

Genetic Diversity in Pakistani Genotypes of *Hyppophae rhamnoides* L. ssp. *turkestanica*

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ABSTRACT

The present investigation was conducted to identify the potential genotypes of Seabuckthorn for breeding better varieties in terms of fruit yield, fruit quality, nitrogen fixing ability and better adaptation in various mountain ecologies of Pakistan. The initial studies included the collection of basic information regarding the cultivation and genetic diversity of Seabuckthorn among the local genotypes with morph-molecular tools consisting of phenotypic characters and the genotypic characters using SDS-PAGE. The phenotypic as well as genotypic studies revealed great variation among the local genotypes with immense potential for future improvement even using conventional breeding techniques. However, in order to improve its overall potential including: nutritional, medicinal and nitrogen fixing qualities and to breed high yielding and thornless varieties, use of molecular techniques would be more meaningful.

Key Words: Seabuckthorn; *Hyppophae rhamnoides* ssp *turkestanica*; Genetic diversity; Genotypes

INTRODUCTION

Seabuckthorn belongs to Elaeagnaceae family constituting six species. Among them, important species is *Hyppophae rhamnoides* L., which comprises of 10 sub species. The only sub species found in the Northern Areas of Pakistan is *turkestanica*. According to the worldwide distribution of *Hyppophae*, *turkestanica* is widely found in the Central and the Western Asia. Northern Areas of Pakistan are just in center of the distribution of this sub species. Seabuckthorn normally starts fruiting at 3 to 4 years of age. A typical plant is usually bearing clusters of juicy fruits, rich in nutrients, such as, carbohydrates, organic acids, amino acids and vitamins. Another feature of Seabuckthorn fruit is its oil; which is extracted both from fruit and seeds. Fruit contains 60 to 80% juice with vitamin C ranging from 200 to 1500 mg/100g which is 5 to 100 times higher than any other known fruit or vegetable. The oil contents range from 1.5 to 3.5% in fruit pulp and 9.9 to 19.5% in seeds. The oil has much more quantity of B-carotene and vitamin E, therefore, is an effective medicine for many diseases. The fruit also contains more than 100 types of nutrients and bioactive substances and more than 22 minerals. High quality wines, jams, jelly's, squash, juice powder, butter, ferments, tea and other healthful foods and syrups, are some main products, prepared from the fruit of Seabuckthorn (Rongsen, 1992). Presently, there are over 200 Seabuckthorn processing factories producing over 100 types of products in China (Rongsen, 1998). Russians and Tibetans prepare drugs from Seabuckthorn oil for various diseases including inflammation, bacterial infections, pain, promising regeneration of tissues and for skin grafting,

cosmetology, operational treatment of corneal wounds, oedema, fever, chill, furuncle, abscess, obstruction by sputum and stomach tumour (Anonymous, 2001). The drugs from Seabuckthorn products produced by Chinese because of their potency in curing heart diseases are very well received internationally (Rongsen, 1998). Similarly, treatment of 30-40 persons having diseases like hepatitis, cancer and ulcer with Seabuckthorn oil has been reported from Pakistan (Shigri, 2001).

Seabuckthorn is also important for fuel wood, fencing, fodder, soil fertility and shelter purposes. It has been reported that a five years old plant can check 90% run-off water and 95% soil erosion. The newly emerged soft twigs and leaves are used as fodder for animals. An adult Seabuckthorn plant can withstand temperature ranging from 55-43°C, and can grow well at pH range from 5.5-9.5. It can withstand atmospheric drought at an annual rainfall of 250-800 mm. The plant has very strong root system. Five years old plant has taproots of up to 1.1 m deep and horizontal roots of up to 2.58 m wide. A symbiotic micorhizal fungus, *Frankia*, has been found on Seabuckthorn roots, which form nodules and fixes maximum amount of nitrogen present in the atmosphere. Its capacity to fix nitrogen is twice than that of soybean (Rongsen, 1992).

The mountainous regions of Pakistan including Azad Kashmir and Northern Areas are at high risk of erosion because of the loss of forests and changing climatic conditions. The holdings in this region are very small and economic conditions of farmer are very poor. Reforestation of pines and other slow growing plants is generally not a suitable option. The use of quickly growing vegetation has

been perceived as a major and most promising tool to control land degradation under different ecological conditions especially mountains (Ahmad & Chauhdry, 1995). Seabuckthorn is one of the species successfully used on a large scale, particularly in Northern China to control desertification, to conserve land and water resources, and to integrate economic exploitation with ecological rehabilitation (Anonymous, 2002). The plant, therefore, can also be utilized for the ecological and economic development of mountainous regions of Pakistan including Northern Areas and Azad Jammu & Kashmir.

