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Morphological and biochemical variation in Sea buckthorn *Hippophae rhamnoides* ssp. *turkestanica*, a multipurpose plant for fragile mountains of Pakistan

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Sea buckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) a member of family Elaeagnaceae, is a very important multipurpose plant in the northern areas of Pakistan. It is an ideal plant for preventing soil erosion and land reclamation, can withstand extremes of temperature ranging from –43°C to 55°C and grows well under drought conditions and variable soil pH. The fruit is rich in nutrients and medicinal compounds such as vitamins, carotene, flavonoids, essential oil, carbohydrates, organic acids, amino acids, and soluble sugars. The plants are also important as fuel wood, fencing, fodder, soil erosion control, to make soil fertile by nitrogen fixation in roots and for the purpose of shelterbelts. In order to compare various populations of Sea buckthorn for morphological and biochemical composition, ten populations from different areas of northern Pakistan were compared using plant and fruit characters. The purpose of the investigation was to identify the variable populations for different valuable characteristics to develop improved varieties for commercial cultivation and easy fruit harvesting. The comparison indicated a significant amount of variability on morphological and biochemical basis. The variability will be utilised to develop commercial varieties of the plant utilising the conventional techniques of selection and hybridisation for economic activities on degraded land of mountainous regions of Pakistan.

Introduction

Sea buckthorn is a general term given to the shrub or small tree of the genus *Hippophae*. The genus belongs to the family Elaeagnaceae that consists of six species and ten subspecies, among which the most economically important one is *Hippophae rhamnoides* L., commonly known as Sea buckthorn (Rongsen 1992). A total of about 1 400 000 hectares of natural Sea buckthorn stands are distributed widely throughout the world, mainly in China, the former USSR and the Himalayan countries, between 2–123°E longitude and 27–69°N latitude (Rongsen 1992).

Sea buckthorn is a native plant species of northern Pakistan, with about 3 000 hectares of wild forests, annually producing 1 200–2 250 tons of Sea buckthorn fruit (Rongsen 1992). The only sub-species found in the northern areas of Pakistan is *Hippophae rhamnoides* ssp. *turkestanica*, widely found in central Asia and west Asia; including Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, Kirghisistan, Xinjiang province of China and northern India. Northern areas of Pakistan are in the centre of the distribution of *H. rhamnoides* ssp. *turkestanica* (Ahmad and Kamal 2002). It is the only sub-species which can withstand the harsh bio-physical conditions characterised by arid, hot summers and cold winters (Rongsen 1992).

Sea buckthorn is a winter-hardy, deciduous bush bearing clusters of juicy fruits, generally about the size of a small pea, which are greenish in colour in the beginning turning orange, red or yellow, as they mature (Ahmad and Kamal 2002). The plants have very strong root systems, five-year-old plants having tap roots of up to 1.1m deep and horizontal roots of up to 2.58m wide. A symbiotic mycorrhizal fungus, *Frankia*, has been found on Sea buckthorn roots, which form nodules and fix maximum amounts of atmospheric nitrogen (Stewart and Pearson 1967). Its capacity to fix nitrogen is twice than that of soybeans. The plant can withstand extremes of temperature ranging from –43°C to 55°C (surface) and grows well under drought conditions of 250–800mm annual rainfall. It also withstands the soil pH of 5.8–9.5 (Rongsen 1992, Ahmad and Kamal 2002).

