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REVIEW

Biotechnological interventions in sea buckthorn (*Hippophae* L.): current status and future prospects

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Abstract Sea buckthorn (*Hippophae* L., Elaeagnaceae) is an economically and ecologically important medicinal plant comprising of species which are winter hardy, dioecious, wind-pollinated multipurpose shrubs bearing yellow or orange berries with nitrogen-fixing ability. It grows widely in cold regions of Indian Himalayas, China, Russia, Europe and many other countries. It is commonly known as 'cold desert gold' due to its high potential as a bio-resource for land reclamation, reducing soil erosion and its multifarious uses. The wild populations are being used for harvesting economic benefits with negligible plantation efforts. Although this plant has many excellent traits, it is still in an early phase of domestication. This woody plant is prone to many pests and diseases which destroy the plants and halt its commercial production. Limited progress has been made for improvement of sea buckthorn through breeding programs due to long juvenile period and lack of QTL linkage map, which makes screening of mapping populations a time-consuming and labor-intensive task. Conventional propagation methods, i.e. seeds, softwood and hardwood cuttings, and suckers are in place but are cumbersome and season dependent. Therefore, application of modern tools of biotechnology needs to be standardized for harnessing maximum benefits from this nutraceutical plant. Improvement of this genus through genetic transformation requires an efficient regeneration system, which is yet to be standardized. Taxonomic status of the genus is controversial and requires more inputs. Taxonomic delineation of species and subspecies and also the breeding programs can be more robustly addressed using molecular markers. This review summarizes the progress made and suggests some future directions of research for this important fruit species.

Keywords Breeding · Characterization · Crop improvement · *Hippophae* · Micropropagation · Nutraceutical value · Taxonomy

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Introduction

Genus *Hippophae* ($2n = 24$), commonly known as sea buckthorn or Leh berry (India; as it grows in Leh region in Trans Himalayas) or Siberian pineapple (Russia; due to its taste and juiciness), is a shrub/small tree of the family Elaeagnaceae. The name is derived from its habit of growing near the sea, and from the possession of many spines or thorns that are reminiscent of some buckthorn species (of the genus *Rhamnus*). In ancient Greece, sea buckthorn leaves and young branches were added to the

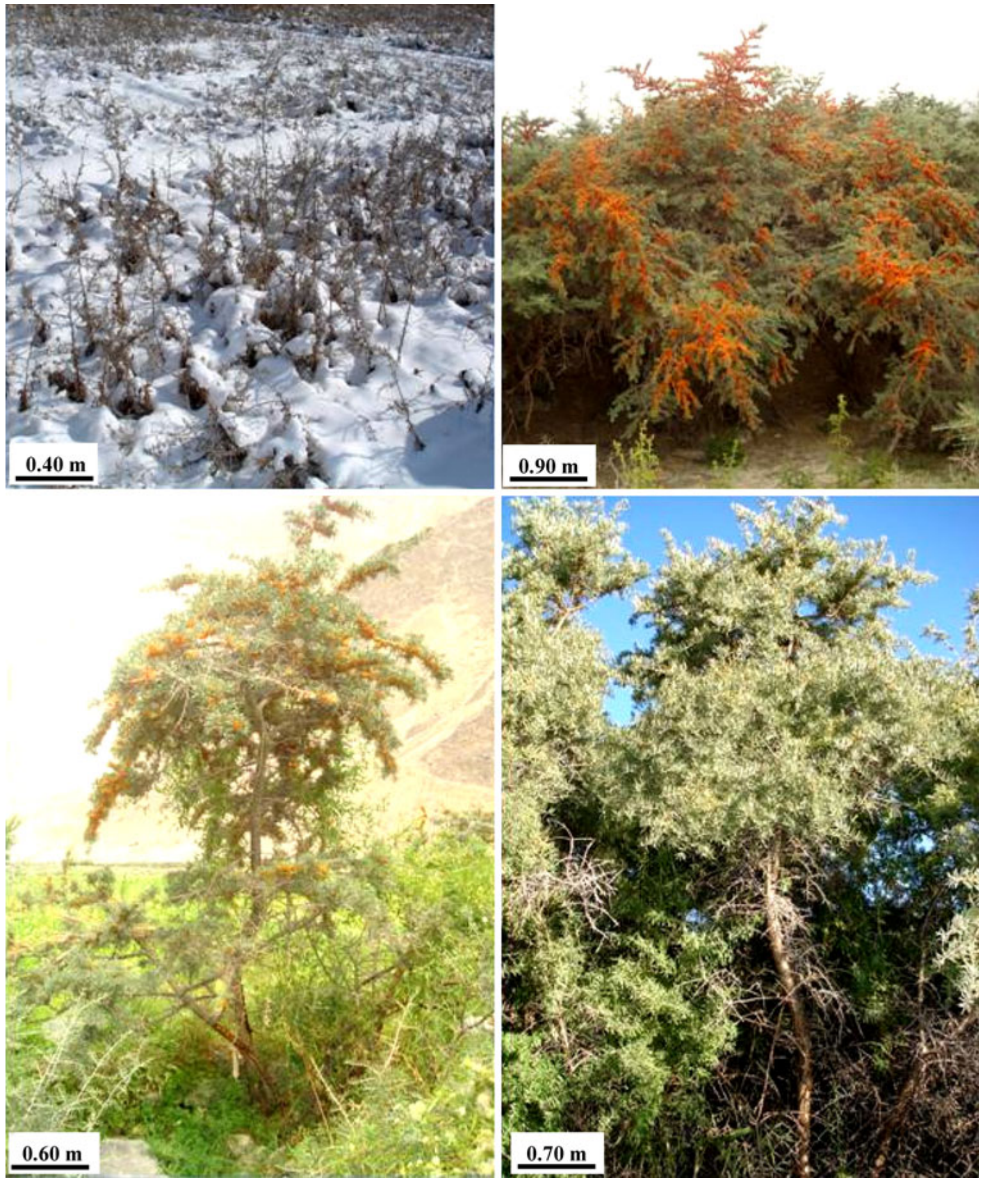

Fig. 1 Variation in *Hippophae rhamnoides* ssp. *rhamnoides* habitat—small bush, hedge, shrub and tree

fodder for horses which resulted in rapid weight gain and a shiny coat for the horse. This, in fact, gave the generic name to the plant, in Latin '*Hippo*' means horse and '*phaos*' means to shine (Rongsen 1992).

Sea buckthorn is a fast-growing, frost-, wind- and heat-resistant plant. It is highly adaptable to extreme climatic conditions, including temperature ranges of −43 to +40°C, drought, high altitude, salinity, alkalinity and inundation (Ruan and Li 2002). The genus *Hippophae* is distributed between 27–69° N latitude and 7°W–122°E longitude (Rousi 1971). This fascinating plant having multifarious and multidimensional uses has worldwide distribution. However, in this genus, only *H. rhamnoides* has an extremely wide distribution throughout the Indian Himalayas but occur fragmentally in Europe and Asia, from China, Mongolia, Russia, Kazakistan, Turkey, Romania, Switzerland, Germany, France and Britain, and northwards to Finland, Sweden and Norway (Rousi 1971; Zeb 2004). The remaining species in the genus have a rather limited distribution and occur only in China and some neighboring countries along the Himalayan Mountains (Rousi 1971; Liu and He 1978; Lian 1988).

