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Research and Biotechnology in Sea Buckthorn (*Hippophae* spp.)

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ABSTRACT

Sea buckthorn (*Hippophae* spp.), which holds environmental importance and is a new commercial berry crop, is naturally distributed from Asia to Europe. Its berries are rich in phytonutrients and bioactive substances, products often used for medical and pharmaceutical purposes. This review summarizes recent research and biotechnology in this genus, including the improvement of ecological environments, chemical composition and nutritional values, medicinal uses and sea buckthorn products, selection and breeding for improving yield and quality, tissue culture, genetic relationship and diversity at the molecular level (isozymes, RAPD, AFLP, ISSR, cpDNA and ITS). Good adaptability, fast-growth, protection against wind and preventing sand from drifting, use in soil and water conservation and improvement of soil by efficient nitrogen fixation make sea buckthorn to be planted at a large scale in China and other countries. Sea buckthorn contains different kinds of nutrients and bioactive substances such as vitamins, carotenoids, flavonoids, polyunsaturated fatty acids, free amino acids, elemental components, and others. These components vary substantially among populations, origins or subspecies, and their presence is more important for the health of individual. The clinical trials and scientific studies during the 20th century confirm the medicinal and nutritional value of sea buckthorn. The present review describes some areas of research that have been important points, for example in cancer therapy, cardiovascular diseases, treatment of gastrointestinal ulcers, skin disorder and as a liver protective agent. Mutation, crossing and conventional breeding were widely used in its selection and in breeding for improving yield and quality. Based on the differences in agronomic traits among two different subspecies, crosses between *H. rhamnoides* ssp. *sinensis* (good adaptability, more thorns and small fruits) and *H. rhamnoides* ssp. *mongolica* (few thorns, big fruits and a long fruit stalk) yielded several fine hybrids. The genetic relationship and diversity based on molecular approaches in sea buckthorn provides a potential for improving the breeding strategy. Dried-shrink disease (*Plowvigneia hippophaeos*) and drought are the main problems restricting the sustainable development of sea buckthorn, and molecular biotechnology may be able to provide for a solution to overcome these problems and limitations. Plants have been successfully regenerated using different organs on different media, and regularly clonal propagation using hardwood and softwood cuttings in the greenhouse and field has been used. Currently, we are cloning genes related to resistance to dried-shrink disease, and expecting to get fine resistant lines by genetic transformation and marker-assisted selection.

Keywords: breeding, eco-environmental function, genetic diversity, genetic relationship, tissue culture

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Invited Review

INTRODUCTION

Sea buckthorn (*Hippophae*), an environmentally important plant used as a new commercial berry crop, is a deciduous, perennial shrub or tree belonging to the Elaeagnaceae family. The genus has six species and 12 subspecies worldwide (Bartish *et al*. 2002; Sun *et al*. 2002; Swenson and Bartish 2002); all species are diploid (2n = 24) and are restricted to the Qinghai-Xizang plateau and adjacent areas except for *Hippophae rhamnoides* L., which is naturally distributed from Asia to Europe, and has been introduced into South and North America (Heinz and Barbaza 1998; Roy *et al*. 2001). Sea buckthorn adapts well to extreme conditions, including temperatures ranging from -43°C to +40°C, drought, high altitudes, salinity and alkalinity. Commercially, sea buckthorn is a hardy, multi-purpose plant with orange, red or yellow berries, and has substantial agricultural, ecological, nutritional, medicinal and ornamental value (Vikberg and Itamies 1999; Ruan *et al*. 2000; Zadernowski *et al*. 2002; Cheng *et al*. 2003; Tsydendambael and Vereshchagin 2003). This review summarizes recent research and biotechnology in sea buckthorn, including the improvement of ecological environments, selection and breeding for improved yield and quality, tissue culture, genetic relationship and diversity at a molecular level (isozyme, RAPD, AFLP, ISSR, cpDNA and ITS). The problems of research and biotechnology in sea buckthorn are discussed and its development is prospected.

HIPPOPHAE RHAMNOIDES: IMPROVING THE ECO-ENVIRONMENT

Water, nutrients, and bio-diversity of *H. rhamnoides* plantation

H. rhamnoides has a fine water ecological adaptability, and its extensive root-system can absorb deep soil water to enhance water supplement. As *H. rhamnoides* ages, soil physical characteristics and fertility are gradually improved, as is soil water-holding capacity; restoring soil water 1-1.5 m deep in woodland works very well in the dry season, and the soil moisture content (7.6%) of the woodland becomes more than that of natural grasslands covering a barren mountain (6.8%) (Ruan *et al*. 2000); the soil bulk density of the woodland 1.28 g/cm^3 is lower than that of barren mountain (1.29 g/cm^3), and the woodland permeability with the first infiltration rate of 2.8 4mm/min and stable infiltration rate of 1.54 mm/min is higher than that of barren mountain with the first infiltration rate of 1.56 mm/min and stable infiltration rate of 0.79 mm/min (Hou *et al*. 1995). The content of water-stable aggregates of soil in *H. rhamnoides* woodland is 85% (that of barren mountain is 53%) (Li 2004). Based on these reasons, a plantation consisting of a low wet layer formed by *H. rhamnoides* could improve the water ecological environment of the woodland, because it could store more precipitation by improving its own permeability and water-holding capacity.

The root nodules of *H. rhamnoides* can fix nitrogen; in addition, it can improve the soil structure and assist the return of nutrient elements to the soil through the decomposition of litter and plant parts. These two factors maintain the nutrient balance in woodland soil. In 2-13 year-old, artificial *H. rhamnoides* plantation at Wuqi county, Shaanxi Province, China, the mean annual net primary productivity above ground was 3820.00 kg/ha (in barren grassland it was 665.79 kg/ha), the mean annual nitrogen accumulation was 92.25 kg/ha (that of barren grassland was 49.04 kg/ha), and the mean annual phosphorus accumulation was 8.25 kg/ha (that of barren grassland was 6.38 kg/ha) (Ruan and Li 2002).

After having been planted for 7-8 years, *H. rhamnoides* could form a shrub-grass community, with thick forest and lush grass and an overall coverage of 80%. After artificial plantation for 13 years, natural shrubs and grass species in the *H. rhamnoides* plantation numbered 80 species more than the pre-plantation phase, while introduced plant species also numbered over 40. The mainly wild weed species in the sea buckthorn woodland growing at the Jianping county of China have *Cleistogenes songorica*, *Thymus vulgaris*, *Lespedeza daurica*, and *Stipa bungeana*, etc., and companion species have *Potentilla chinensis*, *Bidens pilosa*, *Setaria viridis* and *Artemisia lavandulaefolia*, etc. (Li 2004). Natural vegetation could be restored under suitable environmental conditions, while artificial vegetation was established classified into either geophiles, dendrophiles, grassphiles or agriphiles. The new ecosystem formed by the artificially constructed and naturally restored *H. rhamnoides* plantation has a more stable structure, and a more harmonious relationship among different species. Environmental reconstruction and restoration gradually increases the number of wild animals, with 10-30 wild animals perching in the *H. rhamnoides* plantation, including over 3 snake species (*Enhydris chinensis*, *Bungarus parvus* and *Agkistrodon acutus*), many kinds of birds (e.g. *Phasianus colchicus*, *Alectoris chukar* and *Crossoptilon mantchuricum*), *Vulpes vulpes*, rats, rabbits, badgers and others, which form a stable ecosystem and enhance its biodiversity, biostability and bioharmony.

Protection against wind, prevention of sand drift, soil and water conservation

After having been planted for 4-5 years, *H. rhamnoides* became the crown canopy, with 10-20 species of lush weeds growing under forest stands, such as *Ranunculus japonicus* Thunb, *Thalictrum minus* Linn. var. *hypoleucum*, *Bupleurum chinense*, and *Stipa bungeana* Trin., etc. The thickness of the litter layer in an over 5 years-old *H. rhamnoides* plantation was about 2-6 cm, while the water-holding capacity was as much as over two times its own weight. The extensive root system of *H. rhamnoides* is mainly distributed in a 20-80 cm soil layer and forms a root network. The flourishing canopy layer, rich undergrowth of herbaceous plants and a well-developed root system in the *H. rhamnoides* plantation form good water ecological environments and forest eco-structures, with subsequent implications for improved forestland protection. An *H. rhamnoides* plantation growing on a barren mountain and gully was able to not only intercept precipitation thus keeping the soil surface from direct exposure to rain, but was also able to reduce rainstorm intensity, alleviating runoff velocity and intercepting silt. *H. rhamnoides* plantation is firmly capable of improving soil by enhancing soil permeability, and reducing erosion and scouring. At the Ansai research station of the Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, runoff from a 2, 3 and 4 year-old *H. rhamnoides* plantation was 66.2%, 65% and 78.0% of that on a natural barren-slope, respectively, reducing soil erosion by 39%, 37% and 47%, respectively (Ruan and Li 2002). In *H. rhamnoides* plantations over 5 years of age, gully erosion and landslides are rare. The experimental results of planting *H. rhamnoides* in a wind-sand zone of Inner Mongolia and the Shaanxi Province of China showed that an *H. rhamnoides* plantation could effectively control the disastrous effect of sandstorms, fixing drift-sand and improving soil conditions (Ruan *et al*. 2000).