Genetic diversity is the prerequisite tool for breeding and varietal improvement in plants. Morphological characterization is a conventional technique used for evaluating the plant genetic diversity, whereas molecular techniques are relatively new but more effective in this context. The techniques were employed to evaluate variability in *H. rhamnoides* genotypes with varying morphological traits. Seabuckthorn berries have attractive colors, varying from yellow, orange to red. The size of berries varies from 4-60 g/100 berries among genotypes in natural populations, and exceeds 60 g in some Russian cultivars. Seabuckthorn shows diverse fruit shapes, from flattened spherical, cylindrical, ovate, elliptic to many irregular shapes. Normally, Seabuckthorn has terminal and lateral thorns. Observations show large variation in terms of density, shape and sharpness in natural populations. The plant height and other characters are also found to be variable in different genotypes (Yao & Tigerstedt, 1995; Yao & Tigerstedt, 1994). Molecular/Biochemical techniques are also routinely used these days for determining the genetic variability among the species, genotypes and the population (Moller & Spoor, 1993; Ashour *et al.*, 1995). Waines and Payne (1987) analysed glutenin through SDS-PAGE in the A genome of 497 diploid wheat and in 851 landraces of bread wheat. Ciaffi *et al.*, (1993) used SDS-PAGE by studying 315 populations to identify markers for variability. Yao and Tigerstedt (1993) used isozyme analysis to identify variability in Seabuckthorn genotypes. DNA based RAPD (Random Amplified Polymorphic DNA) variation was recently studied in 10 populations of *H. rhamnoides* in Mongolica to study population structure and to find a marker linked to gender determination (Jeppsson *et al.*, 1999). The techniques are quick and accurate to identify the variability in natural populations of the plants, especially to evaluate the wild germplasm for its use in the development of economically viable varieties. Research on Seabuckthorn started in Siberia in the 1930s (Kalinina & Panteleyeva, 1987). Breeding projects have, later on, been initiated also in Germany (Albrecht, 1990), Finland (Yao & Tigerstedt, 1994), China (Huang, 1995), Canada (Li & Schroeder, 1996) and Sweden using conventional-breeding methods, including germplasm evaluation, hybridization and selection (Trajkovski & Jeppsson, 1999). In Pakistan, the studies for evaluating the potential of Seabuckthorn started in 1996,

however, the present investigation was of its kind to initiate systematic improvement program. Main objective was to evaluate the genetic diversity among genotypes and land races of Seabuckthorn from Pakistan using conventional and molecular techniques for the development of suitable varieties.

MATERIALS AND METHODS

The investigation was carried out using four genotypes of Seabuckthorn ssp. *turkestanica* found in Baltistan during the year 2001. The four genotypes were designated as SBT-01, SBT-02, SBTP-03, and SBT-04 on the basis of fruit morphology i.e. color and size of the fruits. The genotypes were investigated for morphological and genetic diversity. Five comparable plants from five different localities were randomly selected for each genotype. The average values were calculated for each character using different replications.

Morphological characters investigated were: plant height, number of main branches per plant, number of sub branches per main branch of plant, number of thorns on main branch of each plant, number of fruits on main branch of each plant, plant canopy and stem girth in each genotype. The data were analyzed using computer based statistical program.

Molecular/Biochemical investigations were carried out by comparing total seed proteins in SDS-PAGE. Total seed proteins were extracted by using an established method routinely used in Plant Breeding & Genetics laboratory of University College of Agriculture, Rawalakot. Preparation and polymerization of gels and electrophoresis was carried out by a little modification in the standard method given by Laemmli (1970). Photograph of gels were taken after staining and destaining gels for reference. The distance covered by different protein bands were closely visualized on a light box. The pattern was also drawn on a paper for later reference. Comparisons were made between the common and variable protein-banding pattern of four genotypes to evaluate genetic variability and relatedness among genotypes.

RESULTS AND DISCUSSION

Morphological as well as the genetical investigations were carried out to determine the variability that exists among the different genotypes of Seabuckthorn sub sp. *turkestanica*. The genetic diversity in the genotypes of the sub sp. *turkestanica* would mean the variability in the chemical constituents of various natures including nutritional, medicinal, and nitrogen fixing ability of a multipurpose plant.