Mature plants of sea buckthorn are extremely variable in height, from a small bush less than 50 cm to a tree more than 20 m high (Fig. 1, Rousi 1971; Zeb and Khan 2008a). It is an air-pollinated dioecious species with male and female flowers on separate trees. Gender of sea buckthorn seedlings can be determined only during flowering. Flower buds are differentiated during the previous growing season, so the number of fruits produced in any one year depends on the growing conditions of the preceding year. The staminate and pistillate flowers are inconspicuous appearing

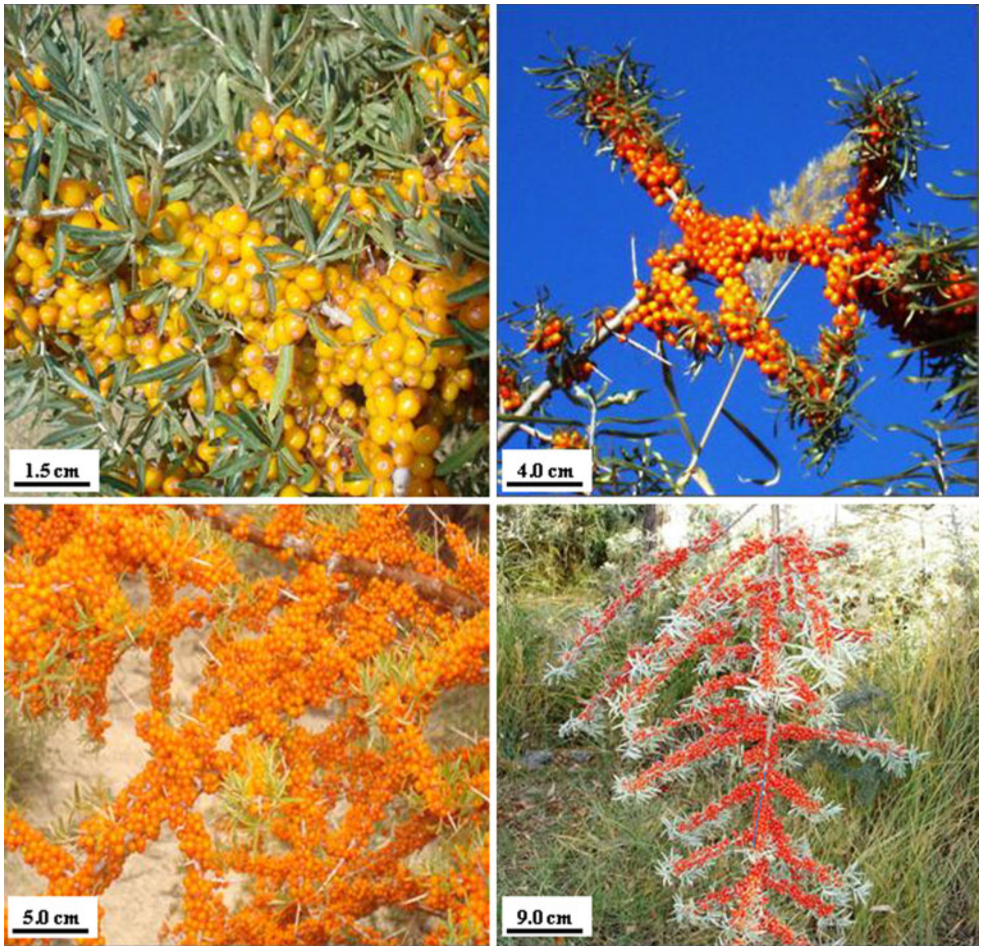

Fig. 2 Variation in color of mature berries (*light yellow to red*) in *Hippophae rhamnoides* ssp. *rhamnoides*

before the leaves. The berries have attractive colors, varying from yellow, orange to red (Fig. 2). The size of berries varies, and the weight ranges from 4 to 60 g/100 berries among genotypes in natural populations, and exceeds 60 g in some Russian cultivars (Yao 1994). Sea buckthorn berries remain on the bushes throughout the winter season due to lack of abscission layer. The fruits have a distinctive sourish taste and a unique aroma reminiscent of pineapple.

Economic importance

The multifarious medicinal values of sea buckthorn were known as early as 5000 BC, as mentioned in the ancient classic Indian system of medicine. The medicinal properties of this wonder plant were recognized by Tibetan doctors as early as the eighth century as mentioned in the Tibetan medical classic '*rGyud bzhi*'. In modern times, however, these came into light only in the twentieth century. The sea buckthorn industry is thriving in Russia since 1940 (Li 2002) and health products for cosmonauts were prepared in 1960–1970s in Russia. Sea buckthorn got further world attention in 1986, when its fruit oil was used for protecting people from radiation leak due to explosion in a nuclear plant at Chernobyl, Ukraine (Singh 2008). Hundreds of products have been developed from the berries, oil, leaves and bark extracts of sea buckthorn. Processed products include oil, juice, alcoholic beverages, candies, ice-cream, tea, jam, biscuits, vitamin C tablets, food colors, medicines, cosmetics and shampoos (Wu 1991). Cosmetic applications for sea buckthorn include moisturizing, dandruff control and hair loss prevention lotions, anti-aging skin creams and lotions, and sun care cosmetics (Parimelazhagan et al. 2004).

The most important pharmacological functions attributed to sea buckthorn oil are anti-inflammatory, antimicrobial, pain relief, and promoting regeneration of tissues (Li 1999). Seed oil contains vitamin K (1.1–2.3 mg g^{-1}) which promotes blood coagulation because of its catalytic role in forming prothrombin (Rongsen 1992). Studies have indicated its potential in the treatment of AIDS also (Shuunguang Lu and Chaode Ma 2001). Exceptional nutrient content and antioxidant qualities have given it the commercial status of a novel 'super-fruit'.

Table 1 Biochemical characterization of *Hippophae* L.

Species	Germplasm collected from	Achievements	References
H. rhamnoides L.	Eastern Anatolia	Examined biochemical relationships between ten selected genotypes	Ercisli et al. (2008)
	Pakistan	Analyzed palmitic, palmitoleic, stearic, oleic, vaccenic, linoleic and α and γ-linolenic acids in seed and pulp	Abid et al. (2007)
	India	Analyzed nutritional value, biochemical constituents (acidity, fat, lignin, carbohydrate, reducing sugar, starch, protein, etc.) and mineral components from fresh berries and seeds	Dhyani et al. (2007)
	China	Isolated four monomeric flavan-3-ols: catechin, epicatechin, gallocatechin and epigallocatechin, along with two dimeric procyanidins, catechin (4α-8) catechin and catechin (4α-8) epicatechin from seed	Fan et al. (2007)
	Canada	Identified campesterol, clerosterol, lanosterol, sitosterol, β-amyrin, sitostanol, Δ^5-avenasterol, $\Delta^{24(28)}$-stigmasta-en-ol, α-amyrin, $\Delta^{5,24(25)}$-stigmastadienol, lupeol, gramisterol, Δ^7-sitosterol, cycloartenol, cycloeucalenol, Δ^7-avenasterol, erythrodiol, 28-methylobtusifoliol, 24-methylenecycloartanol, citrostadienol, uvaol and oleanol aldehyde from seed oil	Li et al. (2007b)
	Finland	Analyzed sugars (d-fructose, D-glucose, ethyl-D-glucose), fruit acids (malic, quinic, and ascorbic acids), vitamin C and pulp oil in different varieties (chuiskaya, avgustinka, raisa, botanicheskaya, trofimovskaya)	Tiitinen et al. (2005, 2006)
	Turkey	Analyzed essential oil and fatty acid composition of the fruits	Cakir (2004)
	Russia	Compared vitamin C content of two Russian cultivars (Ruet and Dar Katuni)	Kallio et al. (2002)
	Uzbekistan	Analyzed carbohydrates and related compounds in certain species of Elaeagnaceae family	Bekker and Glushenkova (2003)
	China	Detected 18 free amino acids in fruit juice	Chen (1988), Tong et al. (1989)
H. rhamnoides, *H. salcifolia*, *H. tibetana*	India	Studied fatty acids, tocols and carotenoids in the pulp oil	Singh et al. (2006)
H. rhamnoides ssp. *sinensis*	China	Analyzed sugar (glucose, fructose, xylose), vitamin C and organic acids (L-malic acid, D-malic acid, citric acid, tartaric acid, succinic acid)	Ma and Cui (1989)
	China	Analyzed vitamin C, L-malic acid, 18 free amino acids and 10 mineral elements	Zhang et al. (1989)
H. rhamnoides ssp. *turkestanica*	Pakistan	Evaluated physiochemical parameters (ash, moisture and vitamin C content, thousand seed mass) and elemental components of seed.	Zeb and Malook (2009)
	Pakistan	Estimated ascorbic acid, vitamin C, oil content, minerals, iron and phosphorus content	Shah et al. (2007)
	Pakistan	Compared mineral composition and nutritive value (anthocyanins, vitamin C and pytosterol content of seed oil) of eight populations	Sabir et al. (2005)
	Pakistan	Compared ten populations for biochemical composition	Sabir et al. (2003)