Adjustment to microclimates

In an *H. rhamnoides* plantation, the flourishing canopy layer and rich undergrowth (i.e. herbaceous layer) could effectively intercept precipitation. The average intercepting rate of canopy on rainfall in a 7-10 year-old *H. rhamnoides* plantation was 8.5%; Intercepting maximum of litter layer of a 5-10 year-old *H. rhamnoides* plantation was 0.89 mm in single a rainfall (Chen and Chen 2003). In addition, the thicker litter layer could accumulate precipitation well, and the litter interception of *H. rhamnoides* forest following 0-5 mm, 11-20 mm, 31-40 mm and >40 mm precipitation in a rain event were 0-1.5 mm, 2.1-5.7 mm, 2.4-6.3 mm and

8.2-11.0 mm, respectively (Wei *et al.* 1998). These two factors make precipitation become redistributed, and stabilize air humidity and soil moisture inside forests.

In Wuqi and Guyuan Counties, China, air humidity inside an *H. rhamnoides* plantation was 10-20% higher than that of 10-15 m outside the forestland, and its range of change was less than outside the forest. The average soil moisture of the *H. rhamnoides* plantation was 3-4% higher than that of 10-15 m outside the woodland.

CHEMICAL COMPOSITION AND NUTRITIONAL VALUES

Sea buckthorn berries are among the most nutritious and vitamin-rich fruits found in the plant kingdom. The vitamin C concentration in berries varies from 360 mg/100g of berries for the European subspecies *rhamnoides* (Rousi and Aulin 1977; Wahlberg and Jeppsson 1992; Yao *et al.* 1992) to 2500 mg/100 g of berries for the Chinese subspecies *sinensis* (Yang *et al.* 1988; Zhao *et al.* 1991; Yao and Tigerstedt 1994). **Table 1** shows that the content of vitamin C in sea buckthorn juice varies from 3.8 mg/100 g to 1505.5 mg/100 g. The carotene content ranges from 30 to 40 mg/100 g of berries (Bernath and Foldesi 1992; Wolf and Wegert 1993). **Table 2** shows that the carotenoid content in the seed oil of *H. rhamnoides* ssp. *sinensis* of different provenance in China varies from 24.22 mg/100 g to 133.30 mg/100 g, pulp oil from 365.8 mg/100 g to 1375.0 mg/100 g. The carotenoid contents in the seed and pulp oil of *H. neurocarpa* were 51.25 mg/100 g and 353.75 mg/100 g, respectively, in ssp. *mongolica* from Mongolia was 24.22 mg/100 g and 145.32 mg/100 g, respectively and from Russia was 36.1 mg/100 g and 230.20 mg/100 g, respectively. Vitamin E concentration can be as much as 160 mg/100 g of berries (Eliseev 1989; Ma and Cui 1989; Zhang *et al.* 1989; Wahlberg and Jeppsson 1992). **Table 3** shows that vitamin E content in the seed oil of *H. rhamnoides* ssp. *sinensis* from 12 different provenances in China varies from 1.1676 mg/100 g to 2.1188 mg/100 g, it is higher than that of Russian sea buckthorn, but lower than that of Canada; Vitamin E content in the dry pulp from three different provenances in China varies from 0.5468 mg/100 g to 1.4525 mg/100 g, which is lower than that of sea buckthorn in Russia. **Table 4** shows that the contents of unsaponifiable matter and phytosterol in sea buckthorn oil of different species. Otherwise, sea buckthorn is also rich in flavonoid (vitamin P) and contains appreciable amounts of water soluble and fat soluble vitamins (Schapiro 1989; Solonenko and Shishkina 1989; Zhang *et al.* 1989).

Sea buckthorn berries contain up to 13% soluble sugars, mainly glucose, fructose and xylose, and 3.9% organic acids, mainly malic and succinic acid (Ma and Cui 1989). Sea buckthorn is rich in proteins and free amino acids. A total of 18 amino acids have been found in sea buckthorn fruit (Mironov 1989; Zhang *et al.* 1989). There are at least 24 chemical elements present in sea buckthorn juice, e.g. nitrogen, phosphorous, iron, manganese, boron, calcium, aluminum, silicon and others (Tong *et al.* 1989; Zhang *et al.* 1989; Wolf and Wegert 1993).

Table 5 shows that difference among seed and dry-pulp oil content of sea buckthorn in different provenance and species or subspecies are bigger, and there are big differences among different varieties of the same provenance or different provenance of the same varieties. Seed oil content varies from 5.62% to 19.51%, and pulp oil from 2.02% to 34.26% (**Table 5**). Oil from the juice and pulp is rich in palmitic and palmitoleic acids (C16:0 and C16:1), while the oil from the seed contains unsaturated fatty acids of C18 type

Table 1 Vitamin C content in sea buckthorn (*Hippophae rhamnoides*) of different provenance (mg/100 g).

Species	Provenance	Juice	Fresh fruit
Hippophae rhamnoides ssp. *rhamnoides*	Weiyuan Gansu	1371.7	1117.84
	Xiahe Gansu	1505.5	1313.6
	Youyu Shanxi	702.3	–
	Wutai Shanxi	962.6	–
	Jiaokou Shanxi	1302.6	–
	Xiaojin Tibet	1380.9	–
H. tibetana	Xiaohe Gansu	157.6	88.64
	Hongyuan Tibet	159.9	–
H. salicifolia	Cuona Tibet	1479.9	–
H. rhamnoides ssp. *yunnanensis*	Zhongxun	1129.1	–
H. rhamnoides ssp. *turkestanica*	Huocheng	472.9	–
	West Pamirs	327.6	–
H. rhamnoides ssp. *mongolica*	Buerjing	383.3	–
	Aertai	394.9	–
H. gyantsensis	Zedang	22.3	–
H. rhamnoides ssp. *stellatopilosa*	Daocheng	3.8	–

"–": it was not determined. Data cited from Lian *et al.* 2000.

Table 2 Carotenoid content in *H. rhamnoide*s ssp. *sinensis* oil of different provenance (mg/100 g).

Provenance	Oil kind	Carotenoid	β-carotene
Qingan Gansu	Seeds	78.13	2.50
Jianping Liaoning	Seeds	104.17	1.30
	Pulp	368.50	12.56
Datong Qinghai	Seeds	51.25	0.97
Liangcheng Neimeng	Seeds	24.22	0.85
Nanxian Shaanxi	Seeds	50.00	3.17
	Pulp	632.50	80.73
Wutai Shanxi	Seeds	133.30	31.40
	Pulp	1375.00	5.44
Huanglong Shaanxi	Seeds	50.00	3.77
Weixian Hebei	Seeds	35.71	1.68
Wenshui Shaanxi	Pulp	365.80	–

"–": it was not determined. Data cited from Zheng and Ruan 2002.

Table 3 Vitamin E content in *Hippophae rhamnoides* oil of different provenance (mg/100 g).

Species	Provenance	Oil kind	a-V_E	V_E
ssp. *sinensis*	Qinan Gansu	Seeds	0.7228	1.8002
	Jianping Liaoning	Seeds	1.2869	2.1188
	Datong Qinghai	Seeds	0.6358	1.3966
	Doulan Qinghai	Seeds	0.6788	1.1985
	Liangcheng	Seeds	0.5464	1.3619
	Nanxian Shanxi	Seeds	0.4554	1.1821
		Dry-pulp	1.1385	1.4525
	Wutai Shanxi	Seeds	0.7806	1.6327
		Dry-pulp	0.5756	0.7563
	Youyu Shanxi	Seeds	0.7934	1.9195
	Huanglong Shaanxi	Seeds	0.7802	1.549
	Zhulu Hebei	Seeds	0.5249	1.1676
	Chicheng Hebei	Seeds	0.5875	1.3940
	Laiyuan Hebei	Dry-pulp	0.5468	0.5468
ssp. *mongolica*	Russia	Seeds	–	1.05-1.20
		Dry-pulp	–	1.30-1.60
	Canada	Seeds	0.603	3.077

V_E: Vitamin E; "–": it was not determined. Data cited from Jiang 1991 and Huang and Xiao 1991.

Table 4 The contents of unsaponifiable matter and phytosterol in sea buckthorn oil of different species.

Species	Oil kind	Unsaponifiable matter (%)	Phytosterol (mg/100 g)
Hippophae rhamnoides ssp. *sinensis*	Seeds	2.04	1430.1
	Pulp	1.75	—
H. neurocarpa	Seeds	1.94	804.2
	Pulp	2.28	—
H. tibetana	Seeds	1.67	967.7
	Pulp	3.07	—
H. rhamnoides ssp. *mongolica*	Seeds	1.40	—
	Pulp	2.40	—
H. rhamnoides ssp. *turkestanica*	Seeds	1.95	1237.6
	Pulp	2.14	—

"—": it was not determined. Data cited from Lian *et al.* 2000.

Table 5 Oil content of sea buckthorn.