Sea buckthorn fruit is rich in nutrients, such as carbohydrates, organic acids, amino acids and vitamins. The fruit contains 60–80% juice rich in sugar, organic acids, amino acids and vitamins. Vitamin C content is 200 to 1 500mg $100g^{-1}$ which is five to 100 times higher than any other fruit or vegetable known (Ahmad and Kamal 2002). The carotene content ranges from 30–40mg $100g^{-1}$ of berries (Bernath and Foldesi 1992, Wolf and Wegert 1993). Vitamin E concentration can be up to 160mg $100g^{-1}$ of berries (Zhang *et al*. 1989, Ma and Cui 1989, Eliseev 1989, Wahlberg and Jeppsson 1990, 1992). Sea buckthorn is also rich in flavonoids (Vitamin P) and contains appreciable amounts of water soluble and fat soluble vitamins (Zhang *et al*. 1989, Schapiro 1989). Vitamin A, Vitamin B1, Vitamin K, Vitamin B2, Vitamin P and Vitamin C were found to be 11mg $100g^{-1}$, 0.04mg $100g^{-1}$, 100–200mg $100g^{-1}$, 0.56mg $100g^{-1}$, 1 000mg $100g^{-1}$ and 300–1 600mg $100g^{-1}$ respectively (Xu 1956, Tian 1985, Wang 1987). Sea buckthorn berries contain up to 13% soluble sugars, mainly glucose, fructose, xylose and 3.9% organic acids, mainly malic and succinic acid (Ma and Cui 1989). A total of 18 amino acids have been found in Sea buckthorn fruit (Zhang *et al*. 1989, Mironov 1989). There are at least 24 chemical elements present in Sea buckthorn juice, e.g. nitrogen, phosphorus, iron, manganese, boron, calcium, aluminum, silicon and others (Wolf and Wegert 1993, Zhang *et al*. 1989, Tong *et al*. 1989). The oil content ranges from 1.5–3.5% in fruit pulp and 9.9–19.5% in seeds (Rongsen 1992). The oil contains significant amounts of β-carotene and Vitamin E, making the oil of Sea buckthorn an effective medicine for many diseases (Ahmad and Kamal 2002). Oil from the juice and pulp is rich in palmitic (16:0) and palmitoleic acids (16:1), while the oil from the seeds contains unsaturated fatty acids of C_{18} type oils, linoleic (18:2) and linolenic acid (18:3). The oil from the seeds and juice also contain Vitamin E and carotene (Bernath and Foldesi 1992, Ma and Cui 1989). Biochemical analysis of Sea buckthorn berries has revealed a wide range of variation in Vitamin C, carotene, flavonoid and Vitamin E concentrations among individual genotypes and populations (Yao and Tigerstedt 1994).

Northern regions of Pakistan including Azad Jammu and Kashmir are hilly with variable climatic conditions. The land holdings are small and the farming is uneconomical. In order to increase the farm area the adjoining forests have been destroyed indiscriminately. Due to deforestation the sloping terrains are becoming vulnerable to soil erosion and land degradation. Multipurpose plants like Sea buckthorn can help greatly to rehabilitate the soils in addition to helping the economic activities of the farmers on a sustainable basis. The present project was therefore undertaken to determine the morphological and biochemical variation in different populations of the Sea buckthorn using biochemical techniques to develop new varieties for better production and efficient harvesting on a commercial scale.

Materials and Methods

The present investigation was carried out on the Sea buckthorn (*Hippophae rhamnoides* L. ssp. *turkestanica*) populations from northern areas of Pakistan. Ten populations of Sea buckthorn were collected from 10 different places in the districts Skardu and Khaplu for comparison. Morphological characters, including plant height, number of main branches per plant, number of sub-branches per main branch of the plant, number of thorns per main branch of the plant, stem girth, plant canopy and the weight of 100 berries, were compared from five randomly selected plants from each of the 10 sites. All the collections were made in September 2001. There were five replications and mean values were taken for analysis using the method given by Steel and Torrie (1980).

Morphological characteristics

1. Plant height
The plant height was measured from the base of the plant to the tip of the tallest shoot of each plant in cm, with the help of a metre rod.

2. Number of branches on the main stem
The number of branches on the main stem of each plant were counted.

3. Number of sub-branches on the main branch
Thr number of sub-branches on the main branch of each plant were counted.

4. Number of thorns on the main branches
The number of thorns on the main branches of each plant were counted.

5. Stem girth
The stem girth of each plant was measured from the base to the start of the branches on the main stem in cm.

6. Plant canopy
The canopy of the plant is the space occupied by the plant. Canopies of the plants were measured with the help of a metre rod from the two outermost branches in cm.