Morphological characters including number of main branches per plant, number of sub-branches per main branch of plant, number of berries per main branch, number of thorns per main branch and plant girth were investigated to

see whether any diversity exists amongst the genotypes (Table I). The plant height although was variable in different genotypes but was not found to be significant statistically. Similarly, the characters like the plant canopy were quite variable but did not show significant difference among the genotypes compared. Comparisons between the numbers of branches per plant among different genotypes indicate significant difference among the genotypes. When number of sub-branches per main branch of each genotype were compared the difference was found to be highly significant. Similar results were obtained when girth of plant was compared among the genotypes. The characters like the number of berries per main branch from each genotype were also compared. Highly significant differences were found in these characters among the Seabuckthorn genotypes. Likewise, the fruit color and fruit size was also found to be variable in different genotypes compared. Such variations in fruit color, size and shape were also reported by Yao (1994). The results were highly significant in most of the characters compared among the genotypes except the plant height and plant canopy (Table II). It would mean that immense variation exists among the natural genotypes of Seabuckthorn, which are indicative of the variability in the genotypes found under somewhat similar environmental conditions. The variation, therefore, may not be due to environment only. Similar variations in fruit color, size and shape as well as in presence of thorns, plant height and other characters have been reported among the genotypes of Seabuckthorn in Chinese part and elsewhere (Yao & Tigerstedt, 1994; 1995). It has also been indicated that the phenotypic characters like fruit size and color may not be the measurement of genetic variability always (Rongsen, 1992).

In order to see the variability at genetic level, SDS-PAGE banding pattern of the gel using total seed protein was also investigated. When total seed proteins extracted from seeds of different genotypes were compared (Table III), diversity in most of the bands was indicated by their movement in the gel. In the genotype, SBT-01, five bands were found at the distances of 1.5, 3, 5.3, 6 and 6.3 cm. In SBT-02, seven bands were at the distances of 1.5, 3.5, 4, 4.6, 5.3, 5.8 and 6.8 cm. Whereas, in SBT-03, six bands at the distances of 1, 4, 4.5, 4.9, 5.4 and 6.3 cm were observed and in SBT-04, five bands at the distances of 0.9, 4.3, 4.6, 5.1 and 6.1 cm were observed. Only one band at 5.5 cm was found to be common in all the genotypes compared. In the genotypes SBT-01 and SBT-02, two bands were common at 1.5 and 5.3 cm apart from a shared band in all genotypes. In SBT-01 and SBT-03 one band at 6.3 cm was common, but in SBT-01 and SBT-04 no band was common except the one shared by all genotypes. In the genotypes SBT-02 and SBT-03, two bands at 4 cm and 5 cm were common while in SBT-04 a single band at 5 cm was observed in common to SBT-02 apart from the common band of 5.5 cm shared by all genotypes.

Table I. Average value of some phenotypic parameters in four genotypes of *Hippophae rhamnoides* ssp. *turkestanica*

Genotypes	SBT01	SBT02	SBT03	SBT04
Plant Height	97.44	85.92	96.72	88.32
No. of Main Branches/Plant	3.60	3.00	2.40	4.40
No. of sub-Branches/Plant	30.40	25.80	37.00	42.00
Plant Canopy	40.20	35.20	43.00	49.60
No. of barriers/ Main Branch	642.60	584.60	711.40	756.40
No. of Thorns Main Branch	49.40	62.40	56.40	66.80
Plant Girth	6.00	5.00	7.00	8.10

Table II. Mean Squares of various parameters corresponding to genetic variation in *Hippophae rhamnoides* ssp *turkestanica* genotypes

SOV	DF	NBP	NSBP	Stem Girth (cm)	NBMB	NTMB
Blocks	4	0.32	11.92	3.057	11.92	22.37
Genotypes	3	3.65*	225.07*	8.84*	225.06*	285.11*
Error	12	0.858	20.02	0.79	20.02	25.41

NBP= Number of Branches/ Plant; NSBP= Number of sub-Branches/ Plant; NBMB= Number of berries/Main Branch; NTMB= Number of Thorns/Main Branch; * = Significant at 5% level

In the genotypes SBT-03 and SBT-04, maximum bands (three bands) were shared at 1cm, 4.5 and 5 cm apart from the common band of 5.5 cm shared by all genotypes compared.

Table III. Comparison of fractionation pattern (total seed proteins) in SDS-PAGE from four genotypes of *Hippophae rhamnoides* ssp *turkestanica*

Distance traveled (Cm)	SBT-01	SBT-02	SBT-03	SBT-04
0.5				
1.0			+	+
1.5	+	+		
2.0				
2.5				
3.0	+			
3.5		+		
4.0		+	+	
4.5			+	+
5.0		+	+	+
5.5	+	+	+	+
6.0	+	+		
6.5	+		+	+
7.0		+		

The variability in SDS-PAGE profile indicated genetic diversity in Seabuckthorn genotypes from Pakistan to a large extent, however, their inter-relationship was also established by sharing at least one common band among all. Such seed proteins variability in SDS-PAGE is very frequently used to identify the genotypes/populations of various plant species (Ciaffi *et al.*, 1993; Yong *et al.*, 1995).