Species	***Hippophae rhamnoides* ssp. *sinensis***											
Provenance	Jianping Liaoning		Sichuan		Menyuan Qinghai		Shanxi		Gansu		Hebei	
Oil kind	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp
Oil content (%)	7.8-11.9	9-18.2	9.87	2.02	9.57	2.38	10.37	8.44	7.97	7.03	10.68	12.07
Species	***H. rhamnoides* ssp. *neurocarpa***				***H. rhamnoides* ssp. *mongolica***							
Provenance	Sichuan		Qilian Qinghai		Altai		Qinghe Xinjiang		Hasake		Xiboliya	
Oil kind	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp
Oil content (%)	16.12	8.60	5.62	34.26	11-13.1	2.8-7.8	7.46	16.28	9.00	23.01	12.31	8.00
Species	***H. gyantsensis* ssp. *yunnanensis***				***H. rhamnoides* ssp. *turkestartica***							
Provenance	Jiangzi Tibet		Sichuan		Urumqi							
Oil kind	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp						
Oil content (%)	5.97	20.32	10.21	2.59	8.04	22.57						
Species	***H. salicifolia***				***H. tibetana***							
Provenance	Linzhi Tibet		Qilian Qinghai		Sichuan							
Oil kind	Seeds	D-pulp	Seeds	D-pulp	Seeds	D-pulp						
Oil content (%)	7.59	4.91	14.57	15.26	19.51	3.50						

D-pulp: Dry-pulp, data cited from Lu 1988, Ma 1988, Zhang *et al*. 1989, Lian *et al*. 2000, Zheng and Ruan 2002.

oils (linoleic and linolenic acid). In addition, sea buckthorn berries, leaves and bark contain sitosterol, tocopherol and many other bioactive compounds (Mironov 1989).

MEDICINAL USES AND SEA BUCKTHORN PRODUCTS

Medicinal uses

Medicinal properties of sea buckthorn has been ascribed to its important phytochemicals, like flavonoids, carotenoids, fatty acids, etc. It has been found that β-carotene (Sadek and Abou-Gabal 1999), flavonoids, fatty acids etc, are important medicinal phytochemicals. Medicinal uses of sea buckthorn are well documented in Asia and Europe. Investigations on modern medicinal uses were initiated in Russia during the 1950's (Gurevich 1956). In the United Kingdom and Europe, sea buckthorn products are used in aromatherapy. Sea buckthorn was formally listed in the "Pharmacopoeia of China" in 1977 (Xu 1994).

Preparations of sea buckthorn oils are recommended for external use in the case of burns, scalds, ulcerations, infections, bed sores, and other skin complications induced by confinement to a bed or treatment with X-ray or radiation (Zhang *et al*. 1988). It is an ingredient in sun block. *Hippophae* oil has UV-blocking activity as well as emollient properties and is an aid in promoting regeneration of tissues (Zhao 1994; Goel *et al*. 2002).

Sea buckthorn is traditionally used for the treatment of stomach and duodenal ulcers and laboratory studies confirm the efficacy of the seed oil for this application (Xing *et al*. 2002). The antiulcerogenic effect of a hexane extract from *H. rhamnoides* was tested on indomethacin- and stress-induced ulcer models. As a result hexane extract from *Hippophae* was found to be active in preventing gastric injury (Suleyman *et al*. 2001).

Research in the late 1950's and early 1960's reported that 5-hydroxytryptamine (hippophan) isolated from sea buckthorn bark inhibited tumor growth (Pukhalsskaia 1958; Sokoloff *et al*. 1961). Clinical studies on the anti-tumor functions of sea buckthorn oils conducted in China were positive (Zhang *et al*. 1989). A reports on the potential of a *Hippophae* extract (an alcohol extract mainly containing flavonoids) to protect the bone marrow from damage due to radiation showed that the extract might help faster recovery of bone marrow cells (Agrawala and Goel 2002). In China, a study was done to demonstrate faster recovery of the hemopoietic system after high dose chemotherapy in mice fed sea buckthorn oil (Chen 2003). The seed oil was found to enhance non-specific immunity and to provide anti-tumor effects in preliminary laboratory studies (Yu 1993).

Sea buckthorn oil, juice or the extracts from oil, leaves and bark have been used successfully to treat high blood lipid symptoms, eye diseases, gingivitis and cardiovascular diseases such as high blood pressure and coronary heart disease (Liu *et al*. 1980; Ge *et al*. 1985; Eccleston *et al*. 2002). *Hippophae* is used as an anti-cardiovascular medicine (Chai *et al*. 1989; Yang and Kallio 2002). In a double-blind clinical trial, 128 patients with ischemic heart disease were given total flavonoids of sea buckthorn at 10 mg each time, three times daily, for 6 weeks. The patients showed a decrease in cholesterol level and improved cardiac function; also they had fewer anginas than those receiving the control drug. No harmful effect of sea buckthorn flavonoids was noted in renal functions or hepatic functions (Zeb 2004). The blockade of activation of NF-κ-B might be a potential access to the improvement in myocardial function with the use of total flavonoids of *Hippophae* for treatment of hypertension and chronic cardiac insufficiency (Xiao *et al*. 2003). Dried *Hippophae* emulsion could adjust the abnormal blood fat and have anti-oxidation function (Yang 1995). It was found that antioxidants rich sea buckthorn juice affects the risk factors (plasma lipids, LDL oxidation, platelet aggregation and plasma soluble cell adhesion protein concentration) for coronary heart disease in humans (Eccleston *et al*. 2002).

A clinical trial demonstrated that sea buckthorn extracts helped normalize liver enzymes, serum bile acids and immune system markers involved in liver inflammation and degeneration (Gao *et al*. 2003). In addition, sea buckthorn oil protects the liver from damaging effects of toxic chemicals, as revealed in laboratory studies (Cheng 1990). A combination of an antiviral drug and sea buckthorn in treating patients with chronic hepatitis-B could shorten the duration for the normalization of serum ALT (Huang *et al*. 1991).

Sea buckthorn products

Since the discovery of the nutritional value of sea buckthorn, hundreds of sea buckthorn products made from the berries, oil, leaves, bark and their extracts have been developed. In Europe sea buckthorn juice, jellies, liquors, candy, vitamin C tablets and ice-cream are readily available (Morzewski and Bakowska 1960; Bernáth and Földesi 1992; Wolf and Wegert 1993). It is also used in Eastern Europe as a food colorant and a fabric dye. Examples of commercial products available are: Biodoat® sold in Austria, Exsativa® a vitamin supplement sold in Switzerland, sea buckthorn syrup in France, liqueurs in Finland, and 'Homoktovis Nektar', an apple-based fruit juice sold in Hungary. Sea buckthorn jams and jellies are produced on a small scale in Saskatchewan. Most of the product is made and utilized by individuals; one Saskatchewan gourmet food processor has been test marketing sea buckthorn jelly (Bep Hamer pers. com).

At present, the largest producers and consumers of sea buckthorn products are China, Russia, and Mongolia. They all have large scale processing facilities. Raw and processed products include: seed oil, fruit oil, raw juice, concentrate juice, juice, alcoholic beverages, candies, ice-cream, tea, jam, biscuits, vitamin C tablets, flavone powder, flavone-soft and life-soft capsule, seed oil-soft capsule, fruit oil-soft capsule, pulp powder, dry fruit, fruit dew, fruit juice, fruit wine, food colors, medicines, cosmetics, beauty capsules and shampoos (Iirkina and Shishkina 1976; Pan *et al*. 1989; Huang *et al*. 1990; Niu 1991; Wu 1991; http://www.shaji.cn/En_B_1.asp).

Oils and oil extracts are the most important sea buckthorn products produced in Russia. These oils are processed and sold as essential oils for numerous medicinal and therapeutic uses. Fruit drinks were among the earliest sea buckthorn products developed in China (http://www.shaji.cn/En_B_1.asp). These drinks have had strong market demand and excellent consumer acceptance. They have rapidly gained a reputation as both a satisfying drink as well as a nutritional beverage that enhances stamina and vitality. Sea buckthorn based juices are also popular in Germany and Scandinavia (H. Albrecht pers. com.).

Cosmetic applications for sea buckthorn are well known in Russia and China. In Russia, sea buckthorn berries are often used in home made cosmetics. Recipes for moisturizing lotions, dandruff control and hair loss prevention are widely known and used in Russia (Pashina 1993). Sea buckthorn oils contain high concentrations of palmitoleic acid. This rare fatty acid is a component of skin fat and can support cell tissue and wound healing. It is generally accepted in the cosmetic industry that sea buckthorn oils have unique anti-aging properties and as a result are becoming an important component of many facial creams manufactured in Asia and Europe. In addition, the UV-spectrum of the oil shows a moderate absorption in the UV-B range which makes sea buckthorn derived products attractive for sun care cosmetics (Quirin and Gerard 1994). The Body Shop, a well established cosmetic chain, uses sea buckthorn oil in their sun screen products as a sun-blocking and tan-enhancing agent. The potential of sea buckthorn oils for dermatological applications such as face masks, body lotions, sun lotions and shampoos is excellent. Clinical research and development in this area is currently under way in Europe as well as Canada (C. Wells, pers. com.).

