Natl. Acad. Sci. Lett. (November–December 2013) 36(6):603–607
DOI 10.1007/s40009-013-0176-5

RESEARCH ARTICLE

Genotypic and Morphometric Effect on Fruit Oil Content in Seventeen Natural Population of Seabuckthorn (*Hippophae rhamnoides* L.) from Trans-Himalaya

Girish Korekar · Phuntsog Dolkar · Harvinder Singh · Ravi B. Srivastava · Tsering Stobdan

Received: 14 March 2013 / Revised: 1 May 2013 / Accepted: 25 July 2013 / Published online: 29 November 2013

Abstract Seventeen natural population of Seabuckthorn (SBT), which comprised 187 plants from trans-Himalaya, were studied to find out the variability, genotypic and morphometric effect on fruit oil content. The soft part of the fruit was found to be rich in oil content ranging from 3.16 to 17.3 %. A variation of 1–5.5-folds in oil content among the examined fruit and univariate two-way ANOVA analysis underlines the important role played by genetic background for determining oil content in SBT fruit. At population level, the highest oil content (12.02 %) was observed in TYX while the lowest was found in CHO population (6.84 %). The overall mean oil content was 9.74 %. Oil content in berry was significantly correlated with fruit weight (–0.203, $p \leq 0.05$), fruit width (–0.195, $p \leq 0.05$), tree habit (0.216, $p \leq 0.05$), seed weight (–0.151, $p \leq 01$), seed length (–0.177, $p \leq 0.01$), leaf area (–0.162, $p \leq 0.01$), number of leaf per 10 cm^2 (–0.151, $p \leq 0.01$), secondary branch apex (–0.144, $p \leq 0.01$) and mature stem colour (0.186, $p \leq 0.01$). However, none of the values were highly significant.

Keywords Himalaya · Oil content · Omega · Pulp oil · Sea buckthorn

G. Korekar · P. Dolkar · R. B. Srivastava · T. Stobdan (✉)
Defence Institute of High Altitude Research, DRDO, Leh 194101, Jammu and Kashmir, India
e-mail: ts_mbb@yahoo.com

H. Singh
Jaypee University of Information Technology, Waknaghat 173215, Solan, Himachal Pradesh, India

Introduction

The actinorhizal plant Seabuckthorn (*Hippophae rhamnoides* L., Elaeagnaceae) is dioecious and wind pollinated. The female plant bears red, orange or yellow berries on two-year-old thorny twigs. Seabuckthorn (SBT) berries are among the most nutritious of all fruits and have immense medicinal properties. Concentrations of vitamins B_2, B_3, B_5, B_6, B_{12}, C and E are much higher than other fruits such as apricot, banana, mango, orange and peach [1]. SBT extracts possess antibacterial activities [2] and have shown protective effect against the toxic effect of mustard gas, a chemical warfare agent. Many of the claims associated with SBT are related to high nutritive value in terms of vitamins, organic acids, flavonoids, macro- and micro-nutrient elements. The nutritional attributes, medicinal and therapeutic potential have recently been reviewed [3, 4]. The shrub serves as a storehouse for researchers in the field of biotechnology, nutraceutical, pharmaceutical, cosmetic and environmental sciences [5].

SBT oil is a valuable product used in medicine, as nutraceutical supplement, and in cosmetic. Oil extracted from seed and pulp differs considerably in fatty acid composition. Oil from juice and pulp is rich in palmitic (16:0) and palmitoleic (16:1) acids, while the oil from the seed contains the essential fatty acids, which are linoleic (18:2) and linolenic (18:3) acids. One of the many special features of SBT fruit is the exceptionally high content of tocopherols and tocotrienols. Total content of tocotrienols varied from 130 to 290 mg kg^{-1} in seed and 40 to 120 mg kg^{-1} in flesh part of the berry [6]. The content of these bioactive compounds are among the crucial criteria defining the quality of SBT. Oil derived from juice contains more vitamin E (216 mg/100 g of fruit) than seed (64.4–92.7 mg/100 g of seed) [7]. The tocopherol and tocotrienols fraction in soft part of the berry consists of 70–80 % α-tocopherol, 4–9 % β-tocopherol, 2–6 % γ-tocopherol and 2–4 % γ-tocotrienol [6]. Presence of high content of α-tocopherol has significant healthful effect, which acts as natural antioxidant in the human body. Carotenoids content of SBT oil ranges from 314 to 2,139 mg/100 g [8]. The seed oil is characterized by its high oleic acid content (17 %) and its one to one ratio of omega-3 (alpha linoleic) and omega-6 (linoleic) at approximately 34 and 31 %, respectively. The relationship of equivalence between the two omegas is critical because they self check each other in a delicate balance to regulate thousands of metabolic functions through prostaglandin pathways. Nearly every biological function is interconnected with balance between omega-6 and omega-3 [3].