Sea buckthorn berries are among the most nutritious and vitamin-rich fruits found in the plant kingdom. The wide chemical and phytochemical composition of sea buckthorn has been reviewed recently (Zeb and Khan 2008b; Tiitinen et al. 2005). In general, the flesh of berries contains a diverse complex of vitamins: A, B, C, D, E, F, K and P; mineral substances such as sodium salts, potassium, calcium; carbohydrates, sugars, proteins, amino acids, organic acids, pectins and tannins; triterpenoids, phospholipids, caumarin, catechins, leucoenthocyans, flavonols, alkaloids, serotonin as well as unsaturated fatty acids and other compounds (Abid et al. 2007; Fan et al. 2007; Li et al. 2007a). Apart from being nourishing, the juice has a freezing point of −22°C allowing it to remain liquid even at sub-zero temperatures. The leaves are an equally rich source of important antioxidants including beta carotene, vitamin E, flavonoids, catechins, elagic acid, ferulic acid, folic acid and significant values of calcium, magnesium and potassium. In addition to its carotenoid and vitamin E content, the oil from the sea buckthorn berry contains on average 35% of the rare and valuable palmitoleic acid (16:1n-7; omega-7 series fatty acid) (Singh et al. 2006). This rare fatty acid is a component of skin fat and is known to support cell, tissue and wound healing. The various reports regarding biochemical characterization of nutraceutical compounds in sea buckthorn are summarized in Table 1.

The aggressive root system of sea buckthorn binds the soil together, prevents erosion and gets it ready for other plants. The wide adaptation, fast growth, strong coppicing and suckering habits coupled with efficient nitrogen fixation ability make sea buckthorn well adapted for soil conservation, soil improvement and marginal land reclamation. It has a highly efficient relationship with actinomycete fungus *Frankia*, (Akkermans et al. 1983). Study conducted by Kumar and Sagar (2007) revealed the presence of 26 fungal species in its rhizosphere. These fungal endophytes (i.e. *Aspergillus niger*, *Glomus fasciculatum*, *Glomus macrocarpum* and *Gigaspora margariata*) have also been isolated from different plant parts (root, stem, leaves and bark) and soil samples, respectively.

Problems to be addressed in sea buckthorn

In spite of being an economically and ecologically important plant, sea buckthorn is in an early phase of domestication and limited information is available regarding the genus. Some important aspects which need to be addressed immediately are summarized.

Taxonomic status of the genus

All the species of the genus *Hippophae* are native to Eurasia and are called sea buckthorns. But exactly how many species are there is still unclear. The classification of genus *Hippophae* has been modified over the years. Originally, it consisted of only one species, *H. rhamnoides*, with three sub species, *rhamnoides, salicifolia* and *tibetiana* (Servettaz 1908). Rousi (1971) re-classified this genus and recognized three species, *H. rhamnoides* L., *H. salicifolia* D. Don, and *H. tibetana* Schlecht., based on morphological variations while Liu and He (1978) added the fourth species *H. neurocarpa* Liu and He, to the list given by Rousi. The most widespread, *H. rhamnoides* has been divided into nine geographically separated subspecies *carpathica, caucasica, gyantsensis, mongolica, sinensis, turkestanica, yunnanensis, rhamnoides* and *fluviatilis* (Rousi 1971) but some specialists think that some of these deserve the rank of species. Racial divergence does exist, even within each subspecies (Rousi 1971; Li and Beveridge 2003). Lian (1988) upgraded *H. rhamnoides* ssp. *gyantsensis* to an independent species, while Lu (1999) introduced a sixth species, *H. goniocarpa.* Lian et al. (2003) described a new subspecies for *H. rhamnoides* (*H. rhamnoides* subsp. *wolongensis*). Hybrid origin of *H. goniocarpa* has been confirmed using molecular markers (Wang et al. 2008a; Bartish et al. 2002; Sun et al. 2002).

Avdeev (1983) and Mathew et al. (2007) described that the genus *Hippophae* is divided into two sections, Hippophae and Gyantsenses. Each has three important species in commercial terms. Section Hippophae has *H. salicifolia, H. rhamnoides* (with 8 subspecies) and *H. goniocarpa* (with 2 subspecies), and section Gyantsenses includes *H. gyantsensis*, *H. neurocarpa* (with 2 subspecies) and *H. tibetana*. However, Li (2003) has reported that the genus *Hippophae* is composed of 6 species and 12 subspecies. On the contrary, according to Bartish et al. (2002); Swenson and Bartish (2002) and Sun et al. (2002, 2003) this genus has 7 species and 8 subspecies. Bartish et al. (2002) showed that the origin of *H. goniocarpa* is not monophyletic and suggested that the two subspecies, ssp *goniocarpa* and ssp *litangensis*, probably originated from different hybridization events: therefore, they upgraded the two subspecies into independent species. The precise classification of the genus is still debatable due to the variations found in the Himalayas and the adjacent areas of Central Asia where the primary differentiation of the genus took place (Rousi 1971).

In spite of many molecular, taxonomic and phylogenetic studies, which were made to address the taxonomic problems, the taxonomic and phylogenetic status of *H. rhamnoides* is still not clear and more robust data need to be generated so that taxonomic and phylogenetic revision of the taxon can be done precisely.

Biotic and abiotic stresses

Though sea buckthorn is a multipurpose and vital species for mountain-rural poor, it is one of the underutilized and unexplored plant species. Vulnerability of the sea buckthorn genotypes to a multitude of biotic stresses has restricted their potential yield. The high mountain areas where sea buckthorn natural stands are gregariously found face severe problems (Gupta et al. 2000). The regeneration of the plant is poor due to abiotic factors like extreme cold, human interference, glacial floods, high-velocity wind and improper harvest of the plant. The sea buckthorn forest is also depleting due to fire and open access to grazing and cutting. Biotic factors including susceptibility to a multitude of pests and pathogens further diminish the yield and quality of sea buckthorn.

Diseases and insects/pests which affect almost every stage/part of the sea buckthorn are the major factors affecting its cultivation. At present, few pests and diseases of sea buckthorn have been reported; however, more are likely to be identified as the number of plantations grow. The most damaging insects include green aphids (*Capitophorus hippophae*), leaf roller (*Arhips rosana*), gypsy moth (*Ocneria dispar*), gall tick (*Vasates* spp.), comma-shaped scale (*Chionaspis salicis*), sea buckthorn fly (*Rhagoletis batava*) and caterpillars of moth *Gelechia hippophaeella*. The insect infestations reported from India include death hawk moth (*Acherontia styx*), some defoliating beetles (*Brahmina* spp) and others such as *Holotrichia longipennis* and *Plodia interpunctella* which cause damage at early growth and fruiting stages (Li 2003). In addition, damage to sea buckthorn is caused by deer, birds, mice, rats, pocket gophers and other rodents (Li 2002). The carpenter moth (*Holocerus hippophaecolus*), a wood-boring pest has recently become a major threat to sea buckthorn in China (Zong et al. 2008). Three main species of the root pests are *Holotrichia oblita*, *Pleonomus* sp. and *Gryllotalpa unispina*. The flower and leaf pests include *Yponomeuta* sp., *Malacosoma neustria, Testacea motschulsky* and *Hippophaetrizoa* sp while *Asias haladendri* Pallas and *Cossus* L., are the two stem pests which attack the weak plants and cause up to 93% of stem death (Ming et al. 2008). There are about 72 species belonging to 23 families and 7 orders of pests found in China that cause damage to the sea buckthorn (Luo et al. 2008).