SELECTING AND BREEDING FOR IMPROVING YIELD AND QUALITY

Among the eight subspecies of *H. rhamnoides*, *H. rhamnoides* ssp. *sinensis* and *H. rhamnoides* ssp. *mongolica* are mainly and widely distributed in China, Russia, Mongolia and other countries in Asia and Eastern Europe. To improve their adaptability, tolerance, yield, quality, and commercial aims, selection and breeding programmes for these two subspecies have been running since the 1960s, and some varieties have been successfully bred and selected in China, Russia and Mongolia, India, Germany, Finland, Canada and other countries. In these programmes, many breeding methods were used: conventional breeding, crossing, mutation breeding (radiation, physical and chemical mutation), polyploidy and apomixis breeding, and others, and over 100 fine cultivated varieties in the world were selected and bred. In **Table 6** the breeding method, origin and characters of 41 Russia varieties are shown. According to the data of a breeding programme in Finland, cvs. 'Terhin' and 'Tytin' contain, respectively, 185-250 and 215-360 mg of ascorbic acid per 100 g of unfrozen fruits (Karhu 2000). In Germany, Mr. H-J. Albrecht is the pioneer of *H. rhamnoides* breeding, with his work having resulted in the production of the varieties 'Frugana', 'Askola', 'Hergo', 'Leikora' and 'Dorana', which have come to be known worldwide, and which were allowed to be planted in Brandenburg and Mecklenburg-Vorpommern, Germany but also in Italy and Chile. Russia and China are leading countries in the area of domestication, breeding, and cultivation of *H. rhamnoides*, and many varieties bred by Chinese and Russian breeders have been planted world-wide; only in China are there about 130,000 ha of new artificial sea buckthorn plantations, and the mean fresh fruit yields in natural and artifical *H. rhamnoides* are 180-375 kg/ha/year and 4500-6000 kg/ha/year, respectively, while fine, big-fruit sea buckthorn yield 12000-22,500 kg/ha/year in China (www.engine. cqvip.com/content/f/96567x/2004/000/006/jj36_f3_10013435.pdf; www.sdxnw.gov.cn/city/h z/kjxl.asp).

Breeding in China

In China, *H. rhamnoides* breeding research has markedly progressed since 1985, guided by the principles of multi-purpose hybridization, multi-level selection, advances in introduction, hybridization and plant multiplication by both sexual and asexual means, and implementation through multi-region tests and multi-unit cooperation (Huang 2005).

Varieties from China (*H. rhamnoides* ssp. *sinensis*) have good adaptability to harsh environments (e.g., drought, salinity) and are fast-growing. Five new elite cultivars, such as 'Hongxia' and 'Wucixiong' were created through multi-level selection from Chinese local sea buckthorns (Zhao 1996), the former has a hundred-fruit weight of 28 g and yield of 12,000 kg/ha, while the latter has strong proliferation ability, and no thorns in most situations.

Varieties from Russia and Mongolia (*H. rhamnoides* ssp. *mongolica*) show many promising agronomic traits, such as big fruit, few thorns, a long fruit-stalk, higher contents of some bioactive substances, and resistance to dried-shrink disease (*Plowvigneia hippophaeos*) in some varieties (Du 2002). To supply and improve sea buckthorn germplasm, and to gain commercial benefits, many varieties from Russia and Mongolia have been introduced into China since 1991. Some of them that could adapt to their new habitat and showed certain economic benefits are being planted widely in China. Fifteen cultivars, including 'Wulanshalin', 'Hunjin' and 'Wucifeng', were selected from seedlings of

Fig. 1 Fine F_1 generation selected from the crosses between *H. rhamnoides* ssp. *mongolica* and *H. rhamnoides* ssp. *sinensis*. (Picture from http://www.icrts.org/chinese/news_view.asp?id=354).

Table 6 Varieties and their characters = sea buckthorn (*Hippophae rhamnoides* ssp. *mongolica*) in Russia.

Varieties	Breeding methods	Characters
Aertaixinwen	Selection from free pollination of the seedlings of Katong population.	Height ~4 m; no thorns; length = fruit stalk ~3 mm; mean weight = fruit 0.5 g; mean fresh fruit yield per plant = 3.2 kg; fruit with dry matter = 14.2%; sugar = 5.5%; acid = 1.7%; oil = 5.5%; V_c = 47 mg/100 g; carotene = 0.43 mg/100 g; resistance to dried-shrink disease.
Katuniliping		Height ~3 m; middle thorns; length = fruit stalk ~4.5 mm; mean weight = fruit 0.4 g; mean fresh fruit yield per plant = 14.0-16.7 kg; fruit with sugar = 5.49%; acid = 1.7%; oil = 6.5-6.9%; V_c = 69.5 mg/100 g; carotene = 2.8 mg/100 g; tolerance to cold.
Jinhui		Few thorns; mean weight fruit 0.5 g; mean fresh fruit yield per plant = 15.2-16.4 kg; fruit with sugar = 5.8%; acid = 1.45%; oil = 7.4-8.3%; V_c = 66 mg/100 g; carotene = 2.8 mg/100 g; susceptive to cold and drought.
Youyong		Few thorns; length = fruit stalk ~4.7-5.8 mm; mean weight fruit 0.37 g; mean fresh fruit yield per plant = 11.2 kg; fruit with sugar = 4.09%; acid = 1.6%; oil = 7.2%; tanning = 0.059%; V_c = 64 mg/100 g; carotene = 7.6 mg/100 g; resistance to dried-shrink disease.
Weishengsou		No thorns; length = fruit stalk ~4.0 mm; mean weight fruit 0.5-0.6 g; mean fresh fruit yield per plant = 7.5-8.0 kg; fruit with sugar = 4.5%; acid = 1.6%; oil = 5.0%; dry matter = 10%; V_c = 112 mg/100 g; carotene = 3.7 mg/100 g.
Jingse	Cross between 'Xieerbingkayihao' and wild sea buckthorn of Katong population.	Height ~2.7 m; few thorns; length = fruit stalk about 2.0-3.0 mm; mean weight fruit 0.8 g; fruit with sugar = 5.4-7.2%; acid = 1.8%; oil = 7.2%; tanning = 0.059%; V_c = 64 mg/100 g; carotene = 7.6 mg/100 g; resistance to dried-shrink disease.
Ebi		Mean weight fruit 0.8 g; mean fresh fruit yield per plant = 10.6-14.0 kg; fruit with sugar = 4.37%; acid = 1.81%; oil = 5.6-8.3%; V_c = 135-170 mg/100 g; carotene = 2.8-4.5 mg/100 g.
Xibeiliya		No or few thorns; mean weight fruit 0.9 g; mean fresh fruit yield per plant = 11.9-15.4 kg; fruit with sugar = 5.28%; acid = 1.7%; oil = 5.89-8.50%; V_c = 116-172 mg/100 g; carotene = 2.14-2.95 mg/100 g.
Juren		Height ~3m; middle thorns; length = fruit stalk ~4.5 mm; mean weight fruit 0.4 g; mean fresh fruit yield per plant = 14.0-16.7 kg; fruit with sugar = 5.49%; acid = 1.7%; oil = 6.5-6.9%; V_c = 69.5 mg/100 g; carotene = 2.8 mg/100 g; tolerance to cold.
Hupo		No thorns; length = fruit stalk ~3-5 mm; mean weight fruit 0.6-0.7 g; mean fresh fruit yield per plant = 9.5-10.0 kg; fruit with sugar = 7.4%; acid = 1.68%; oil = 6.0-6.6%; V_c = 189 mg/100 g; carotene = 6.4 mg/100 g; resistance to cold.
Hunjin		Height ~2.4m; few thorns; length = fruit stalk ~3-4 mm; mean weight fruit 0.7 g; mean fresh fruit yield per plant = 14.5-20.5 kg; fruit with sugar = 5.3%; acid = 1.55%; oil = 6.9%; V_c = 133 mg/100 g; carotene = 3.81 mg/100 g; resistance to cold and drought.
Yousheng		Few thorns; length = fruit stalk ~7 mm; mean weight fruit 0.8 g; mean fresh fruit yield per plant = 7-8 kg; fruit with dry matter = 17%; sugar = 7.6%; acid = 1.6%; oil = 5.5%; V_c = 118.2 mg/100 g; carotene = 2.5 mg/100 g; resistance to cold and drought; susceptible to dried-shrink disease.
Fengshou		Height ~3 m; middle thorns; length = fruit stalk ~3-4 mm; mean weight fruit 0.8-0.85 g; mean fresh fruit yield per plant = 16.0-20.3 kg; fruit with sugar = 6.9%; acid = 1.2%; oil = 4.9%; V_c = 142 mg/100 g; carotene = 2.88 mg/100 g.
Jingse-Xibeiliya		Few thorns; length = fruit stalk ~3-5 mm; mean weight fruit 0.8 g; mean fresh fruit yield per plant = 12.6-22.1 kg; fruit with dry matter = 15.4%; sugar = 6.7%; acid = 1.8%; oil = 6-6.4%; V_c = 116 mg/100 g; resistance to cold; susceptible to dried-shrink disease.
Guangming		Few thorns; length = fruit stalk ~4 mm; mean fresh fruit yield per plant = 10-11 kg; fruit with sugar = 6.6%; acid = 2.59%; oil = 5.5 -6.0%; V_c = 196 mg/100 g; carotene = 3.58 mg/100 g.
Chuyi	Cross between 'Chuyi' and 'Chuyi'.	Height about 2.5 m; few thorns; length = fruit stalk about 2-3 mm; mean weight fruit 0.9 g; mean fresh fruit yield per plant = 9.5-10 kg; fruit with sugar = 6.4%; acid = 1.7%; oil = 6.2%; V_c = 134 mg/100 g; carotene = 3.7 mg/100 g.
Chengse	Cross between wild sea buckthorn of Sayianling population and 'Katuniliping'.	Few thorns; length = fruit stalk ~3-4 mm; mean weight fruit 0.8 g; mean fresh fruit yield per plant = 11.2-15.5 kg; fruit with sugar = 6.6%; acid = 1.7%; oil = 6.6%; V_c = 157 mg/100 g; carotene = 3.1 mg/100 g; resistance to dried-shrink disease.
Aliyi	Cross between 'Aertaixingwen' and Katong population.	Male; no thorns; one inflorescence with 17-24 flowers; high pollen yield; strong pollen viable; flower period as the same as other varieties.
Zhiwuyuanliping	Free pollination of Leningrad population.	Few thorns; length = fruit stalk ~6 mm; mean weight fruit 0.8 g; mean fresh fruit yield per plant = 5-23 kg; fruit with sugar = 1.4%; acid = 1.27%; oil = 3.4%; V_c = 65 mg/100 g; carotene = 60.9 mg/100 g; resistance to cold and dried-shrink disease.
Fangxiang		Middle thorns; length = fruit stalk ~4-5 mm; mean weight fruit 0.5 g; mean fresh fruit yield per plant = 6.0 kg; fruit with sugar = 1.7%; acid = 1.3%; oil = 6.1%; V_c = 125 mg/100 g; carotene = 6.4 mg/100 g.
Moscow Ren		Height ~2.0 m; few thorns; length = fruit stalk ~4 mm; mean weight fruit 0.76 g; mean fresh fruit yield per plant = 18 kg.
Xiaohujiao		Few thorns; length = fruit stalk ~5 mm; mean weight fruit 0.7 g; mean fresh fruit yield per plant = 6-7 kg; fruit with sugar = 4.2%; acid = 2.5%; oil = 3.5%; V_c = 200 mg/100 g; carotene = 7.1 mg/100 g.
Huaqiu	Cross between Leningrad sample and Basel sample.	Height ~2-2.5 m; few thorns; length = fruit stalk ~5-6 mm; mean fresh fruit yield per plant = 6.5 kg.
Zhuwuyuanfangxiang		Few thorns; length = fruit stalk ~5.0 mm; mean fresh fruit yield per plant = 18.0 kg.
Luomengruosuofu	Cross between Leningrad kind and Altai kind.	Height ~2.5 m; few thorns; length = fruit stalk ~6.3 mm; mean weight fruit 0.6-0.65 g; mean fresh fruit yield per plant = 14 kg.
Xieerbingkayihao	Free pollination of Sayanling population.	Height ~2.5 m; no thorns; mean weight fruit 0.78 g; fruit with sugar = 7.38%; acid = 1.83%; oil = 2.7%; V_c = 95 mg/100 g; carotene = 0.7 mg/100 g.
Aokahebin	Cross between 'Aertai xingwei' and 'Tongjingsi 40'	Few thorns; mean weight fruit 0.6-0.65 g; mean fresh fruit yield per plant = 5-7 kg.