The oil content of SBT in seed and pulp has been extensively studied. *H. rhamnoides* contains higher amount of oil than for *H. tibetana* and *H. salicifolia* [9]. Fleshy part of the berry is the main source of oil. On an average, only 10–20 % of the oil is in the seed, the proportions of which never exceed that of the pulp oil [10]. The oil content ranges from 4.2 to 16.5 % in seeds and 1.7 to 10.5 % in whole berry [10]. However, quantification of the oil content has been studied either in elite selections [6, 11, 12] or within limited number of samples [6, 9, 11, 13]. Therefore, studies involving large population and the importance of genotype have not been investigated. Besides, no detail studies have been conducted to study interrelationship between oil content and morphometric traits. Therefore, the objective of the present study was to investigate genotypic effect on oil content in soft part of SBT fruit from the 187 plants representing 17 natural populations. Attempts have been made to find interrelationship between oil content and 36 morphometric traits.

Materials and Methods

Sample Collection

Seventeen natural populations of *H rhamnoides* L. comprising 187 female plants were sampled across the major distribution site from Indian trans-Himalaya. The altitude of collection site ranged from 2,765.1 to 3,336.1 m a.s.l (Table 1). The mean maximum and minimum temperature in the study site was 18.9 ± 9.5 °C and –5.8 ± 9.8 °C, respectively while the mean maximum and minimum relative humidity was 35.5 ± 7.3 % and 25.0 ± 3.7 %, respectively during 2001–2011 [14]. Mean maximum temperature during cropping season (May–September) was 28.4 °C while the minimum was 4.5 °C during the last decade. Samples were collected with fruiting twigs and berries were removed manually in the Laboratory. Seed was separated and the remaining soft part of the fruit was lyophilized in a Laboratory freeze dryer (ALPHA 2–4 LD plus, Fisher Bioblock Scientific, France).

Morphological Characterization

Thirty-nine morphometric traits were analyzed in the 187 plants. Characters such as plant height and canopy width were measured in the field while fruit, seed and leaf characters were evaluated in the laboratory.

Oil Extraction

Oil was extracted in Soxhlet extraction unit as described by Conkerton et al. [15] with minor modification. Briefly, 6 g freeze dried samples per thimble was used. Hexane was used as solvent to extract oil. The solvent-to-sample ratio was 10:1. The extraction temperature was 68 °C and condenser was maintained at 15 °C. Extracted oil was desolvenized in a vacuum oven at 50 °C to determine yield. The oil content was determined in terms of fresh weight taking into consideration the initial fresh weight and weight of sample after freeze drying.

Statistical Analysis

All the experiments were performed in triplicate. Influence of populations on oil content was analyzed using univariate General Linear Model (GLM). One way ANOVA with two-sided Tukey's HSD at $p \leq 0.05$ and two-tailed Pearson correlation was done using SPSS 17.0 version for Windows.

