) was recorded in GC-2 (Mixed stand).). The 100 seed weight was observed to be maximum (2.99 g) in Schilling gene pool area, followed by Mane (2.71 g), whereas minimum seed weight was shown by Hurling (1.61 g) gene pool area. On the other hand growing condition showed significant variation. Crop land showed maximum 100 seed weight (2.57 g).

Table 3: Morphological traits variation in populations of Seabuckthorn (*Hippophae rhamnoides* L.) under different growing conditions among different major gene pool areas, in Spiti Valley of Himachal Pradesh.

Major gene pool areas	Plant height (cm)				Branch length (cm)				Leaf density (no. of leaves/ 10 cm)				100 fruits weight				100 seed weight			
	Growing conditions			Mean	Growing conditions			Mean	Growing conditions			Mean	Growing conditions			Mean	Growing conditions			Mean
	GC1	GC2	GC3		GC1	GC2	GC3		GC1	GC2	GC3		GC1	GC2	GC3		GC1	GC2	GC3	
GPA1 (Rangreek)	119.20	115.20	112.00	115.47	47.80	48.00	52.00	49.27	47.80	48.00	52.00	49.27	13.13	11.13	15.12	13.13	2.12	1.93	2.31	2.12
GPA2 (Kaza)	122.60	105.60	115.00	114.40	44.20	45.00	48.20	45.80	44.20	45.00	48.20	45.80	16.07	14.14	18.16	16.12	2.42	2.31	2.81	2.51
GPA3 (Sheigo)	130.80	118.80	123.60	124.40	47.00	44.40	45.60	45.67	47.00	44.40	45.60	45.67	18.82	16.05	20.76	18.54	2.97	2.76	3.25	2.99
GPA4 (Schilling)	117.40	103.20	102.80	107.80	46.20	45.40	53.40	48.33	46.20	45.40	53.40	48.33	16.77	15.08	18.52	16.79	2.53	2.41	2.91	2.61
GPA5(Poh)	111.80	103.60	108.60	108.00	43.80	39.60	39.60	41.00	43.80	39.60	39.60	41.00	12.13	11.04	14.09	12.42	2.04	1.81	2.11	1.99
GPA6(Tabo)	113.00	100.00	105.00	106.00	41.40	40.20	41.00	40.87	41.40	40.20	41.00	40.87	14.11	12.08	16.57	14.25	2.23	2.10	2.52	2.29
GPA7(Lari)	114.16	109.00	114.00	112.39	41.60	41.60	43.80	42.33	41.60	41.60	43.80	42.33	11.05	10.33	13.12	11.50	1.82	1.52	2.52	1.95
GPA8(Mane)	119.40	110.80	112.40	114.20	39.00	36.60	37.40	37.67	39.00	36.60	37.40	37.67	17.58	15.19	19.10	17.29	2.70	2.52	2.90	2.71
GPA9(Hurling)	98.20	96.00	98.60	97.60	45.00	43.80	41.20	43.33	45.00	43.80	41.20	43.33	11.02	10.04	13.04	11.36	1.62	1.41	1.81	1.61
Mean	116.28	106.91	110.22		44.00	42.73	44.69		44.00	42.73	44.69		14.52	12.79	16.50		2.27	2.08	2.57	
cd0.05																				
Gene pool areas (GPA)	12.836				NS				1.009				0.137				0.030			
Growing conditions (GC)	7.411				NS				0.583				0.079				0.017			
GPA × GC	NS				NS				NS				0.237				0.051			

Table 4: Seed oil chemical traits variation in populations of Seabuckthorn (*Hippophae rhamnoides* L.) under different growing conditions among different major gene pool areas, in Spiti Valley of Himachal Pradesh.