Table 6 (cont.).

Varieties	Breeding methods	Characters
Achula	Free pollination of wild sea buckthorn in Yagangeer	Height ~1.5 m; middle thorns; length = fruit stalk ~2.3 mm; mean weight fruit 0.4-0.5 g; mean fresh fruit yield per plant = 5.5-6.0 kg; fruit with sugar = 6.8%; acid = 1.9%; oil = 7.6%; V_c = 228 mg/100 g; carotene = 4.2 mg/100 g.
Ayaganka	Free pollination of wild sea buckthorn in Jiemokerao river	Middle thorns; length = fruit stalk ~2-3 mm; mean weight fruit 0.5-0.6 g; mean fresh fruit yield per plant = 4.5-10 kg; fruit with sugar = 9.5%; acid = 2.0%; oil = 5-6.3%; V_c = 185.0 mg/100 g; carotene = 5.3 mg/100 g; resistance to cold and drought.
Caoyuan		Middle thorns; mean weight fruit 0.3 g; mean fresh fruit yield per plant = 4 kg; fruit with sugar = 6.6%; acid = 2.1%; oil = 6.3%; V_c = 196 mg/100 g; carotene = 5.4 mg/100 g.
Telafeim=u	Cross between Leningrad sample and Aertai population	Few thorns; length = fruit stalk ~4.5-5.0 mm; mean weight fruit 1.1 g; mean fresh fruit yield per plant = 6-7 kg; fruit with sugar = 1.76%; acid = 3.46%; oil = 3.85%; V_c = 183 mg/100 g; carotene = 5.8 mg/100 g.
Zhiwuyuan		No thorns; length = fruit stalk ~5-5.5 mm; mean weight fruit 0.8 g; mean fresh fruit yield per plant = 8.0kg; fruit with sugar = 3.1%; acid = 2.5%; oil = 5.6%; V_c = 61 mg/100 g; carotene = 5.1 mg/100 g; resistance to dried-shrink disease and cold.
Woluobiyuefu	Free pollination of Jialininggelei population	Height ~3 m; few thorns; length = fruit stalk ~5-5.5 mm; mean weight fruit 0.9 g; mean fresh fruit yield per plant = 7.0-8.0 kg; fruit with acid = 3.0%; oil = 4-4.5%; V_c = 170 mg/100 g; carotene = 8.6 mg/100 g; resistance to dried-shrink disease.
Bayuexian	Cross between 'Telafeimofu 17397-3' and Jialininggelei population	Mean weight fruit 0.6 g; mean fresh fruit yield per plant = 16 kg.
Daxuesheng	Cross between 'Liningrad 19397-6' and 'Jialininggelei 9343'	Height ~1.5-2.0 m; few thorns; mean weight fruit 0.69 g; mean fresh fruit yield per plant = 12 kg.
Zazhongxiaohuqiu	Cross between 'xiaohuqiu 19397-39' and 'Aertai 8715'	Few thorns; mean weight fruit 0.65-0.8 g; mean fresh fruit yield per plant = 18.5 kg; fruit with sugar = 3.0%; V_c = 223.3 mg/100 g.
Moscow Jinyan	Cross between "Liningrad 17397-6' and Aertai 8715'	Height ~2.5 m; no thorns; length of fruit stalk about 6.5-7.3 mm; mean weight fruit 0.85 g; mean fresh fruit yield per plant = 14 kg.
Moscow Bolo	Free pollination from "17397-6' selected from Liningrad population	Height ~3 m; few thorns; length = fruit stalk ~4.5 mm; mean weight fruit 0.6 g.
Niweilianna	Free pollination of Liningrad population	Height ~2.5 m; few thorns; length = fruit stalk ~5.0 mm; mean weight fruit 0.8 g; mean fresh fruit yield per plant = 30 kg.
Xieerkabin 2	Free pollination of Sayanling population	Height ~2.2 m; few thorns; length = fruit stalk ~3.0-5.0 mm; mean weight fruit 0.82 g; mean fresh fruit yield per plant = 10.0 kg; fruit with dry matter = 14.4%; sugar = 7.0%; acid = 1.8%; oil = 2.5-2.8%.
Xieerkabin 3	Free pollination of Tongjinsi population	Height ~1.5 m; no thorns; mean weight fruit 0.5-0.6 g; mean fresh fruit yield per plant = 6-12 kg; fruit with dry matter = 13.7%; sugar = 5.9%; acid = 2.86%; oil = 2.0%; V_c = 150 mg/100 g; carotene = 0.61 mg/100 g; resistance to cold.

introduced sea buckthorns, having no or few thorns, large fruit and longer fruit stalk, and yields of 10,000-20,000 kg/ha (Zhan *et al*. 2005). For 'Wucifeng', the length of the fruit stalk is about 4.0-5.0 mm, with the following means: weight of fruit, 0.85 g; fresh fruit yield per plant, 20 kg; fresh fruit yield per ha, 23250 kg/year; pulp with sugar, 7.72%; acid, 1.25%; vitamin E, 0.61 mg/100 g; β-carotene, 8.42-26.39 mg/100 g; seed with vitamin E, 9.48 mg/100 g; flavonoids 7.92 mg/100 g (Huang and Zhao 2004).

Varieties from China have small fruit, more thorns, and a short fruit stalk. Varieties from Russia and Mongolia have weak adaptability to harsh environments (e.g., drought, salinity), slow-growth and are prone to dried-shrink disease in some varieties. Introduced varieties in China have unstable adaptability and unreliable yield and quality. One breeding approach to overcome these disadvantages is to crosses *H. rhamnoides* ssp. *sinensis* with *H. rhamnoides* ssp. *mongolica*. Significant heterosis in the F_1 generation of the crosses between *H. rhamnoides* ssp. *mongolica* and *H. rhamnoides* ssp. *sinensis* was identified in hybridization experiments, and three fine lines (**Fig. 1**) were selected from 1500 F_1 individuals, which had some better characters than the parents, e.g. the average plant height of F_1 fine lines was 222 cm (that of *mongolica* and *sinensis* parents was 132 cm and 161 cm, respectively), the hundred-fruit weight was 30.6-41.2 g (that of *mongolica* and *sinensis* parents was 28.0 g and 14.1 g, respectively), fast-growth, strong adaptability, bigger fruit, higher yield per individual (F_1 fine lines was 4.4-5.2 kg, *mongolica* and *sinensis* parents were 1.5 g and 2.4 g, respectively), richer nutrient components in fruit [vitamin C content of F_1 fine lines was 117.0-313.4 mg/100 g (that of *mongolica* and *sinensis* parents was 132.5 mg/100 g and 320.6 mg/100 g, respectively), vitamin E was 6.2-17.5 mg/100 g (that of *mongolica* and *sinensis* parents was 18.2 mg/100 g and 5.8 mg/100 g, respectively), β-carotene was 1.9-6.1 mg/100 g (that of *mongolica* and *sinensis* parents was 1.6 mg/100 g and 7.9 mg/100 g, respectively)] and a stronger resistance to disease (Lu *et al*. 2005; http://www.icrts.org/chinese/news_view.asp?id=354). Several hybrid clones, including 'Hualin 1' and 'Tianshui 1', were selected from the descendant populations of various parent combinations (Huang 2005).