The major fungal diseases reported on sea buckthorn include verticillium wilt, fusarium wilt, damping off, brown rot and scab. Dried-shrink disease, which has been named as cancer for sea buckthorn is a serious disease which decreases 20–40% of yield and leads to large-scale plantation deaths. In China alone it eliminates over 10,000 hectares plantations annually and is a major bottleneck restricting sea buckthorn industry. The other common pathogenic fungi include the species of *Fusarium*, *Alternaria*, *Pythium*, *Fomes, Monillia*, *Stigmina hippophaes* and *Valsa* (Li 2003).

Since sea buckthorn is a new cultivated crop, there are no registered pesticides or fungicides. Limited research related to the disease and pest control in sea buckthorn has been reported till date. The damages caused by the insects and pests can be controlled to some extent by physical, chemical and biological methods (Renjun et al. 2008).

Thorniness of the bush

The transformation of the raw sea buckthorn berry into a sophisticated product requires appropriate harvest, transportation, holding, and storage procedures. Berries persist on the branches all winter due to the absence of an abscission layer which poses enormous difficulties in harvest. Thorniness of the bush further limits harvesting. Almost all *Hippophae* species start to develop thorns (2–5 cm) on 2- to 3-year-old plants. The total labor cost estimated for harvesting a sea buckthorn orchard of 4 ha was 58% of the total cumulative production costs over 10 years (Li 2002). The common harvesting technique is to remove the entire branch which leads to destruction of the shrub. This method reduces future yields, and harvest can be obtained only every alternate year because the flower buds appearing in the previous growth season transform into fruits. Also, the prototype harvesters available commercially are based upon the principle of cutting off the entire fruiting branch. Therefore, efforts are required for improving harvesting ease. Preliminary results indicated that ethephon, an ethylene generator, can reduce berry removal force marginally in sea buckthorn if it was applied 10 days before harvest. It reduces berry removal force in sour cherries and many other fruit crops. Another successful method of mechanical harvesting involves shaking the individual branches of the shrub in situ to dislodge the berries and causing them to fall into a catcher placed around the base of the tree (Li 2002). Development of thornless varieties can further improve the harvesting methods and automation of the process.

The thornless Russian and Mongolian varieties are being used to introgress thornlessness in fast-growing Chinese varieties with strong adaptability for enhancing commercial benefits (Ruan and Li 2005).

Breeding

Formal breeding program of sea buckthorn was started in 1933 by Lisavenko Research Institute of Horticulture, Siberia. At present, more than 60 cultivars have been described (Trajkovski and Jeppsson 1999). To improve local adaptation, the Russian material was crossed with Swedish collections (Jeppsson and Trajkowski 2003). In addition to *H. rhamnoides* ssp. *mongolica* and ssp. *turkestanica* of central Asian origin, the European ssp. *rhamnoides* and *carpatica* and ssp. *caucasica* from Minor Asia have been utilized for breeding purposes (Bartish et al. 2000b). Cultivated varieties from China have strong adaptability and are fast growing, but bear small fruits, more thorns; short fruit stalk, have lower content of bioactive substances, and are more prone to dried-shrink disease. However, the cultivated varieties from Russia and Mongolia show many promising agronomic traits, such as big fruits, few or no thorns, long fruit stalk, high content of bioactive substances and resistance to dried-shrink disease (some varieties), but show weak adaptability and are slow-growing (Ruan and Li 2005). Many cultivated varieties from Russia and Mongolia have been introduced into China since 1991 to improve local sea buckthorn germplasm for enhancing commercial benefits. Efforts are in progress to produce thornless, disease-resistant, fast-growing varieties having strong adaptability and higher content of bioactive substances through conventional and molecular breeding so as to harness maximum economic gains from sea buckthorn.

Since sea buckthorn is dioecious, identification of superior paternal parents is a prerequisite for achieving the breeding goals. However, selecting parents is still a major problem (Ruan and Li 2005). The conventional breeding programs for improving quantitative traits require large inputs of labor, land and financial resources. Therefore, plant breeders are motivated to identify promising lines as early as possible in the selection process. Marker-assisted selection (MAS), which uses DNA markers to select optimal parental genotypes, is an excellent tool for selecting beneficial genetic traits in crops and forest trees at an early stage as well as for assessing the genetic potential of specific genotypes prior to phenotypic evaluation. However, non-availability of mapping populations and substantial time needed to develop such populations may prove to be a major limitation in the identification of molecular markers for specific traits. Additional limitations to this technology include limited availability of gene pool, restricted distribution of species and subspecies, unstable adaptability and unsteady yield and quality of introduced varieties, lack of knowledge about extent of hybridization possible between species, non-availability of linkage maps and lack of markers associated with important QTLs for quality characters. Recently, four ISSR markers have been associated with dried-shrink disease resistance in sea buckthorn which may prove useful in selecting resistant lines during breeding programs (Ruan et al. 2009).

Identification of sex of plants

Since sea buckthorn is dioecious, plant breeding projects aim at producing both female and male cultivars. However, breeding objectives for female and male cultivars differ and, generally, there are more quality criteria to be met in a female cultivar (Jeppsson et al. 1999). Unfortunately, gender of sea buckthorn seedlings cannot be determined until flowering, which usually takes place after 3–4 years in the field. This represents a serious problem for plant breeders who have to retain large number of superfluous males for several years. An easily scored genetic marker, which could be used at an early stage for screening sea buckthorn seedlings and discarding the males, would be very useful in plant breeding programs. Much of work and money could thus be saved if a large proportion of the males could be discarded at an early stage in the evaluation process (Jeppsson et al. 1999).

In general, gender is genetically determined in dioecious plants, either by the occurrence of distinguishable sex chromosomes or, more commonly, by the expression of alleles at one or several autosomal loci (Irish and Nelson 1989; Durand and Durand 1990), but environmentally induced sex determination has also been demonstrated (Irish and Nelson 1989). The occurrence of distinguishable sex chromosomes in sea buckthorn has been reported with the males being heteromorphic, suggesting that gender is determined by an *X/Y* system (Shchapov 1979), although this has not been substantiated in other studies. Recently, Persson and Nybom (1998) and Jeppsson et al. (1999) studied the usefulness of RAPD markers for gender determination in sea buckthorn. However, the mechanism governing gender determination or markers linked to sex have not been established yet in sea buckthorn.

Conventional propagation methods

Conventionally, sea buckthorn is propagated by seeds, cuttings (hardwood, softwood, layering) and through suckers. Although propagation using seeds is an easy and inexpensive method, it is impossible to distinguish the male and female plants until they start to flower. Also, some desirable traits of the parents may not be expressed in seed-propagated plants (Li and Schroeder 2003). Clonal multiplication of selected elite germplasm is meaningful for maintenance of superior traits which otherwise will be lost due to open pollination and out crossing. Also, it is a dioecious species and female plants are more valuable; therefore, vegetative propagation methods are most appropriate for its commercialization. The conventional vegetative propagation methods are not very successful being season-dependent, space requiring and cumbersome in nature. Another bottleneck is the limited availability of initial planting material. Therefore, we need to sort to alternate methods like micropropagation for rapid multiplication of planting stock material.

Progress made using modern biotechnological tools

Vulnerability of sea buckthorn to an array of biotic and abiotic stresses, need for elite planting material for commercial plantations, limitations of conventional breeding and propagation methods, lack of proper estimation of available diversity, conservation strategies and controversial taxonomic status of the genus necessitates the application of plant biotechniques encompassing plant tissue culture and genetic engineering, and application of molecular markers for diversity assessment and marker-assisted breeding programs for improvement of sea buckthorn. An outline of tentative biotechnological interventions required in sea buckthorn have been summarized in Fig. 3. The progress made in the application of the modern biotechnological tools in sea buckthorn is summarized below.