7. Weight of 100 berries
The weight of 100 berries was measured on a digital balance in grams.

Biochemical analysis

1. Ascorbic acid content of berries
Ascorbic acid was determined using the phenol indophenol dye method (AOAC 1984). 10g of the fresh samples were blended with metaphosphoric-acetic acid extracting solution to homogenous slurry. 5ml of the filtrate extract was then titrated with standard indophenol to pink end point. Three replications were taken for each determination. All reagents were analytical grade and purchased from E Merck.

2. Lipid content of berries
Oil contents from the berries/fruits of different populations of Sea buckthorn were extracted in a Soxhlet apparatus (AACC 1983). Samples were dried in an oven at 105°C for 6–12h. 10g of dried sample was used for extraction of oil in a Soxhlet apparatus (30–40°C) for 6h using diethyl ether as the solvent. The solvent was removed under vacuum and the residual oil dried over anhydrous Na_2SO_4. Three replications were taken for each determination. Analytical grade chemicals were used for extraction of the oil.

3. Sterol determinations
Sterol estimation was carried using the Lieberman-Burchard method (Said *et al*. 1995). Samples of 1g oil were made to

10ml with chloroform as the solvent. Samples were stirred till completely dissolved and diluted to 10 times. 3ml of diluted sample solutions were taken and their absorbance was determined on a spectrophotometer after adding 2ml of chloroform and Lieberman-Burchard reagent, containing 0.5ml of sulphuric acid dissolved in 10ml of acetic anhydride. Lieberman-Burchard reagent reacts with the sterol to produce a characteristic green color whose absorbance was determined on a Spectronic 20-D (Milton Roy Company) spectrophotometer at 640nm. 10mg of standard cholesterol was dissolved in 10ml chloroform. Choles-5-en-3-β-ol was used as standard cholesterol whose minimum purity was 95%. The result was drawn by plotting the calibration curve using the different concentrations of sterol against absorbance.

Statistical analyses

All comparisons between the different parameters of the morphological characters were done using ANOVA. The results of the biochemical analyses were expressed as a mean of three determinations ± SD.

Results and Discussion

The mean values for different morphological traits including plant height, number of main branches per plant, number of sub-branches per main branch, number of thorns per main branch, stem girth, plant canopy and weight of 100 berries for 10 populations of Sea buckthorn are compared in Table 1. The Table indicates significant variation ($P \leq 0.01$) among all the traits but one, i.e. stem girth for which the values were non significant. It implies that the populations of Sea buckthorn (*Hippophae rhamnoides* L. ssp. *turkestanica*) from different areas of northern Pakistan vary greatly among themselves. It has been very well reported that the ssp. *turkestanica* is distributed in central and west Asia, including Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, Kirghisistan, Xinjiang province of China, northern India and Pakistan (Rongsen 1992, Ahmad and Kamal 2002). These areas are geographically connected by land but are isolated by high mountain peaks, rivers and variable climatic conditions. During the process of its spread and establishment many changes took place in the genetic make up of the subspecies due to natural selection and adaptation (Ahmad *et al*. 2003). Northern areas of Pakistan also vary in topography and micro-climate, therefore, the variation in different traits among the population could be due to the same physical factors. Such variation in Sea buckthorn populations has also been reported in earlier investigations (Yao *et al.* 1992, Rongsen 1992, Yao and Tigerstedt 1994, Ahmad and Kamal 2002). They described that the fruit color in Sea buckthorn varies from yellow and orange to red in different populations, our observations were also in line with these earlier studies. The plants of Sea buckthorn were found to be extremely variable in height, from a small bush, less than 50cm, to a tree more than 20m high (Rousi 1971, Yao and Tigerstedt 1994). The present study found plant height to range from 75.6–110cm among the populations compared, which is on the low side. Lower plant height may be due to the high altitude (over 3 000m asl) of the northern regions of Pakistan. Yao and Tigerstedt (1995) reported this phenomenon in their studies, where variation of Sea buckthorn in growth rhythm, hardiness and height were recorded according to geographic distribution, i.e. the higher the latitude, the shorter the growth period and plant height. The weight of berries has been observed to vary from 4–60g 100^{-1} berries among genotypes in natural populations, andwas found to exceed 60g in some Russian cultivars (Yao 1994). In this investigation the range was found to be 0.25–2.04g 100^{-1} berries, which is again on the low side. Yao and Tigerstedt (1993) described variable density and sharpness of thorns in Sea buckthorn population as has been observed in this investigation.

