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Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Seabuckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya

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ABSTRACT

Seventeen natural population of Seabuckthorn (SBT), which comprised 187 plants from trans-Himalaya, were studied to find out variability and genotypic effect on total phenolic content (TPC), total antioxidant capacity (TAC), ascorbic acid and carotenoids content in fruit pulp. The fruits were found to be rich in TPC ranging from 964 to 10,704 mg gallic acid equivalent/100 g. The free radical-scavenging activity in terms of inhibitory concentration (IC_{50}) ranged from 0.7 to 9.1 mg/ml and ferric reducing antioxidant potential (FRAP) from 180 to 1355 $FeSO_4 \cdot 7H_2O$ μg/ml. The ascorbic acid and carotenoids content ranged from 56 to 3909 mg/100 g and 0.1–14.4 mg/100 g, respectively. A variation of 1–11 fold in TPC, 1–14 folds in IC_{50} by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and 1–8 fold in ferric reducing antioxidant potential, 1–70 fold in ascorbic acid content and 1–206 fold in carotenoid content among the examined fruit across 17 populations underlines the important role played by genetic background and the geographical location for determining the health promoting compounds. Significant correlation was observed between TPC, IC_{50}, FRAP, carotenoids, ascorbic acid, fruit lightness (L*) and plant height. Among the 20 morphological traits studied, fruit colour and plant height showed positive correlation with the health promoting compounds.

1. Introduction

Natural products for food and nutritional supplements have gained increased attention in recent years. In this context, there is an increasing interest in the beneficial health effects of plant derived compounds. Epidemiological studies have demonstrated that there is a positive relation between intake of antioxidant rich diet and lower incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts (Ames, Shigenaga, & Hagen, 1993; Gordon, 1996; Halliwell, 1996). Along with other antioxidant components, phenolics, carotenoids and ascorbic acid present in fruit and vegetable have been reported to play a major role in disease prevention. These results have stimulated research to characterize different types of plants with regards to their health promoting compounds.

Hippophae (commonly known as Seabuckthorn) is an actinorhizal plant having symbiotic association with *Frankia*. The genus *Hippophae* comprises of seven species. All species are diploid ($2n = 24$), wind pollinated, and dioecious, and are restricted to the Qinghai Plateau and adjacent areas, with the exception of the species *Hippophae rhamnoides* L. that occurs widely but sporadically in Asia and Europe (Stobdan, Korekar, & Srivastava, 2013). 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 (Stobdan et al., 2010). SBT extracts possess antibacterial activities and have shown protective effect against the toxic effect of mustard gas, a chemical warfare agent (Vijayarahgavan et al., 2006).

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; FW, fresh weight; IC_{50}, inhibitory concentration; L*, colour in lightness; PCA, principal component analysis; PR, phosphotungstate reagent; SBT, Seabuckthorn; TAC, total antioxidant capacity; TPC, total phenolic content.

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Many of the claims associated with SBT are related to high nutritive value in terms of vitamins, organic acids, flavonoids, macro- and micronutrient elements. The major phenolic compounds detected in SBT juice (mg/l) are gallic acid (1220), proanthocyanidins (351), isorhamnetin 3-*O*-rutinoside (181), isorhanetin 3-*O*-glucoside-7-*O*-rhamnoside (75), isorhamnetin 3-*O*glucoside (75), catechin (19), quercetin 3-*O*-rutinoside (14.5), quercetin 3-*O*-glucoside (9.0), epicatechin (2.8), protocatechuic acid (1.5), isorhamnetin (1.4), isorhamnetin 7-*O*-rhamnoside (1.3) (Rosch, Bergmann, Knorr, & Froh, 2003). Phenolic acids present in SBT berry are 2,5-dihydrobenzoic acid, gallic acid, pyrocatechuic acid, protocatechuic acid, salicylic acid, syringic acid, vanillic acid, veratric acid, caffeic acid, meta-, ortho- and para-coumaric acid, ferulic acid, hydroxycaffeic acid, p-hydroxyphenyl-lactic acid and quinic acid (Zadernowski, Naczk, Czaplicki, Rubinskiene, & Szalkiewicz, 2005). The carotenoids consist of zeaxanthin, β-carotene, β-cryptoxanthin, litein, lycopene and γ-carotene (Anderson, Olsson, Johansson, & Rumpunen, 2009). The nutritional attributes, medicinal and therapeutic potential have recently been reviewed (Geetha & Gupta, 2011; Stobdan et al., 2013). The shrub serves as a storehouse for researchers in the field of biotechnology, nutraceutical, pharmaceutical, cosmetic and environmental sciences (Stobdan, Angchuk, & Singh, 2008).