Micropropagation

Micropropagation or in vitro culture has immense potential in mass propagation and genetic improvement programs in dioecious species like sea buckthorn. Moreover, the modern biotechnological tools can be exploited to improve the quality, content, flavor and shelf-life of fruit crops only

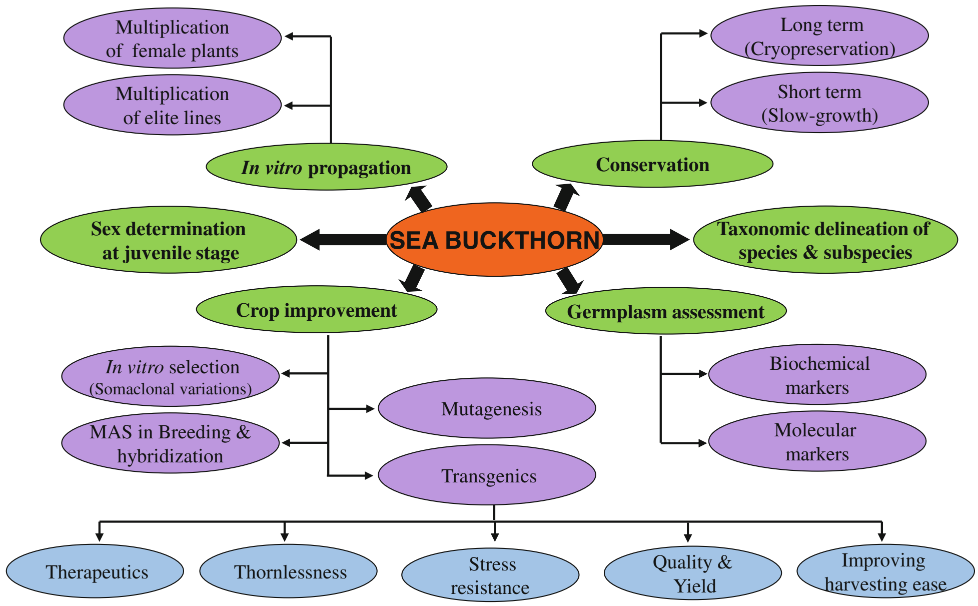

Fig. 3 An outline of tentative biotechnological interventions required in sea buckthorn

Table 2 Micropropagation of *Hippophae* L.

Species	Explants	Medium + PGR's	References
Organogenesis			
H. rhamnoides L.	Nodal segments, leaves, cotyledons, hypocotyls	MS + BAP + TDZ	Sriskandarajah and Lundquist (2009)
	Roots from seedlings	WPM + BA + GA_3 + IAA	Sriskandarajah and Lundquist (2009)
	Cotyledon, hypocotyl	WPM + BAP	Knyazev et al. (2003)
	Nodal segments	MS + BAP	Montpetit and Lalonde (1988)
	Apical meristem	–	Burdasov and Sviridenko (1988), Guo Chunhua et al. (2000), Yang et al. (2004)
	–	–	Nilov and Tretyakova (1993), Yao (1994), Mou (1995)
H. rhamnoides ssp. *carpathica*	Shoot apices	MS + BAP + IAA	Vantu (2007)
H. rhamnoides ssp. *sinensis*	Nodal segments	MS + TDZ	Lummerding (2001)
H. rhamnoides ssp. *turkestanica*	Nodal segments with active and dormant buds	MS + BAP + IAA	Gupta and Singh (2003), Singh and Gupta (2008)
Embryogenesis			
H. rhamnoides L.	Leaves	MS + CPPU + BA + NAA	Sriskandarajah and Lundquist (2009)
	Cotyledon, leaves, hypocotyls	SH + KIN + IAA	Liu et al. (2007a)

when we have a protocol for regeneration of complete plantlets in vitro. Two distinct patterns of in vitro differentiation, i.e. organogenesis and somatic embryogenesis have been used for micropropagation of sea buckthorn (Table 2). Organogenesis has been induced through axillary shoot production using explants from seedlings as well as from mature plants; however, only two reports are available regarding somatic embryogenesis in sea buckthorn. Genetic transformation studies have not yet been initiated in sea buckthorn due to lack of standardized micropropagation protocols and field trials.

Factors controlling micropropagation

Successful micropropagation, especially for difficult and recalcitrant species, is largely governed by the quality of explants, and the response of the explants is primarily determined by the genotype, physiological state of the tissue and the time of the year when the explants are collected and cultured. Sriskandarajah and Lundquist (2009) found that the performance of leaf segments taken from seedlings was better in comparison with those obtained from mature tree for shoot formation and vice versa for somatic embryogenesis. Further, among the seedling-derived explants, cotyledon and hypocotyl explants were not as responsive as leaf explants for organogenesis and somatic embryogenesis. Similarly, Liu et al. (2007a) reported that leaf, cotyledon and hypocotyls taken from the seedlings of sea buckthorn showed more or less similar response towards somatic embryogenesis.

In sea buckthorn, a number of media have been used for plant regeneration. But mostly MS (Murashige and Skoog 1962) medium was used for shoot formation (Table 2). In a few studies, however, other media have also been used for organogenesis and somatic embryogenesis. Not much work has been done on strength of media that support better in vitro growth of explants except the report of Sriskandarajah and Lundquist (2009) who reported that half MS rather than full MS was better for somatic embryogenesis from leaf explants taken from seedlings.

Development of in vitro culture protocols for sea buckthorn has resulted in varying degrees of success. Gupta and Singh (2003) indicated that sea buckthorn explants are specific about their requirements for plant growth regulators (PGRs) and nutrient composition of the culture medium. When high levels of PGRs such as BA (6-benzyladenine), kinetin and 2, 4-D (2,4-dichlorophenoxy acetic acid) were used, hypertrophy and mortality were observed. Most studies on sea buckthorn have used cytokinins such as BA and kinetin and obtained limited regeneration (Montpetit and Lalonde 1988; Knyazev et al. 2003; Yang et al. 2004; Singh and Gupta 2008). In studies with *H. rhamnoides* ssp *sinensis* cv. Indian summer, another cytokinin TDZ (Thidiazuron) did not promote axillary bud growth and the cultures eventually died (Lummerding 2001). Other growth regulators including GA_3(Gibberellic acid), growth-enhancing medium additives such as carbon sources and CPPU [*N*-(2-chloro-4-pyridyl) *N*-phenylurea] also had significant effect on shoot initiation and multiplication (Sriskandarajah and Lundquist 2009).

Root initiation in sea buckthorn occurred on MS basal medium without any growth regulators (Montpetit and Lalonde 1988; Vantu 2007), although Gupta and Singh (2003) and Singh and Gupta (2008) used IBA (Indole-3-butyric acid) for rooting. Montpetit and Lalonde (1988) achieved rooting of shoots on medium containing one-quarter strength MS salts.

Constraints in micropropagation

A few studies have reported in vitro culture of sea buckthorn using axillary buds; however, some problems have been highlighted during clonal propagation, including low micropropagation efficiency, browning of explants, poor rooting frequency and genotypic differences among cultivars (Montpetit and Lalonde 1988; Nilov and Tretyakova 1993; Yao 1994; Knyazev et al. 2003; Yang et al. 2004). Lummerding (2001) established the in vitro cultures of *H. rhamnoides* ssp. *sinensis* but the cultures died during subculturing. Similarly, Liu et al. (2007a) reported that only 30–50% somatic embryos converted into normal plantlets due to the formation of several abnormal embryos during regeneration. Gupta and Singh (2003) and Singh and Gupta (2008) while working on micropropagation of Indian sea buckthorn (*H. rhamnoides* ssp. *turkestanica*), observed high levels of variations in vitro when cultures were established from different cultivars of this species. Therefore, genotype is one of the main factors that influence the organogenic response of cultures. The existence of strong genotype specificity in the regeneration capacity of the different cultivars represents an important limiting factor that makes development of a standard regeneration protocol a problem, and therefore, specific regeneration protocols are required for each cultivar. Recently, Sriskandarajah and Lundquist (2009) reported the problem of vitrification and browning of the cultures during organogenesis and somatic embryogenesis from juvenile and adult tissues of *H. rhamnoides*. Therefore, further systematic scientific studies are necessary to focus on improving the multiplication protocol and survival rates of the plantlets in the greenhouse to evaluate its efficiency of multiplication on commercial scale. In spite of many reports on micropropagation of sea buckthorn, field performance of tissue-culture raised plants has not yet been initiated.