Breeding in Russia

In Russia, conventional breeding programmes of *H. rhamnoides* started in 1933 by the LRIHS (Lisavenko Research Institute of Horticulture for Siberia), and at that time seeds were collected from the natural *H. rhamnoides* forest near the Katun river, and three varieties ('Altainews', 'Katouniniping' and 'Jinsuishaji') were created in 1964. For 70 years, more than 40 varieties of *H. rhamnoides* have been bred at the LRIHS, such as 'Novost Altaya', 'Maslichnaya', 'Dar Katuni', 'Zolotoy Pochatok', 'Vitaminnaya', 'Zivko', 'Chuyskaya', 'Chulishmanka', 'Chechek', 'Tenga', 'Inya', 'Elizaveta', 'Altayskay', 'Avgustina' and 'Agurnaya', etc. Their yield ranges from 7.5 to 18.0 t/ha, with a 100-berry mass of 62-120 g (maximum 140 g), pedicel length of 3-6 mm, 4.0-8.0% seed oil content, 15.0-48.0 mg carotenoids/100 g of fruit, 5-10% sugars, and no or few thorns in most varieties (Zubarev 2005). Selection was done by selecting the best seedlings from the best starting forms by hybridization, using a small amount of chemical mutagens (0.2% dimethyl sulfide and 0.01% *N*-nitroso-*N*-methylurea by treating seeds for 24 h). The total annual volume of selection results in about 100 cross combinations, and about

50,000 hybrid seeds are produced. After strong selection only 50-60 new hybrids are used in further work. At present, there are 45,000 hybrid sea buckthorn cultivars in the LRIHS (Zubarev 2005).

During 1975-1980, using conventional breeding, seven varieties were selected and bred by researchers in the Experimental Station of Fruit Tree and Berry of Breyat, including 'Ayagang', 'Achula', 'Grass', 'Bayangguoer', 'Nalang', 'Beijiaerlubing' and 'Dabatechaoxia' (Miahahoba 1998); Some fine lines were selected and bred by crosses between 'Vitamin' and 'Tongjin', 'Masili' and 'Tongjin', and 'Altainews' and 'Tongjin', which have no or few thorns, low plant height, 100-fruit weight of 50-90 g and yield per tree of 5-10 kg (Miahahoba 1998).

In addition, at the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, the following berry cultivars were developed: 'Zyrianka', 'Druzhina', 'Podruga', 'Zolotoi Cascad', 'Siberskii rumianets', 'Krasnyi fakel', 'Ivushka', 'Ognistaya', 'Capris', which have universal and table applicability for industrial and amateur gardening, with a high carotenoid content in fruits and good transportability and packaging (http://www.sbras.nsc.ru/expo-en/expo-view.asp?id1=628).

TISSUE CULTURE IN *HIPPOPHAE RHAMNOIDES*

Tissue culture in *H. rhamnoides* including callus and organ culture has seen great progress in many aspects since 1988, such as the selection and sterilization of explants, selection of optimal media to induce callus, buds and roots, prevent browning and transplant tube seedlings; however a large-scale production system has still not yet been constructed, and micropropagation techniques are still only focused at the experimental level. There are no reports on the somatic embryogenesis and on cryopreservation/long-term presservation of germplasm or synthetic seed production.

Organ culture, plant regeneration and acclimatization

Micropropagation is the asexual propagation of plants from growing tissue – be it whole plant tissue (cuttings) or callus (loose, unorganized cellular growth) (Lummerding 2001). In organs culture of sea buckthorn, different explants were selected in different studies, including the root apex (Yao *et al*. 1995), apical meristem (Burdasov and Sviridenko 1988; Sun 2005), axillary meristem (Yao *et al*. 1995; Guo and Xu 2000; Li *et al*. 2001; Zhou *et al*. 2006), stem segments (Li *et al*. 2001; Lummerding 2001; Kang *et al*. 2002), axillary buds (Xu and Liang 2001; Lu *et al*. 2002), leaves (Zhou *et al*. 2006), and different organs of aseptic seedlings (Montpetle and Lalonde 1988; Xu and Liang 2001). **Table 7** shows the optimal components and concentrations of initiation, multiplication and rooting media in organ culture of *H. rhamnoides* applicable for different explants and varieties, or even for different lines within the same variety. Among the reported studies of organ culture (**Table 7**), there were only a few reports of successful plant regeneration, and the regeneration rate was lower, even less than 10%. Yao (1995) built a micropropagation system of *H. rhamnoides* using root apices and stem explants; WPM (Woody plant media, Lloyd and McCown 1981) was the optimal initiation and multiplication media. Lummerding (2001) successfully micropropagated *H. rhamnoide* ssp. sinensis varieties (S4 and S5 lines) using chokecherry media (SBM, Lummerding 2001) with 50% macro-elements of MS basic media, but only 11 of the 427 rooted plants could survive transplanting.

Clonal propagation using vegetative cuttings of sea buckthorn is widely reported in the greenhouse and field, including hardwood (Hai and Niu 2006) and semi-hardwood (or softwood) (Shan 2006) cuttings. In **Table 8** we show the different kinds of cuttings, basic media or soil, cutting treatment, management of seedling cuttings, and survival rate of sea buckthorn cuttings. To make seedling cuttings in the field, two methods may be used: the first is by not removing seedlings and leaving them *in situ* through winter. In this case, the cutting period is prior to the middle ten days of July in which new branches are strong with a 0.3 cm diameter, with over three full buds at the middle or top parts, an extensive root system 0.4 cm in diameter. The second method involves pre-planting. In this case, cutting time is after the middle ten days of July, in which new branches are weak, there are no full buds, and there is slight rooting (Zhou 2005).

Table 7 Tissue and organ culture in *Hippophae rhamnoides*.

Varieties or lines	Explants	Optimal initiation medium	Optimal multiplication medium	Optimal rooting medium	Reference
Kaniya, 90-3-2, 90-2-14 (*H. rhamnoides* ssp. *mongolica*)	Apical meristem	MS + NAA 0.03 mg/L ~0.05 mg/L + KT 0.3 mg/L ~ 0.5 mg/L		MS + NAA 0.002 mg/L + KT 1.0 mg/L	Sun 2005
92-1-18, 92-5-15, 92-3-1, 92-7-8 (*H. rhamnoides* ssp. *mongolica*)	Meristem	MS + IBA 0.01 mg/L + BA 0.2 mg/L			Guo and Xu 2000
H. rhamnoides ssp. *sinensis*	Stem segments, lateral bud	½B5 + IBA 0.4 mg/L + sucrose 1.5 % + agar 0.46 %		½B5 + IBA 0.2 mg/L + IAA 0.2 mg/L	Zhao *et al*. 1989
	Cotyledon, hypocotyl, and meristem of aseptic seedling	¼MS + 6-BA 0.30 mg/L + NAA 0.002 mg/L	¼MS + NAA 0.05 mg/L + IBA 0.2 mg/L	¼MS + NAA 0.05 mg/L + IBA 0.2 mg/L	Xu and Liang 2001
Yousheng (*H. rhamnoides* ssp. *mongolica*)	Stem segments	½MS + 6-BA 1.0 mg/L + IAA 0.5 mg/L	⅓MS + 6-BA 0.5 mg/L + NAA 0.2 mg/L	¼MS + NAA 0.03 mg/L + IBA 0.1 mg/L	Kang *et al*. 2002
H. rhamnoides ssp. *sinensis*, Hybrids of Qiuyisikke (*H. rhamnoides* ssp. *mongolica*) and *H. rhamnoides* ssp. *sinensis*	Stem segments	½B5 + 6-BA 0.5 mg/L + IAA 0.5 mg/L	½B5 + 6-BA 0.5 mg/L + IAA0.5 mg/L	½B5 + IAA (0.3-0.5 mg/L)	Yang *et al*. 2004
Shiyou 1 (*H. rhamnoides* ssp. *mongolica*)	Meristem	½MS + 6-BA 1.0 mg/L + IAA 0.5 mg/L	½MS + 6-BA 2.5 mg/L + IAA 0.2 mg/L		Zhou *et al*. 2006
	Hydroponic leaves	½MS + 6-BA 0.5 mg/L + KT 0.2 mg/L + NAA 0.02 mg/L	½MS + 6-BA 0.5 mg/L + KT 0.2 mg/L + NAA 0.02 mg/L	½B5 + 6-BA 0.3 mg/L + IBA 0.4 mg/L	
	Bud	B5 + 6-BA 0.5 mg/L + NAA 0.5 ~ 1.0 mg/L	½B5 + 6-BA 0.5 mg/L +NAA 0.5 mg/L	½B5 + 6-BA 0.1 mg/L +NAA 1.0 mg/L	Zhou *et al*. 2005

Media: MS = Murashige and Skoog (1962); B5 = B_5 basic media (Gamborg 1968).
Plant growth regulators: 6-BA = 6-benzyladenine; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; KT = kinetin; NAA = α-naphthaleneacetic acid.