Sea buckthorn displays great adaptation to variable climatic conditions, hence occupies diverse regions of the world. The species grow under variable temperatures (–34°C to 55°C, variable soil pHs (5.5–9.5) and drought (Rongsen 1992, Yao and Tigerstedt 1994, Yao and Tigerstedt 1995, Ahmad and Kamal 2002).

When the populations of Sea buckthorn were compared bio-chemically on the basis of Vitamin C, fatty oil and phytosterol content a wide range of variation was observed among the populations (Table 2). The highest concentration of Vitamin C was found in SBT-3 (250mg $100g^{-1}$) while the lowest concentration was observed in SBT-7 (100mg $100g^{-1}$) as shown in Table 2. The concentration of Vitamin C, although highly variable among the populations, as reported previously (Karhu *et al*. 1999), was lower compared to some other studies. Rongsen (1992) reported Vitamin C concentrations of 200–1 500mg $100g^{-1}$ in Sea buckthorn fruits. The carotene content was found to range from 30–40mg $100g^{-1}$ of berries (Bernath and Foldesi 1992, Wolf and Wegert 1993). Vitamin E concentrations as high as 160mg $100g^{-1}$ of berries have been reported (Zhang *et al*. 1989, Ma and Cui 1989, Eliseev 1989). Zhang *et al*. (1989) and Schapiro (1989) also mentioned appreciable amount of water soluble and fat soluble vitamins in Sea buckthorn berries. The lower concentration of Vitamin C in the present investigation may be due to the specific geographic nature of the area, where short reproductive season prevails (Yao and Tigerstedt 1995).

Oil content in the fruits of Sea buckthorn were also found to vary in the different populations compared (Table 2). The highest concentration of oil content was detected in the SBT-4 sample at 4.5g $100g^{-1}$ (4.5%) of the fruit, far greater than any other fruit. The lowest concentration was detected in SBT-6, 1g $100g^{-1}$ (1%) of the fruit. Fatty oil content ranged from 1–4.5%, the highest reported to date. Rongsen (1992) reported oil content of 1.5–3.5% in the fruit pulp. It has previously been reported that the oil from the juice and pulp of Sea buckthorn is rich in palmitic and palmitoleic acids and contains Vitamin E and carotene (Bernath and Foldesi 1992, Ma and Cui 1989). The higher oil concentrations in ssp. *turkestanica* may be very important in its use in medicines.

Phytosterols are plant sterols, with a structures similar to cholesterol, which on consumption are capable of lowering plasma cholesterol in humans. Elevated blood cholesterol is one of the well established risk factors for coronary heart disease, and lowering this indicator can impact on the inci-

Table 1: Comparison of mean values and their standard deviations for some morphological traits in Sea buckthorn populations