Major gene pool areas	Seed oil (%)				Acid value (mg KOH/g)				Saponification value (mg KOH/g)				Iodine value (g I ₂ / 100 g)			
	Growing conditions		Mean		Growing conditions		Mean		Growing conditions		Mean		Growing conditions		Mean	
	GC1	GC2	GC3		GC1	GC2	GC3		GC1	GC2	GC3		GC1	GC2	GC3	
GPA1(Rangreek)	4.87	4.83	4.91	4.87	4.20	4.12	4.31	4.21	210.07	205.76	214.05	209.96	146.34	145.86	147.64	146.61
GPA2(Kaza)	5.02	4.96	5.09	5.02	4.43	4.24	4.56	4.41	217.8	210.45	221.98	216.74	147.21	146.37	148.95	147.51
GPA3(Sheigo)	5.21	5.13	5.36	5.23	4.79	4.57	4.96	4.77	231.47	226.28	236.79	231.51	151.27	151.02	151.83	151.37
GPA4(Schilling)	5.15	5.06	5.17	5.13	4.52	4.41	4.67	4.53	225.37	221.09	234.42	226.96	150.41	149.82	151.57	150.60
GPA5(Poh)	4.61	4.51	4.68	4.60	4.01	3.94	4.2	4.05	193.46	189.31	198.34	193.70	139.67	138.46	140.43	139.52
GPA6(Tabo)	4.73	4.65	4.89	4.76	4.29	4.15	4.35	4.26	207.62	202.34	214.06	208.01	144.68	143.83	144.92	144.48
GPA7(Lari)	4.39	4.29	4.52	4.40	4.15	4.02	4.24	4.14	196.75	193.16	206.94	198.95	141.65	140.43	142.55	141.54
GPA8(Mane)	5.08	4.94	5.13	5.05	4.35	4.25	4.49	4.36	214.26	210.47	229.47	218.07	149.96	148.41	150.67	149.68
GPA9(Hurling)	4.29	4.04	4.41	4.25	3.96	3.82	4.09	3.96	185.63	175.28	193.47	184.79	137.36	136.85	138.74	137.65
Mean	4.82	4.71	4.91		4.30	4.17	4.43		209.16	203.79	216.61		145.39	144.56	146.37	
cd _{0.05}																
Gene pool areas (GPA)	0.124				0.224				2.286				0.687			
Growing conditions (GC)	0.040				0.050				0.850				0.243			
GPA × GC	0.239				0.269				3.591				0.824			

For seed oil evaluation Maximum per cent seed oil (5.23) was recorded in GPA-3 (Sheigo) while minimum plant height (4.25) was recorded in GPA-9 (Hurling) gene pool area. On the other hand, Maximum per cent seed oil (4.91) was recorded for GC-3 (crop land) which shows significant variation among different growing conditions. Maximum acid value(4.77 mg KOH/g), saponification value (231.51 mg KOH/g), and iodine value (151.37 g I₂/ 100 g) was recorded for GPA-3 (Sheigo) major gene pool area, and 4.91 mg KOH/g, 216.61 mg KOH/g and 146.37 g I₂/ 100 g respectively for GC-3 (crop land) growing condition.

However, an overall consideration revealed that GPA-3 (Sheigo) excelled over other major gene pool areas for morphometric traits and seed oil chemical traits among different major gene pool areas. Over all, GC-3 (crop land) was found superior for all morphological traits among different growing conditions under different major gene pool areas. Superiority might be because of genetic makeup. Causes of variations are attributed to (i) genetic characters of different populations (Beweley and Black, 1994) [6] or (ii) impact of mother plant environment (Anderssin and Milberg, 1988) [2] or (iii) wider habitat conditions (Friis, 1992) or (iv) diverse altitudinal gradients (Rawat *et al.*, 2006) [9]. These major gene pool areas are geographically connected with land but are isolated by high mountain peaks, and variable climatic conditions. During its spread and establishment, many changes took places in genetic makeup of *Hippophae rhamnoides* due to natural selection and adaptation (Ahmad and Kamal, 2003) [1].

Conclusion

On the basis of present investigation, eventually, it may be concluded that GPA-3(Sheigo) found superior for morphometric traits and seed oil traits among different major gene pool areas. Over all, GC-3 (crop land) was found superior for all morphometric traits among different growing conditions and in different major gene pool areas. It emerged from the present study that a large variability exists in the population of *Hippophae rhamnoides* growing under different growing conditions among different major gene pool areas in Spiti valley of Himachal Pradesh, particularly for morphological traits, which can be harnessed for improved

genetic gain. This is the first preliminary research report of its own kind on this aspect of applied action oriented research on Seabuckthorn in Indian conditions, more particularly, in cold deserts of Himalayas, although good amount of work has been reported and undertaken on this species for various aspects in China, Russia, Mongolia and most parts of North Europe as well as other parts of the world. The variability of different characteristics could be utilized for selection of genotypes suitable for the plantation and utilization in making different non wood forest products and also for utilizing ecological and economical gains in cold deserts of Himachal Pradesh.

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