Genetic diversity assessment

Characterization of germplasm is essential for identifying individual genotypes as well as the extent of variability existing among the accessions. It is the basis for plant adaptation, evolution and breeding. Germplasm characterization and evaluation is the first step in a breeding program. The comprehensive information obtained from such an exercise would help breeders, geneticists and conservationists to effectively utilize the valuable genetic resources. Some of the important investigations related to sea buckthorn germplasm characterization using various markers are presented in Table 3.

Morphological markers

Traditionally, diversity within and between populations is determined by assessing differences in morphology. However, morphological determinations need to be taken by an expert in the species as they are subject to changes due to environmental factors and may vary at different developmental stages. Moreover, the morphological characters are limited in number. In most woody plants, leaf morphological and physiological characteristics are extremely variable across environmental gradients.

Table 3 Diversity assessment of *Hippophae* L.

Tools	Achievements	References
Morphological markers		
Shoot height, leaf N concentration, leaf carbon isotope concentration, specific leaf area, stomatal density	Found differences in sex-related morphological and physiological responses in *H. rhamnoides* along the altitudinal gradient in Wolong region of China	Li et al. (2007a)
Seed and fruit characters	Analyzed morphological traits of seeds and fruits to reconstruct the relationship among the taxon of different regions to test association between morphological characters and environment	Aras et al. (2007)
Habitat, plant growth habit, stem, leaf, fruit, flower, seed and biochemical parameters	Developed a set of morphological descriptors for the genus *Hippophae* after thorough study of the germplasm collected from different parts of India	Mathew et al. (2007)
Growth form, leaf morphology, and fruit properties	Compared ten populations of Pakistan for morphological characters	Sabir et al. (2003)
Pollen	Reported that morphological characteristics of pollen of ssp. *caucasica* in Turkey showed different features while pollens from Trabzon showed hybrid features;	Aras and Türkiye'nin (1995)
Biochemical markers		
Isozymes	Provided information on genetic variation, differentiation and evolution in seed samples of 25 populations from China, Finland and Russia	Yao and Tigerstedt (1993)
SDS–PAGE	Evaluated seed storage protein profile of cultivated and naturally grown plants in northern Pakistan and revealed the presence of only low molecular weight proteins in range of 15–50 kDa	Zeb and Malook (2009)
FAME	Examined biochemical relationships between 10 selected genotypes of Eastern Anatolia	Ercisli et al. (2008)
Molecular markers		
RAPD	Examined genetic relationships between 10 selected genotypes found in Eastern Anatolia	Ercisli et al. (2008)
	Analyzed genetic diversity and relationship among and within species of *Hippophae*	Sheng et al. (2006)
	Studied genetic variation among 17 morphotypes growing in cold arid region of Ladakh (India)	Singh et al. (2006)
	Studied genetic variation in *H. rhamnoides* ssp. *sinensis*, and found no association between genetic distance and geographical distribution	Sun et al. (2006)
	Evaluated genetic relationships among 14 cultivated varieties from China, Russia and Mongolia	Ruan et al. (2004)
	Generated diversity map for 55 cultivars and accessions of 5 ssp. of *H. rhamnoides* L. and intraspecific hybrids between different ssp.	Bartish et al. (2000a)
	Studied genetic variations in 10 populations from native stands of Netherlands and Sweden	Bartish et al. (1999)
	Studied population structure and identified markers linked to gender determination	Jeppsson et al. (1999)
	Marker development for sex determination	Persson and Nybom (1998)
AFLP	Evaluated phylogenetic relationship among 25 wild ecotypes from Pakistan	Shah et al. (2009)
	Analyzed genetic relationships among cultivars from China, Russia and Mongolia	Ruan (2006)
	Analyzed DNA fingerprint patterns and genetic relationships among 15 cultivated varieties from China, Russia and Mongolia	Ruan and Li (2005)

Table 3 continued

Tools	Achievements	References
SSRs	Developed 26 microsatellite markers of which 9 were polymorphic among 12 distantly distributed individuals	Wang et al. (2008b)
ISSR	Assessed genetic stability and established genetic relationships between four propagated cultivars	Li et al. (2009)
	Reported four ISSR markers significantly correlated with resistance to dried-shrink disease among 52 accessions of 16 sea buckthorn varieties.	Ruan et al. (2009)
	Genetic diversity analysis of *H. rhamnoides* L. populations at varying altitudes in the Wolong natural reserve of China	Chen et al. (2008)
	Reported that Russian sea buckthorn genotypes had a higher polymorphism ratio than China's	Liu et al. (2007b)
	Analyzed 300 individuals of fifteen natural populations of China	Tian et al. (2004)
Chloroplast DNA	Sequences of cpDNA were used to establish double maternal origin of diploid hybrid, *H. goniocarpa*	Wang et al. (2008a)
	Analyzed 15 taxa in *Hippophae* based on cpDNA and combined data of morphological characters and cpDNA	Bartish et al. (2002)
ITS sequences	Reported hybrid origin of the diploid species *Hippophae goniocarpa* using ITS markers of nuclear rDNA	Sun et al. (2003)
	Analyzed phylogenetic relationships among 15 taxa of the genus by comparing sequences of ITS and region of nuclear rDNA	Sun et al. (2002)

Aras et al. (2007) analyzed morphological traits of seeds and fruits, considered as diagnostic characters, of *H. rhamnoides* L. ssp. *caucasica* in Turkey (Sivas, Trabzon, Ilgaz, Ürgüp) to reconstruct the relationship among the collections made from different regions and to test whether there is a significant association between the morphological characters measured and their environment. In a previous report, Aras and Türkiye'nin (1995) observed that pollen of ssp. *caucasica* in Turkey showed different morphological features and also pollen grains collected from Trabzon showed hybrid features. Moreover, the dimensions of the seeds from Trabzon and Sivas were similar, but surface ornamentations of testa and also some critical wood anatomical characteristics were different. Based on these results, it was concluded that these differences were not due to ecological conditions, but there were some taxonomic problems. He also proposed that *H. rhamnoides* growing in Turkey would probably form a different taxon or races of the taxa.

A set of morphological descriptors for the genus *Hippophae* have also been developed after thoroughly studying the germplasm obtained from explorations in different parts of India (Mathew et al. 2007). The pattern of variability in morphological characteristics associated with habitat, plant growth habit, stem, leaf, fruit, flower, seed and biochemical parameters was taken into consideration. The suitability of these parameters in the characterization of *Hippophae* germplasm was demonstrated successfully and it was reported that *H. rhamnoides*, *H. salicifolia* and *H. tibetiana* formed separate clusters in a dendrogram based on unweighted hierarchical cluster analysis (Fig. 4).