Traits	SBT-1	SBT-2	SBT-3	SBT-4	SBT-5	SBT-6	SBT-7	SBT-8	SBT-9	SBT-10	*SL
Plant height (cm)	96.8 ± 16.6	84.6 ± 8.67	96.7 ± 10.13	88.3 ± 7.12	75.6 ± 23.7	81.6 ± 9.61	98 ± 16.04	104 ± 12.9	100 ± 7.9	110 ± 7.9	**
No. main branches per plant	3.6 ± 1.14	3 ± 0.707	2.4 ± 0.54	4.4 ± 0.89	4.4 ± 1.51	6 ± 1.58	3 ± 1.41	5 ± 1.58	6 ± 1.58	4 ± 1.22	**
No. sub-branches per main branch	30.4 ± 2.70	25.8 ± 5.63	37 ± 2.91	42 ± 4.94	12.8 ± 3.4	19.4 ± 5.77	17 ± 2.54	23 ± 4.69	19 ± 4.47	35 ± 4.06	**
Plant canopy (cm)	40.2 ± 8.14	35.2 ± 10.7	43 ± 4.0	49.6 ± 9.61	79.8 ± 23.5	145 ± 36.1	85 ± 3.16	54 ± 2.0	92 ± 2.91	58 ± 2.45	**
No. thorns per main branch	49.4 ± 3.84	69.2 ± 6.46	56.4 ± 4.15	66.8 ± 4.96	65.2 ± 15.5	55 ± 1.58	61 ± 2.91	52 ± 3.16	54 ± 1.58	65 ± 3.67	**
Stem girth (cm)	6 ± 0.71	5 ± 1.0	7 ± 0.61	8.1 ± 1.88	5.5 ± 1.27	9.3 ± 7.51	6.5 ± 0.46	8.5 ± 0.85	5.7 ± 0.33	8.8 ± 0.59	NS
100 berries wgt (gm)	0.25 ± 0.01	0.57 ± 0.02	0.81 ± 0.006	0.49 ± 0.01	3.96 ± 0.03	1.97 ± 0.01	2.04 ± 0.18	0.86 ± 0.03	1.37 ± 0.02	1.2 ± 0.01	**

* SL = Significance level
SBT = Sea buckthorn
** = Significant at 0.01 level of probability
NS = Non significant

Table 2: Comparison of Vitamin C, oil content in berries/fruits and sterol content in the oils of Sea buckthorn populations

Populations	Vitamin C (mg $100g^{-1}$)	Oil (g $100g^{-1}$)	Sterol (% of oil)
SBT-1	170 ± 0.40	4 ± 0.20	1.30 ± 0.20
SBT-2	143 ± 0.16	3.5 ± 0.15	1.60 ± 0.02
SBT-3	250 ± 0.56	3.5 ± 0.23	1.51 ± 0.1
SBT-4	222 ± 0.39	4.5 ± 0.26	1.28 ± 0.01
SBT-5	222 ± 0.40	3.4 ± 0.10	1.61 ± 0.02
SBT-6	217 ± 0.10	1.0 ± 0.17	2.00 ± 0.26
SBT-7	100 ± 0.30	1.5 ± 0.25	1.40 ± 0.10
SBT-8	120 ± 0.17	2.5 ± 0.24	1.35 ± 0.03
SBT-9	200 ± 0.26	3.0 ± 0.17	1.65 ± 0.01
SBT-10	160 ± 0.50	2.8 ± 0.17	1.80 ± 0.17

dence of heart disease (Thurnham 1999). Phytosterols are the major constituents of the unsaponifiable fraction of Sea buckthorn oils. The major phytosterol in Sea buckthorn oil is sitosterol (β-sitosterol), with 5-avenasterol second in quantitative importance. Other phytosterols are present in relatively minor quantities. The total quantity of phytosterol is quite high in Sea buckthorn and may exceed soybean oil by 4–20 times. The sterol content in different varieties ranged from 1.3–2% (Table 2). The highest concentration of sterol was reported in SBT-6 (2%) and the lowest sterol concentration was reported in SBT-1 (1.3%).

Conclusions

This investigation is based on morphological and biochemical characterisation, i.e. the concentration of Vitamin C, fatty oil and phytosterol in berries/fruits of Sea buckthorn *Hippophae rhamnoides* L. ssp. *turkestanica*. The variability among the populations of Sea buckthorn will help to breed better varieties using conventional methods of breeding. The large amount of the oil found in the subspecies will be of commercial importance and will assist the local communities with additional income from the marketing of their farm produce. Due to the high Vitamin C content Sea buckthorn berries can be used for in the production of fruit juices and other beverages. As Sea buckthorn oil is a concentrated source of phytosterols, which compete with the absorption of cholesterol in body, use of Sea buckthorn oil will decrease the incidence of heart diseases.

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