Result and Discussion

Oil Content

Oil content in pulpy soft part surrounding the seed is presented in Table 1. SBT fruits were found to be rich in oil content ranging from 3.16 to 17.3 % between individuals in the studied population. Therefore, a variation of 1–5.5-folds in oil content was observed. At population level, the highest oil content (12.02 %) was observed in TYX while the lowest was found in CHO population (6.84 %). The overall mean oil content was 9.74 %. In comparison, Tiitinen et al. [11] reported 0.7–3.6 % pulp oil in ssp *mongolica* of Russia origin grown in Finland and 2.9–3.3 % in ssp. *rhamnoides* of Finnish origin. Ranjith et al. [9] reported 2.99–3.61 % pulp oil in SBT from India. In contrast, Sabir et al. [13] reported 17.8–29.1 % oil in softer pulp in eight populations from Pakistan. High amount of oil in the Pakistan origin is probably because the fruits were dried initially and the expression may be in terms of dry weight basis. In the present study, the oil content in the soft part of berry in terms

Table 1 Locations of 17 populations of *H. rhamnoides* L. from Indian trans-Himalaya: oil content in soft part of SBT berry within 17 populations and the effect of genetic background within a population (% FW)

Sampling Localities	Population ID	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Sample size	% Oil		
						Mean	Min	Max
Choglamsar	CHO	34°06′.7″	77°35′.0″	3213.8	14	*6.84 ± 1.85[a]	3.31	9.50
Chuchot	CHU	34°05′.3″	77°35′.6″	3219.9	12	*11.51 ± 2.34[bc]	7.84	14.87
Shey	SHY	34°04′.1″	77°37′.5″	3238.8	07	*10.75 ± 2.10[abc]	7.25	13.23
Shey Forest	SHF	34°05′.2″	77°36′.2″	3222.6	10	*9.82 ± 2.22[abc]	7.25	14.74
Phey	PHY	34°08′.2″	77°29′.0″	3185.5	17	*11.84 ± 1.90[c]	8.19	15.21
Shey Picnic	SHP	34°03′.6″	77°37′.5″	3239.4	35	*8.00 ± 2.21[abc]	3.16	12.53
Skuru	SKR	34°40′.1″	77°17′.5″	3125.4	07	*11.62 ± 2.47[bc]	8.53	15.02
Tyaxi	TYX	34°53′.2″	76°48′.3″	2765.1	07	*12.02 ± 2.07[c]	9.61	15.08
Turtuk	TRK	34°50′.5″	76°49′.5″	2869.4	17	*10.79 ± 3.06[abc]	6.06	17.30
Bogdang	BGD	34°48′.1″	77°2′.4″	2987.0	03	*9.67 ± 1.95[abc]	7.70	11.59
Changlung	CHG	34°55′.4″	77°28′.2″	3303.7	06	*9.37 ± 1.47[abc]	7.49	10.92
Panamik	PNK	34°47′.4″	77°31′.5″	3196.1	09	*9.92 ± 2.50[abc]	6.16	12.13
Sumur	SMR	34°37′.6″	77°36′.3″	3108.0	04	*7.59 ± 1.96[ab]	5.77	10.06
Skuru Forest	SKF	34°41′.1″	77°16′.1″	3044.6	14	*10.04 ± 2.29[abc]	6.13	13.68
Hunder	HUN	34°35′.0″	77°29′.5″	3089.7	08	*8.58 ± 2.46[abc]	6.53	13.34
Thirth	TRT	34°32′.2″	77°39′.2″	3212.9	14	*10.61 ± 2.33[abc]	6.03	13.58
Khalsar	KHA	34°29′.2″	77°42′.1″	3336.6	03	*7.31 ± 2.73[a]	5.63	10.46
Total						*9.74 ± 2.71	3.16	17.30

Values represented as mean ± SD; for each column, different lowercase letters indicate significantly different at $p < 0.05$, as measured by 2-sided Tukey's HSD between SBT populations

* Significant difference between individual samples within a population at $p \leq 0.05$

of dry weight basis ranged from 17.95–31.55 % and the mean value was 25.57 %.

Significant difference in oil content was observed between individual plants within a population in all studied 17 populations at $p < 0.05$ (Table 1). This underlines the importance of genetic background for determining oil content in SBT. Significant variation between plants within a population could be observed as SBT is wind pollinated out-cross species and therefore high genetic variation is expected in natural population.

One-way ANOVA

Authors conducted a GLM model based analysis to determine the significant effect of populations on oil content. Populations significantly affect the oil content (Table 2). The result suggested that choice of population is an important criteria for selecting plant from natural population as a source of oil content in the berry. A large number of populations are needed to screen for their potential source as breeding stock to improve oil content in the berry.