Biochemical markers

To overcome the limitations of morphological traits, other markers have been developed at both protein level and DNA level. Yao and Tigerstedt (1993) provided information on the genetic variation, differentiation and evolution in 25 populations of *Hippophae* from China, Finland and Russia based on isozyme studies. They investigated six loci, out of which four were good markers for identifying species and sub-species. The phylogenetic tree prepared from these data agreed well with botanic classifications of the species and sub species and their geographical distributions. Fatty acid methyl ester (FAME) analysis has also been used to examine biochemical and genetic relationships between sea buckthorn genotypes (Ercisli et al. 2008). Fatty acid composition of sea buckthorn berries was determined using gas chromatography, and the results showed that there were differences between genotypes in

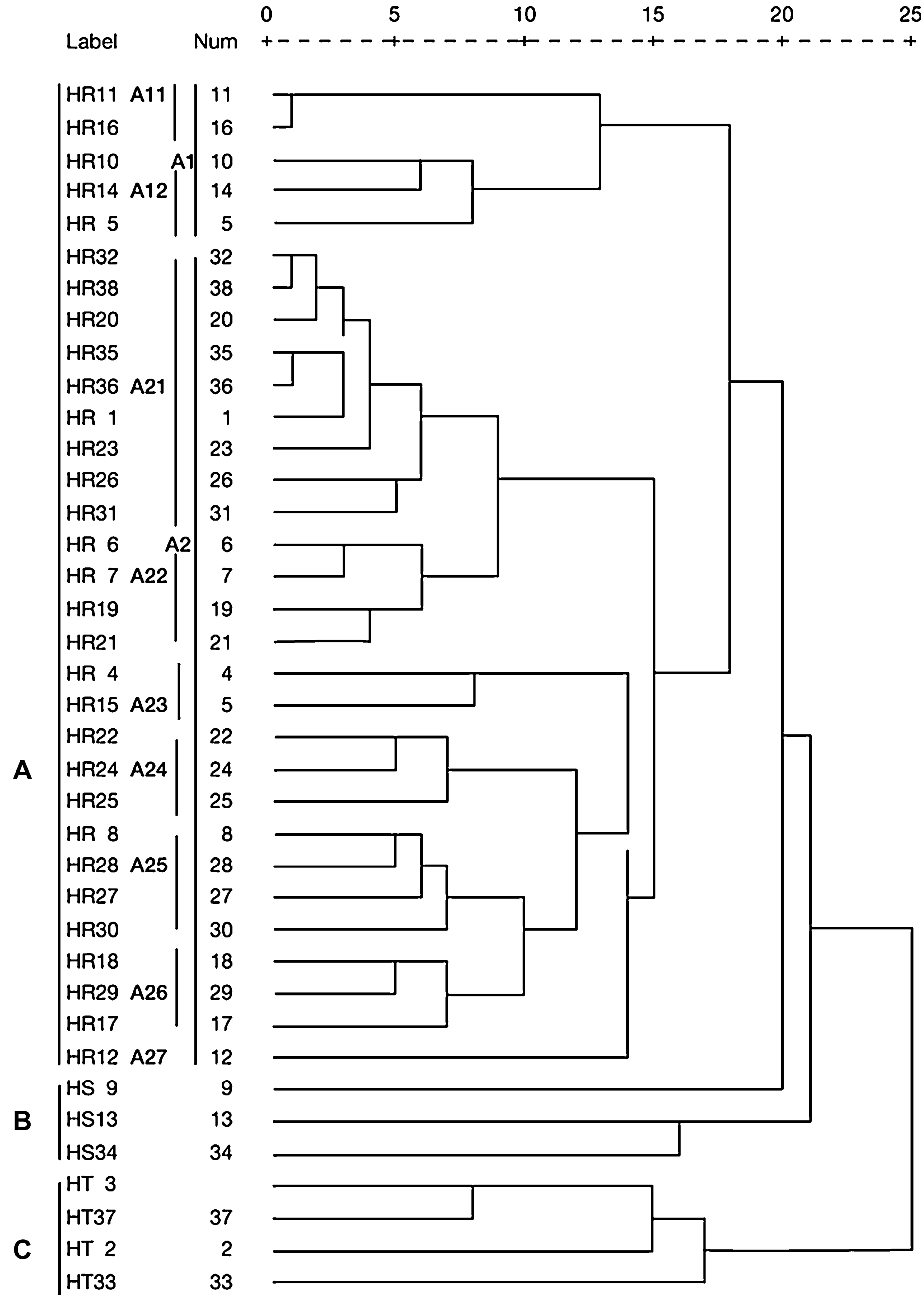

Fig. 4 Genetic distances among *Hippophae* germplasm accessions derived by following the morphological descriptor using hierarchical un-weighted analysis (*HR*, *Hippophae rhamnoides; HS, H. salicifolia*; *HT, H. tibetana*) (adapted from Mathew et al. 2007)

both the percent and presence of fatty acids in the berries. It was concluded that the fatty acid patterns could be useful indicators for the characterization and grouping of sea buckthorn genotypes.

Molecular markers

During the past decades, classical methods to evaluate genetic variation have been complemented by molecular techniques. These are useful for characterizing the genetic diversity among different cultivars or species, for identifying genes of commercial interest and improvement through genetic transformation technology. In recent years, different molecular markers (RAPD, AFLP, SSR, ISSR, cpDNA, ITS sequences) have been employed for the investigations of cultivars, origin and taxonomic relationships of sea buckthorn (Table 3).

RAPD analysis of sea buckthorn plants from native stands showed low level of genetic distance suggesting high level of gene flow or genetic variability which helps the species to evolve. This low genetic differentiation among populations was attributed to the long distance dispersal of seeds facilitated by birds, in addition to its characteristics of out crossing, wind pollination and widespread distribution. No association between genetic distance and geographical distribution was found (Sun et al. 2006). This pattern of population's differentiation may imply the adaptation of subspecies populations to local environment, given that its habitats vary greatly across its distribution.

Persson and Nybom (1998) studied the usefulness of RAPD markers for gender determination in sea buckthorn. Out of 78 primers tested, 4 seemed to yield partitioning between male and female bulks. Similarly, Jeppsson et al. (1999) used RAPD markers to determine genetic variation between and within native populations and to measure relatedness between populations. They also tried to use RAPD analysis to find a marker linked to gender determination.

Ruan and Li (2005) and Ruan (2006) used AFLP markers to study genetic relationships among 15 sea buckthorn varieties from China, Russia and Mongolia. Genetic similarity between ZGSJ and WLGM, and ALY and ZGSJ based on AFLP data was relatively low. Therefore, it was concluded that crossing between ALY and ZGSJ may breed a fine hybrid. Recently, Shah et al. (2009) analyzed 25 plant samples of sea buckthorn (*H. rhamnoides* ssp. *turkestanica*) using AFLP from natural populations in Pakistan. Phylogenetic distance estimation revealed that the ecotypes expressed common heritage for their phylogenetic relationship with a considerable genetic diversity among them as well. Quite a few ecotypes showed close relationship irrespective of their geographic distances and morphological attributes.

Wang et al. (2008b) developed 9 microsatellite loci for *H. rhamnoides* ssp. *sinensis*. They used the biotin capture method to enrich AG/CT/AC/GT/CG/GTG/CCA microsatellites and isolated 26 microsatellites of which 9 were found to be polymorphic among 12 distantly distributed individuals. The number of alleles per locus ranged from 3 to 12 and expected heterozygosity from 0.2659 to 0.4767, respectively. Tian et al. (2004) analyzed 300 individuals of 15 natural populations of sea buckthorn in China using ISSR markers and detected mean genetic diversity in the natural populations of *H. rhamnoides* ssp. *yunnanensis,* ssp. *sinensis,* and ssp. *gyantsensis*. No significant correlation between genetic and geographic distances of the populations was found. Similarly, Chen et al. (2008) observed genetic diversity among *H. rhamnoides* L. populations at varying altitudes in the Wolong nature reserve of China. Liu et al. (2007b) reported that Russian sea buckthorn genotypes had a higher polymorphism ratio than China's, based on ISSR markers. Recently, Li et al. (2009) employed 15 ISSR primers to assess the genetic stability of lines obtained by cuttings of 4 *H. rhamnoides* cultivars and the genetic relationships among them. Cluster analysis based on ISSR data indicated that at a Jaccard coefficient of 0.78, mother plant and lines obtained by cuttings for each cultivar were grouped into different sub clusters, respectively, which could be further sub clustered.

Marker-assisted selection (MAS), which uses DNA markers to select optimal parental genotypes, is an excellent tool for selecting beneficial genetic traits in crops and forest trees at an early stage as well as for assessing the genetic potential of breeding lines prior to phenotypic evaluation. Ruan et al. (2009) were successful in identifying 4 ISSR markers which were significantly correlated with dried-shrink disease of sea buckthorn, which decreases fruit yield by 20–40% and usually appears on plants that are at least 3 years old. These markers will provide a potential tool for selection of dried-shrink disease resistant genotypes during breeding programs, especially when no other genetic information like linkage maps and QTLs are available in sea buckthorn.