Proximate composition of SBT berry has been extensively studied (Chen, 1988; Dhyani et al., 2007; Kallio, Baoru, Peipp, Tahvonen, & Pan, 2002; Korekar, Stobdan, Singh, Chaurasia, & Singh, 2011; Stobdan et al., 2010; Tang & Tigerstedt, 2001; Tong, Zhang, Zhao, Yang, & Tian, 1989; Zhang, Yan, Duo, Ren, & Guo, 1989). However, quantification of the health-promoting compounds has been studied either in elite selections or within a limited number of samples. Total phenolic content (TPC) and total antioxidant capacity (TAC) depend on specific plant genotype and interaction of cultivation condition (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005). Although the importance of genotype in determining TAC in selected fruit has been demonstrated (Connor, 2005; Connor, Luby, & Tong, 2002; Leccese, Bartolini, & Viti, 2012; Panico et al., 2009; Scalzo et al., 2005; Yildirim, San, Koyuncu, & Yildirim, 2010), the effect of genotype in SBT has not been deeply investigated. Ercisli, Orhan, Ozdemir, and Sengul (2007) reported genotypic effect on chemical composition and antioxidant activity of SBT berry based on 10 wild genotypes from a single location in Turkey. However, studies involving large population have not been reported. Besides, limited studies have been conducted in fruit crops to study interrelationship between morphological and biochemical traits. To our knowledge extensive studies have not been conducted in SBT. Therefore, the objective of the present study was to investigate genotypic effect on TAC, TPC, ascorbic acid and total carotenoids content in SBT fruit from 187 plants representing 17 natural populations. Attempts have been made to find interrelationship between the health promoting compound content and 20 morphological traits in the plant.

2. Materials and methods

2.1. Sample collection and chemicals

Seventeen natural populations of *H. rhamnoides* L., which grows in wild without human interference, comprising 187 female plants were sampled across the major distribution site from Indian trans-Himalaya. The altitude of collection sites ranged from 2765 to 3337 m a.s.l. (Table 1). A voucher specimen has been deposited in the herbarium of Department of Medicinal and Aromatic Plants, DIHAR, Leh, India. The mean minimum and maximum temperature recorded at 3500 m a.s.l was −6 ± 10 °C and 19 ± 10 °C, respectively while the mean minimum and maximum relative humidity was 25 ± 4 and 36 ± 7%, respectively during 2001–2011. Mean minimum temperature during cropping season (May–September) was 4.5 °C while the maximum temperature was 28 °C during the last decade. Samples were collected with fruiting twigs and 500–1000 g ripe berries were removed manually from each plant of similar age in the laboratory and pooled to constitute a single sample. Trunk diameter was taken as a proxy age of the plant. Seed was separated and fruit pulp was lyophilized in a Laboratory freeze dryer (ALPHA 2-4 LD plus, Fisher Bioblock Scientific, France). Solvents, sodium tungstate and Folin–Ciocalteu reagent were purchased from Merck, Germany; gallic acid, ascorbic acid, ferrous sulfate hexahydrate, β-carotene, DPPH and TPTZ were purchased from Sigma–Aldrich, USA. Sodium chloride, metaphosphoric acid and sodium carbonate were purchased from HiMedia, Mumbai, India.

Table 1
Locations of 17 natural populations of *H. rhamnoides* L. from Indian trans-Himalaya.

Sampling locations	Population ID	Latitude (N)	Longitude (E)	Altitude (m) ASL	Sample size
Choglamsar	CHO	34°06′0.7″	77°35′0.0″	3214	14
Chuchot	CHU	34°05′0.3″	77°35′0.6″	3220	12
Shey	SHY	34°04′0.1″	77°37′0.5″	3239	07
Shey Forest	SHF	34°05′0.2″	77°36′0.2″	3223	10
Phey	PHY	34°08′0.2″	77°29′0.0″	3186	17
Shey Picnic	SHP	34°03′0.6″	77°37′0.5″	3240	35
Skuru	SKR	34°40′0.1″	77°17′0.5″	3125	07
Tyaxi	TYX	34°53′0.2″	76°48′0.3″	2765	07
Turtuk	TRK	34°50′0.5″	76°49′0.5″	2869	17
Bogdang	BGD	34°48′0.1″	77°20′0.4″	2987	03
Changlung	CHG	34°55′0.4″	77°28′0.2″	3304	06
Panamik	PNK	34°47′0.4″	77°31′0.5″	3196	09
Sumur	SMR	34°37′0.1″	77°36′0.3″	3108	04
Skuru forest	SKF	34°41′0.1″	77°16′0.1″	3045	14
Hunder	HUN	34°35′0.0″	77°29′0.5″	3090	08
Thirth	TRT	34°32′0.2″	77°39′0.2″	3213	14
Khalsar	KHA	34°29′0.2″	77°42′0.1″	3337	03

2.2. Morphological characterization

Twenty morphological characters 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.

2.3. Preparation of extract

Freeze dried samples were defatted with hexane followed by two cycles of extraction with methanol. Each sample (20–40 mg) was extracted ($n = 3$) for 15 min with 1.5 ml methanol in a 2 ml micro centrifuge tube and vortexed at room temperature. The sample was centrifuged at 5600 g for 10 min and the supernatant was recovered. The residue was mixed with 1.5 ml of water and the process was repeated as described above. TPC and TAC were measured directly in the methanolic and aqueous extracts and the values were combined mathematically.

2.4. Determination of total phenolic content

The Folin-Ciocalteu reagent assay was used to determine the TPC (Singleton & Rossi, 1965). An aliquot of the samples (30 μl) was introduced into 96 well ELISA plate followed by 150 μl Folin-Ciocalteu reagent, which was previously diluted with distilled water (1:10) and 120 μl sodium carbonate (75 g/l). The ELISA plates were vortexed, covered with parafilm and allowed to stand for 30 min in ELISA reader. Absorbance at 765 nm was recorded in an ELISA reader (SpectroMax M2 e, Molecular Devices, Sunnyvale, CA,