Correlation Analysis

Detail studies on interrelationship between oil content and morphometric traits have not been reported. A positive correlation between pulp oil content and fruity flavor and a negative correlation with sweetness has been reported [11]. Table 3 displayed the correlation between oil content and 36 morphemetric traits. Oil content in berry was significantly correlated with fruit weight (–0.203, $p \leq 0.05$), fruit width (–0.195, $p \leq 0.05$), tree habit (0.216, $p \leq 0.05$), seed weight (–0.151, $p \leq 0.01$), seed length (–0.177, $p \leq 0.01$), leaf area (–0.162, $p \leq 0.01$), number of leaf per 10 cm^2 (–0.151, $p \leq 0.01$), secondary branch apex (–0.144, $p \leq 0.01$) and mature stem colour (0.186, $p \leq 0.01$). However, none of the values were highly

Table 2 Univariate Two-way ANOVA in GLM to estimate the effect of populations on oil content

Source	Sum of squares	*df*	Mean square	F	$P < 0.05$
Corrected model	483.271	16	30.204	5.787	0.000
Intercept	12063.035	1	12063.035	2311.274	0.000
Population	483.271	16	30.204	5.787	0.000
Error	887.266	170	5.219		
Total	19123.833	187			
Corrected total	1370.538	186			

Table 3 Pearson correlation to estimate the interrelationship between oil content and morphometric traits

Correlation with trait	% Oil	F weight (mg)	F length (mm)	F width (mm)	No berries/10 cm^2	No berries/bunch	F index
	1	−0.203**	−0.111	−0.195**	0.025	0.050	0.076
Correlation with trait	Pedicel length (mm)	Seed Wt (mg)	Seed length (mm)	Seed width (mm)	Seed index	Leaf area (cm^2)	No leafs/10 cm^2
	-.072	−0.151*	−0.177*	−0.083	−0.118	−0.162*	0.151*
Correlation with trait	No thorn/10 cm^2	Plant height (m)	Canopy width (m)	PH/CW	L	a	b
	0.057	−0.136	−0.056	−0.011	0.086	−0.095	0.132
Correlation with trait	Leaf density	Fruit distribution	Density of fruit bearing	Fruit appearance	Tree habit	Tree vigour	No. of shoot
	0.150*	0.099	0.081	0.085	0.216**	−0.024	−0.029
Correlation with trait	Colour of dormant mature stem	Uniformity of fruit	Branching habit	Thorniness	Leafyness on thorn	Secondary branch apex	Stem shape
	−0.016	−0.006	0.049	0.123	−0.034	−0.144*	−0.049
Correlation with trait	Immature stem colour	Mature stem colour					
	0.040	0.186*					

F weight fruit weight, *F length* fruit length, *F width* fruit width, *PH/CW* plant height/Canopy width, *L* Fruit colour in terms of lightness, *a* Fruit colour in terms of blueness, *b* Fruit colour in terms of yellowness

Correlation significant at * $p \leq 0.05$; ** $p \leq 0.01$

significant. The results are in agreement with the observed phenomenon in genera *Ribes*, *Rubus*, and *Arctostaphylos*, according to which the smaller the seeds within one genus, the higher the oil content [16, 17]. However, it deviates from the previous report in *H. rhamnoides* ssp *sinensis* and *mongolica* [6].

Sabir et al. [13] observed that yellow and orange-yellow fruits have higher level of oil than orange and orange-red fruits. However, in the present study fruit colour has no significant effect on oil content. A positive correlation between pulp oil content and fruity flavor and a negative correlation with sweetness has been reported earlier [11].

Conclusions

The influence of plant genetic background on oil content in the pulpy fruit part surrounding the seed was demonstrated. Significant variation was found within and between the 17 natural populations, which underline the role of genetic background for determining the oil content in SBT. Many fold variation in oil content (1–5.5) was observed within the 187 studied plants. Population has significant effect on the oil content in berry. Significant interrelationship between oil content and eight out of 36 morphological traits was observed. Results obtained in this study can be considered for selection of genotype for breeding purpose to increase oil content in the berry.

Acknowledgments One of the author G K is grateful to Defence Research and Development Organisation, New Delhi, India for providing financial support to carry out this work through Senior Research Fellowship. Authors thank Divisional Forest Officer, Leh for permission to survey and collect natural population of seabuckthorn.