The phylogenetic relationships among 15 taxa of *Hippophae* were analyzed by comparing the sequences of the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) (Sun et al. 2002, 2003). They varied in length from 651 to 666 bp. The aligned sequences were 690 bp in length and 269 (39%) were variable sites with 150 being parsimony informative. The amount of polymorphism observed within a taxon was extremely low in most taxa except the putative hybrids. The consensus trees of parismony analysis supported monophyletic origin of the genus *Hippophae*. Similar conclusions were drawn from the results from two parsimony analyses of all 15 recognized taxa in *Hippophae*, one based on chloroplast DNA (cpDNA), and the other based on a combined data set of morphological characters and cpDNA (Bartish et al. 2002). Both the groups refrained from recognizing sections within the genus as have been recognized by some other workers. The ITS sequence data analysis supported the hybrid origin of *H. goniocarpa* and *H. litangensis* as proposed previously (Sun et al. 2002). Sequences of cpDNA have also been used to establish double maternal origin of diploid hybrid, *H. goniocarpa* (Wang et al. 2008a).

Undoubtedly, molecular markers will prove far more efficient than morphological and biochemical markers in unequivocally establishing the phylogeny and taxonomy of sea buckthorn in addition to their role in breeding programs and germplasm characterization.

Conclusions and future prospects

Sea buckthorn is an economically and ecologically important plant species, currently being domesticated in various parts of the world, including Afghanistan, Belarus, Bhutan, Bolivia, Canada, China, Czech Republic, Finland, Germany, Hungary, India, Mongolia, Nepal, Netherlands, Norway, Pakistan, Poland, Romania, Russia, Sweden, Ukraine, United Kingdom and USA, reflecting interest in its long-identified multiple uses (Singh et al. 2003). Although this plant has many excellent traits, it is still in an early phase of domestication. Limited progress has been made in improving the taxon through conventional breeding programs, but some traits which need further improvement include yield (fruit size, quality and maturity period), harvesting ease (thornlessness, fruit abscission), palatability, local climatic adaptation and, disease and pest resistance among others while retaining the desired traits.

Taxonomic delineation of species and subspecies is still controversial and needs to be addressed more rigorously. Efforts have been made to classify the genus based on morphological, biochemical and molecular markers including ITS region sequences and cpDNA sequences, but more inputs are required to confirm the phylogeny and taxonomy of the genus.

Breeding programs for sea buckthorn are being conducted since 1960s in China, Russia and Germany, and few improved varieties and lines have been selected using morphological traits. Conventional breeding programs are time, cost and labor intensive; therefore, MAS must be used for selecting beneficial genetic traits as well as for assessing the genetic potential of specific genotypes prior to phenotypic evaluation. Molecular markers linked with QTL/major genes for traits of interest must be developed. In addition, availability of a broad genetic base is a must for initiating breeding programs in any given crop. Diversity studies have been initiated in localized pockets in sea buckthorn-growing regions of the world which need to be strengthened further. The available genetic base can be broadened using modern tools of biotechnology including in vitro selection, mutagenesis and transgenics. For long-term breeding prospects, it would be advantageous to incorporate a hermaphrodite mode of reproduction into parental stocks, so that selection for berry traits need not occur solely through the maternal parent. This would also obviate the need to use males in commercial plantations. Knowledge of genetic relationships in parental varieties could improve the effectiveness of breeding programs. Robust markers need to be in place for determining the sex of the seedlings at an early stage so that much of the effort and money required for maintaining the germplasm till flowering can be saved.

Sea buckthorn is vulnerable to various types of pests, pathogens and climatic constraints during cultivation like other woody perennials. Therefore, development of genetically engineered plants capable to counter such biotic and abiotic stresses is imperative. However, an efficient regeneration protocol must be in place before genetic transformation studies can be initiated. A few studies on in vitro culture of sea buckthorn have been reported, but low proliferation rate, vitrification, browning, death during subculturing, poor rooting, and genotypic differences among cultivars and high levels of variations in establishing in vitro cultures of different promising cultivars remain to be the main constraints. Somatic embryogenesis has also been reported wherein only 30–50% embryos convert into normal plantlets. The in vitro regeneration techniques need to be improved further so that they can be used for genetic improvement of sea buckthorn through transgenics.

The microbial associates (rhizosphere fungi, VAM spores and endophytes) of sea buckthorn can be exploited for their mass multiplication with the ultimate aim of transplanting the resultant tailored seedlings in degraded and treeless land of cold desert areas. These VAM fungi help to increase the soil root interphase area and hence increase the nutrient uptake. These fungi are also known to increase the phosphate and moisture uptake by the plant (Kumar and Sagar 2007). Stone et al. (2000) have also regarded VAM endophytes as a source of novel metabolites for therapeutics, as potential biocontrol agents helping the establishment of seedlings at new sites by providing disease resistance and enhancing growth. Therefore, in-depth scientific research on the role of microbial associates and their exploitation are imperative, so as to suggest the strategies for growing sea buckthorn on degraded and abandoned land, which will not only help them to improve their ecological environment but also help in the sustainable development of the traditional mountain societies.

Sea buckthorn, despite being a common and widespread species, is deserving of conservation measures. Its distribution pattern has been described as highly fragmented, i.e. it tends to occur as isolated patches, and these are often genetically distinctive. High nutrient and medicinal values of the fruit have led to uncontrolled exploitation and even destruction of sea buckthorn resources in some parts of its natural distribution. Thus, protection and preservation of the valuable germplasm have become urgent. In Hungary, wild sea buckthorn is rarely observed, and the plant is protected as an endangered species. Such protection needs to be extended, especially to the Asian sea buckthorns, which occupy small distinct ranges (Small et al. 2002). Also, the wild plants constitute an invaluable source for selecting superior agricultural traits. In such conditions, conservation of agronomically important cultivars through in vitro methods and cryopreservation must be done. It will be the most promising answer to biological and climatic hazards which may threaten the germplasm maintained in situ in field genebanks and germplasm gardens. Cryopreserved material (stored as seeds, ovules, embryos, callus, etc.) can be used successfully for breeding in the future.

Sea buckthorn is serving as a measure of biodiversity conservation, soil conservation, medicines, food, fodder and fuel wood. In addition to this, seabuckthorn has become a vital source for research and development work due to the presence of more than 300 bioactive agents. Still, there is a need to emphasize more on the standards of certain seabuckthorn-based health food products like juice concentrate, spray-dried powder to retain its α-carotene pigment in purified pulp oil and seed oil. There is a need for novel techniques and approaches for integrated processing of sea buckthorn berries into their nutraceutical and therapeutic products. There is ample scope for further research on the identification of certain bioactive compounds from the deflated crude extracts from seeds to exhibit their functional and synergistic effects against certain degenerative diseases like arteriosclerosis, diabetes, arthritis, cancer and aging. Similarly, berry oil has shown good potential against platelet aggregation and beneficial effects on blood clotting in humans; further studies on dose response effect are needed to assess the practical use of berry oil supplements (Chauhan et al. 2008). Therefore, cost effective and easily available drug delivery systems having no toxicity can be established at large scale.

There is no doubt that the future holds great promise for sea buckthorn. This ancient plant with its powerful and healing synergies has much to contribute to this planet and its inhabitants. The environmental problems being faced in global arid and semi-arid regions are very serious due to which productivity of agriculture, forestry and animal husbandry is decreasing. In such conditions, sea buckthorn has the outstanding capacity to improve the environment and economic development. We can look forward to a continued revelation of sea buckthorn's many gifts through the increasing interest and research into its abundant and valuable properties. Judicious exploitation and utilization of sea buckthorn resources can bring more benefit to mankind throughout the world.

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