Table 8 Hardwood and softwood cutting seedlings of sea buckthorn.

Varieties	Cutting site	Kind of Cutting	Basic media or soil	Cutting treatment	Management	Survival rate
ssp. *mongolica*: 'Hunjin', 'Xiangyang', 'Chuyi'; ssp. *sinensis*: 'Zhong-guowuci'; Hybrid: 'Zhongezajiao'	Greenhouse	Softwood, branches with apical bud, the length of 15-20 cm	Chestnut soil : dust-color forest soil = 1:1	50 mg/kg indolebutyric acid, 50 mg/kg ABT-2 stimulate rooting	Automatic spray, temperature of 25-28ºC, relative humidity of 85-95%	81.4%-93.2%[1]
Hippophae rhamnoides ssp. *sinensis*	Field	Hardwood, middle and basic of 2-3 years branches	Loessial soil and ray warp soil	After dipping cutting 2-4 days with move water, 200 ppm ABT-1	Common management	94.4%[2]
		Hardwood cutting, middle and basic of 1-2 years branches	Common soil: vermiculite:organic manure = 5:4:1	200 mg/kg ABT2	Plastic film house	73.5%[3]
		Softwood from 3-6 years plants, with apical bud and leaves	Fluvial heavy sand	100 mg/kg ABT1	Full illumination spray	97.5%[4]
		Softwood, optimal cutting time from June 20 to July 5	Mixture of ash and slay with sand, or Fluvial heavy sand	50 mg/L ABT1 or ABT2	Full illumination spray	82%[5]
Qiji 92-5-2 (ssp. *mongolica*)	Field	Hardwood cutting of 2 years branches	Soil and organic manure	100 mg/kg ABT2	Hand spray water	50%[6]
Qiuyisike C3, C4, C17 (ssp. *mongolica*)	Field in dry desert region	Hardwood cutting, the length of 20 cm	Soil and sand	Mixture of cutting with sand, and rooting under the condition of inverse insertion	Hand supplied water	40-79%[7]
'Hongxia', 'Sunmiao', 'Jufeng', 'Juda', 'Jida' (ssp. *sinensis*)						22-78%[7]
H. tibetana	Field	Softwood of one year branches	Sand, soil, horse dung	50 mg/L ABT1 dipping cutting 2 h	Common management	86%[8]
Qiji 1 (ssp. *mongolica*)	Greenhouse	Softwood of one year branches	Ash and slag, loessial soil	50 mg/kg indolebutyric acid dipping cutting 12 h	Optimal cutting time is July, automatic spray	91.2%[9]

Note: ABT: a plant growth regulator.
References: 1 = Qi *et al*. 2007; 2 = Lian 1994; 3 = Wang and Yu 2006; 4 = Zhang *et al*. 2001; 5 = Li *et al*. 2006; 6 = Yang *et al*. 2004; 7 = Luo *et al*. 2000; 8 = Li 2002; 9 = Gong *et al*. 2006.

There are no reports on the acclimatization of *in vitro*-regenerated plants, however the acclimatization of seedling cuttings of sea buckthorn is fine-tuned based on our experience. Our convictions are based on the fact that our seedling cuttings in fields at Fuxin, Liaoning province of China could grow normally and set fruit in different cultivated regions (e.g. Shaanxi, Shanxi, Hebei, Beijing, Gansu Province, etc.), and their yields and chemical and bioactive characters are similar to those of its parent clones.

Callus culture

Callus culture is import for *H. rhamnoides*, not only because the seedlings by callus differentiation producing adventitious buds might be used in rapid propagation and screening of useful mutants, but also large-scale callus culture might be used in extracting useful, active ingredients or secondary metabolites. Among the cotyledon, radicle, hypocotyl, and apical meristems of aseptic *H. rhamnoides* ssp. *sinensis* seedlings, the rate of callus induction of cotyledons (90.3%) was the highest on optimal induction medium, i.e. ¼MS + 0.3 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid) (Xu and Liang 2001). For bud explants, optimal callus induction media was of ⅔MS + 0.5 mg/L 6-BA (6-benzyladenine) + 0.05 mg/L NAA (α-naphthalene acetic acid), and the rate of callus induction was as much as 100%; regenerated plants could be further multiplied on medium containing ½MS + 0.3 mg/L 6-BA + 0.05 mg/L NAA and rooted on ½MS + 0.2-0.5 mg/L IAA (indole-3-acetic acid) (Li *et al*. 2001). For hydroponics using the apical meristem, optimal callus induction medium was ½MS + 1.0 mg/L 6-BA + 0.5 mg/L IAA (Zhou *et al*. 2006). For stem segments, the callus induction rate was over 90% on ½B_5 + 0.5-1.0 mg/L 6-BA + 0.5 mg/L NAA (details of B_5 basic media in Gamborg (1968)), and every callus piece differentiated 6.9-10.8 adventitious buds, while the seedling rate was over 90% (Zhou *et al*. 2005).

Prevention of browning

The phenolic content of *in vivo H. rhamnoides* is high, and stem segments, apical buds and leaves brown easily at the initiation of culture, and serious callus browning after subculture affects the capacity of explants to absorb nutrients and may even result in the death of explants (Zhou *et al*. 2006). In *H. rhamnoides* tissue culture, certain procedures are effective for decreasing browning in culture of aseptic seedlings (Kang *et al*. 2002), e.g. dipping explants into vitamin C solution (200 ppm) (Yang *et al*. 2004) and the addition of cysteine (0.05 mg/L) to the medium (Zhou *et al*. 2006). Despite this browning is still one of the main reasons hindering plant regeneration.

GENETIC DIVERSITY AND RELATIONSHIP AT THE MOLECULAR LEVEL

Genetic diversity

Genetic diversity in sea buckthorn provides a good opportunity for plant breeding and selection while clinal variations in growth rhythm, height and hardiness provide guidelines for seed and plant transfer as well as for plant introduction.

Plant breeders can use this information to design their breeding plans to obtain an ideal type with certain growth periods, maturity times and plant height for a particular region or cultivation technology (Yao and Tigerstedt 1994; Tang and Tigerstedt 2001). Studies exist on the genetic diversity of sea buckthorn based on molecular studies (isozymes, RAPD, ISSR) in Europe, Canada and China (Jeppsson *et al*. 1999; Chowdhury *et al*. 2000; Ruan 2004; Tian *et al*. 2004a; Ruan 2006). Yao and Tigerstedt (1993) used isoenzymes (peroxidase, superoxide dismutase, catalase, and glutathionine peroxidase) to demonstrate a within-population genetic diversity of 0.168. They also noted that populations of *H. rhamnoides* ssp. *rhamnoides* were more diverse (as revealed by Nei's genetic distance of 0.037) than *H. rhamnoides* ssp. *sinensis* (0.007) (Yao and Tigerstedt 1993). Based on a 5-enzyme (peroxidase, esterase, amylase, malate dehydrogenase and superoxide dismutase) system study on eight lines (two belonging to *H. rhamnoides* ssp. *sinensis*, and other six of *H. rhamnoides* ssp. *mongolica*), 83.4% of genetic variation was attributed to the differentiation among lines, while the remaining 16.6% resided within each variety (Li 2003). Bartish *et al*. (1999, 2000, 2001) studied ten populations of *H. rhamnoides* ssp. *rhamnoides* from Northern Europe using RAPD markers; in one population of *H. rhamnoides* ssp. *mongolica* they estimated that 85% of the genetic variation was within populations, and 15% between populations. Tian *et al*. (2004b) studied the genetic diversity of *H. rhamnoides* from 300 individuals of fifteen natural populations in China using ISSR (inter-simple sequence repeats) markers. The mean genetic diversities detected in *H. rhamnoides* ssp. *yunnanensis*, *H. rhamnoides* ssp. *sinensis*, and *H. gyantsensis* were 0.194, 0.217, and 0.137, respectively. Based on ISSR statistics, Zhou's (2005) results indicated that the genetic variation coefficient of *H. rhamnoides* ssp. *yunnanensis* and *H. rhamnoides* ssp. *sinensis* populations were 0.219 and 0.213, respectively; gene flow (*N*m) of *H. rhamnoides* ssp. *yunnanensis* and *H. rhamnoides* ssp. *sinensis* populations were 1.784 and 1.851, respectively, which were lower than average of gene flow of outcrossing and wind-pollinated species (*N*m = 5.380).