CONCLUSIONS

The results indicated the importance of the natural germplasm of Seabuckthorn, which need to be included in any program to develop suitable varieties of the plant for economic activity and commercial exploitation in mountainous regions of Pakistan. The work was first activity of its kind in Pakistan; however, these preliminary results are demanding further investigation to enhance the quality and production of such valuable plants and their commercial products.

REFERENCES

- Ahmad, S.D. and M.B. Chaudhry, 1995. Development of Grass-Legume Mixture A Potential Source of Sustainable Development of Agriculture in Hilly Areas of Pakistan. *Sci. Tech. Dev.*, 14: 15–22.
- Albrecht, H.J., 1990. Sortenentwicklung bei Sanddorn. *Gartenbauwissenschaft*, 37: 207–8.
- Anonymous, 2001. History of Seabuckthorn in China. International Centre for Research and Training on Seabuckthorn. <http://www.icrts.org>.
- Anonymous, 2002. Seabuckthorn in China. International Centre for Research and Training on Seabuckthorn <http://www.icrts.org>.
- Ashour, A.A., H.A. Taha and A.E.H. Muhammad, 1995. Comparative SDS-PAGE protein patterns of four ascarided Nematodes. *J. Egyptian. Soc. Parasitol.*, 25: 761–7.
- Ciaffi, M., D. Lafiandra, E. Porceddu and S. Benedettelli, 1993. Storage protein variation in wild emmer wheat (*Triticum turgidum* ssp *dicoccoides*) from Jordan and Turkey. Electrophoretic characterization of genotypes. *Theor. Appl. Genet.*, 86: 474–80.
- Huang, Q., 1995. A review of Seabuckthorn breeding in China. *Proc. Int. Workshop on Seabuckthorn, Beijing, China*, p. 111–7.
- Jeppsson, N., I.V. Bartish, and H.A. Persson 1999. Reprinted from: Perspectives on new crops and new uses. 1999. J. Janick (ed.), ASHS Press, Alexandria, VA.
- Kalinina, I.P. and Y.I. Panteleyeva, 1987. Breeding of Seabuckthorn in the Altai. In: *Advances in Agricultural Science*. Moscow, Russia.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680–5.
- Li, T.S.C. and W.R. Schroeder, 1996. Seabuckthorn (*Hippophae rhamnoides* L.): A multipurpose plant. *Hort. Technol.*, 6: 370–80.
- Moller, M. and W. Spoor, 1993. Discrimination and identification of *Lolium* species and cultivars by rapid SDS-PAGE of seed storage proteins. *Seed Sci. Tech.*, 21: 213–23.
- Rongsen, Lu, 1992. "Seabuckthorn a multipurpose plant for Fragile Mountains", ICIMOD occasional paper no. 20 Kathmandu, Nepal. pp: 6-7: 18–20.
- Rongsen Lu, 1998. Seabuckthorn production in Western Sichuan, China and the analysis of its development and constraints to its development. In: MIE Series No. 98/7, chapter 2&3.
- Shigri, G.N., 2001. "Seabuckthorn" General introduction. NADRI sub-station Skardu. pp 7-12.
- Trajkovski, V. and N. Jeppsson, 1999. Domestication of seabuckthorn. *Botanica Lithuanica Suppl.*, 2: 37–46.
- Waines, J.G. and P.I. Payne, 1987. Electrophoretic analysis of the high molecular weight glutenin subunits of *T. monococcum*, *T. uratu*, and the A genome of bread wheat (*T. aestivum*). *Theor. Appl. Genet.*, 74: 71–6.
- Yao, Y. and P.M.A. Tigerstedt, 1995. Geographic variation of growth rhythm, height and hardiness and their relations in *Hippophae rhamnoides*. *J. Amer. Soc. Hort. Sci.*, 120: 691–8.
- Yao, Y. and P.M.A. Tigerstedt, 1994. Genetic diversity in *Hippophae* L. and its use in plant breeding. *Euphytica*, 77: 165–9.
- Yao, Y. and P.M.A. Tigerstedt, 1993. Isozyme studies of genetic diversity and evolution in *Hippophae*. *Genetic Res. Crop Evol.*, 42: 153–64.
- Yong, Z.X., Y. Xin Ming, D. Yus Shen, 1995. Genetic analysis of wheat germplasm by poly acrylamide gel electrophoresis of gliadin. *Scientia Agriculture Sinica*, 28: 25–32.

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