References

1. Stobdan T, Chaurasia OP, Korekar G, Mundra S, Ali Z, Yadav A, Singh SB (2010) Attributes of seabuckthorn (*Hippophae rhamnoides* L.) to meet nutritional requirements in high altitude. Def Sci J 60:226–230
2. Arora R, Mundra S, Yadav A, Srivastava RB, Stobdan T (2012) Antimicrobial activity of seed, pomace and leaf extracts of Seabuckthorn (*Hippophae rhamnoides* L.) against food borne and food spoilage pathogen. Afr J Biotechnol 11:10424–10430
3. Bal LM, Meda V, Naik SN, Satya S (2011) Sea buckthorn berries: a potential source of valuable nutrients for neutraceuticals and cosmoceuticals. Food Res Int 44:1718–1727
4. Stobdan T, Korekar G, Srivastava RB (2013) Nutritional attributes and health application of seabuckthorn (*Hippophae rhamnoides* L.)—a review. Curr Nutr Food Sci 9:151–165
5. Stobdan T, Angchuk D, Singh SB (2008) Seabuckthorn: an emerging storehouse for researchers in India. Curr Sci 94: 1236–1237
6. Kallio H, Baoru Y, Peipp OP, Tahvonen R, Pan R (2002) Triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols

in berries and seeds of two subspecies (ssp. *sinensis* and *mongolica*) of seabuckthorn (*Hippophae rhamnoides*). J Agric Food Chem 50:3004–3009
7. Li TSC (2002) Product development of Sea buckthorn. In: Janick J, Whipkey A (eds) Trends in new crops and new uses. ASHS Press, Alexandria, pp 393–398
8. Zhang W, Yan J, Duo J, Ren B, Guo J (1989) Preliminary study of biochemical constituents of berry of Sea buckthorn growing in Shanxi province and their changing trend. In: Proceedings of international symposium on Sea buckthorn (*H. rhamnoides* L.). Xia, China. pp 96–105
9. Ranjith A, Kumar KS, Venogupalan VV, Arumughan V, Shawney RS, Singh V (2006) Fatty acids, tocols, and carotenoids in pulp oil of three Sea buckthorn species (*Hippophae rhamnoides*, *H. salicifolia*, and *H. tibetana*) grown in the Indian Himalayas. JAOCS 83:359–364
10. Kallio K, Yang BR, Tahvonen R, Hakala M (1999) Composition of seabuckthorn berries of various origin. In: Proceeding of international symposium on Seabuckthorn (*Hippophae rhamnoides* L.). Beijing., China
11. Tiitinen KM, Hakala MA, Kallio HP (2005) Quality components of Sea buckthorn (*Hippophae rhamnoides*) varieties. J Agric Food Chem 53:1692–1699
12. Zadernowski R, Nacrk M, Amarowicz R (2003) Tocopherols in Sea buckthorn (*Hippophae rhamnoides* L.) berry oil. JAOCS 80:55–58
13. Sabir S, Maqsood H, Hayat I, Khan MQ, Khaliq A (2005) Elemental and nutritional analysis of seabuckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) berries of Pakistani origin. J Med Food 8:518–522
14. Korekar G, Dwivedi SK, Singh H, Srivastava RB, Stobdan T (2013) Germination of *Hippophae rhamnoides* L. seed after 10 years of storage at ambient condition in cold arid trans-Himalayan Ladakh region. Curr Sci 104:110–114
15. Conkerton EJ, Wan PJ, Richard OA (1995) Hexane and heptanes as extraction solvents for cottonseed: a laboratory-scale study. JAOCS 72:963–965
16. Johansson AK, Kuusisto PH, Laakso PH, Derome KK, Sepponen PJ, Katajisto JH, Kallio HP (1997) Geographical variation in seed oils from *Rubus chamaemorus* and *Empetrum nigrum*. Phytochemistry 44:1421–1427
17. Johansson AK, Laakso PH, Kallio HP (1997) Characterization of seed oil of wild, edible Finnish berries. Z Lebensm Unters Forsch A 2004:300–307