Genetic relationship

Knowledge of genetic relationships in parental varieties could improve the effectiveness of breeding programmes (Le Thierry d'Ennequin *et al.* 2000). Ruan *et al*. (2004) studied genetic relationships among 14 sea buckthorn varieties from China, Russia and Mongolia using RAPD markers, and the results showed that genetic similarities ranged from 0.45 to 0.80, with a mean of 0.67, and that genetic distances ranged from 0.23 to 0.80, with a mean of 0.40. Cluster analysis based on RAPD markers identified 'Zhongguoshaji' from China (*H. rhamnoides* ssp. *sinensis*) as the most genetically distinct cultivar. 'Hongguo' (*H. rhamnoides* ssp. *sinensis*), 'Zhongguoyou' (*H. rhamnoides* ssp. *sinensis*), the hybrid 'Liaohuerhao' from China, 'Zeliang' and 'Huoguang 'from Russia (*H. rhamnoides* ssp. *mongolica*) were more distantly clustered. The seven cultivars from Russia and Mongolia (*H. rhamnoides* ssp. *mongolica*) and the hybrid 'Liaohuyihao' from China were grouped into two main clusters. Cluster I contained 'Chengsi', 'Liaohuyihao', 'Xiangyang', 'Chuyi' and 'Wulangemu'. Cluster II consists of 'Nuyou', 'Hongyun' and 'Aleiyi' (Ruan *et al*. 2004). It is difficult to distinguish HG from HY by phenotypic characteristics, however, the RAPD results clearly showed the two to be distinct (Ruan *et al*. 2004). Sun *et al*. (2002) analyzed the phylogenetic relationships among 15 taxa of the genus by comparing sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA). In the strict consensus trees of parsimony analysis, the monophyly of *Hippophae* was supported by a 100% bootstrap value. *H. tibetana* was at the basal position of the genus, and the remaining taxa formed two clades with a high boot strap support. The species *H. tibetana*, *H. neurocarpa* and *H. salicifolia* were all distinctly positioned. The monophyly of *H. rhamnoides* were supported when *H. rhamnoides* ssp. *gyantsensis* was excluded (Sun *et al*. 2002). Based on the cpDNA and morphological data, monophyly of *Hippophae* was strongly supported, and the most widespread species, *H. rhamnoides*, was, in spite of low support, most likely monophyletic and distinguished by a single molecular synapomorphy (Bartish *et al*. 2002). *H. goniocarpa* ssp. *goniocarpa*, and *H. goniocarpa* ssp. *litangensis* were clearly not monophyletic, i.e. a single lineage of evolution, but rather were sister to two different species in the analyses, and they believed that two subspecies were results of two independent hybridizations which were described as species (Bartish *et al*. 2002). The results based on cpDNA *trnL-F*, *trnS-G* and *ncpGS* data indicated that *H. salicifolia* was the most primitive taxon in the genus of *Hippophae*, and that *H. goniocarpa* ssp. *litangensis* was a homoploid hybrid (Zhang 2005). The incongruence between gene trees validated the homoploid hybrid origin of *H. goniocarpa* ssp. *goniocarpa* between *H. rhamnoides* ssp. *sinensis* and *H. neurocarpa* ssp. *neurocarpa*.

Fifteen sea buckthorn cultivated varieties from China, Russia and Mongolia were fingerprinted with AFLP markers (Ruan and Li 2005). The fingerprinting patterns obtained allowed unequivocal identification of each cultivated varieties. Some AFLP primer combinations were better suited to discriminate among cultivated varieties; E+CC/M+AT could detect a higher number of polymorphic loci in all cultivated varieties analyzed. Two RAPD primers OPA11 and OPA13 could distinguish three sea buckthorn germplasms and their female, including Chinese (*H. rhamnoides* ssp. *sinensis*), Russian (*H. rhamnoides* ssp. *mongolia* × *H. rhamnoides* ssp. *turkestanica*) and Mongolian (*H. rhamnoides* ssp. *mongolica*) sea buckthorn (Sheng 2004).

The resulting information of genetic relationships based on molecular data could also be used as a guideline for germplasm collection and the development of crossing strategies in a breeding programme. For example, 'ALY' is adaptable to habitats in Shannxi, Liaoning, Heilongjiang and Jiangsu Provinces, China, and has promising characteristics (e.g., big fruit, and high vitamin C content in seeds and leaves). Genetic similarity between 'ALY' and 'ZGSJ', based on AFLP data, was relatively low (0.585) (Ruan and Li 2005), so crosses between 'ALY' and 'ZGSJ' may be useful in breeding.

PROSPECTS

Tissue culture

As the experimental varieties or lines increase, tissue culture in sea buckthorn has great advantages, however there are some problems seriously restricting the efficiency of tissue culture: (1) Basal media (MS, B_5, WPM, SBM) and their modifications and common hormones have been selected in most experiments; these selections are narrow, and need to be broadened; (2) Explant selection is simple, with mostly researchers selecting the meristem and stem segments as explants. There are few reports that use leaves from plants in the field or from hydroponics, but no reports yet using pollen, anther, embryo or endosperm as explants, all of which have import significance in plant tissue culture, and which need to be explored; (3) It influences the survival rate at transplanting to the low rooting rate of test-tube seedlings, the poor rooting quality and the partial roots deriving from callus. In the future, one way to enhance the self-supporting ability and vigor of rooted seedlings is to produce root nodules using nodulating bacteria to infect roots. This may improve the survival rate of transplanting, because sea buckthorn is a nitrogen-fixing plant, and its roots in symbiosis with actinomycetes could form root nodules with high nitrogen-fixing efficiency (Wang *et al*. 1997; Qu *et al*. 1998; Wu *et al*. 2002).

Table 9 Different purposes need various chemical and nutrient compounds for better sea buckthorn utilization.

Utilization	Chemical or nutrient compounds	Reasons	Reference
Medicine against cardiovascular disease	Varieties with higher content of flavonoids in the seed or pulp oil	Diets containing a rich supply of flavonoids may play a significant role in protection against cardiovascular disease and some cancers.	http://www.icrts.org/news/document/tea.htm
Cardiovascular health	Sea buckthorn tea from the leaves of the varieties with higher content of magnesium	Magnesium may play a very important role in maintaining cardiovascular health post myocardial infarction. Magnesium also plays an important part in ridding blood vessels of cholesterol deposits and maintaining cardiovascular health.	
Medicine against atherosclerosis	Varieties with higher content of flavonoids in seed or pulp oil	Flavonoids may help slow down atherosclerosis (a deposit of cholesterol leading to a narrowing of the blood vessels and restriction of blood flow).	
Medicine against gastric ulcer	Varieties with higher content of amino acids, organic acids, chlorogenic and phenols	A lot of amino acids, organic acids, chlorogenic and phenols can promote the composition of hydrochloric acid, promote rehabilitation of the damaged tissue and secrete of gastric juice with the function of helping digestion, nourishing the stomach, invigorating the spleen, comforting the liver, refreshing the breath, and healing gastric ulcer.	http://www.icrts.org/news/document/oil.htm
Medicine against cancer	Varieties with higher content of leucocyanidin, quassin, coumarin	Leucocyanidin, quassin, coumarin contained in sea buckthorn have a significant function to resist the action of cancer, control and kill cancer cells, stop the growth of cancer-inducing factors, and increase cancer resistance by promoting the patient's immune system, reduce the side effect of short-term treatment. It has a better treatment effect especially on stomach and esophageal cancer, and carcinoma of the rectum.	
Healing dosage of burning, scaling	Varieties with higher content of Vitamin E, carotene, carotenoid, unsaturated fatty acids	There are rich bio-active substances such as Vitamin E, carotene, carotenoids, unsaturated fatty acids, etc., contained in sea buckthorn oil which can promote the metabolism and do good to damaged tissue recovery, block inflammation and promote the healing of ulcers.	
Protection of liver	Varieties with higher content of malic acid and oxalic acid	Organic acids like malic and oxalic acid stored in sea buckthorn have the function of liver protection and relieving the poison and side effects of antibiotics and other drugs. Phosphatide compounds such as lecithin have very high bio-activities, effectively promoting the metabolism of cells, improving the liver function, resistance to fatty liver and liver cirrhosis. In addition, sea buckthorn oil has a significant protecting effect to heart, liver, lungs and bone marrow.	
Sweet juice	Varieties with higher content of sugar, and lower content of acid		Ruan and Li 2000
Cosmetic	Varieties with higher content of vitamin C and superoxide dismutase		

Biotechnology in sea buckthorn breeding

The great success of sea buckthorn is explained by its great eco-environmental function, rich biochemical composition, especially, by the presence of unique oil in fruits. Because of these, the main aims for breeding in many countries are to increase the contents of oil in berries and improve its adaptability to harsh environments. In commerce, different purposes need various chemical and nutrient compounds (**Table 9**), and breeding is one of the keys to achieving this in the future. In improving the environment and sustainable development sea buckthorn, gradually breeding fine lines with resistance to dried-shrink disease, carpenter moth (*Holcocerus hippophaecolus*) and drought are being developed. Sea buckthorn varieties in Altai accumulate a high quantity of carotenoids, and therefore this oil finds its basic application in medicine and is unsuitable for cosmetic purposes (Zeb 2004). At the same time, varieties in Europe are more suitable for cosmetics (Zubarev 2005). As a rule, varieties with high oil content have small berries; they are difficult to harvest and are only moderately productive. On the other hand, varieties with moderate oil contents of fruits (5.0-8.0%) (**Tables 5, 6**), but distinguished by high yield and productive harvesting. However, up to now, methods in sea buckthorn breeding have been traditional or conventional, such as selection, crossing, physical and chemical mutation, and radiation. Though these methods bred many fine varieties, they take time to breed, and the efficiency is limited in some aspects (e.g. improving resistant to salinity, drought, and cold, breeding new varieties with the resistance to disease, and breeding new varieties with a high content of objective traits). Molecular biotechnology may be able to provide some solutions to the present problems restricting sustainable development of sea buckthorn, such as resistance to dried-shrink disease and carpenter moth, drought intolerance, and others.

In order to select and breed varieties of sea buckthorn resistant to dried-shrink disease, the following molecular biotechnologies in our lab are being or will be used: (1) Marker-assisted selection (MAS). First, double pseudo-test-crosses between lines with resistance to dried-shrink disease and lines susceptible to dried-shrink disease are being performed, and two gene pools of resistant and susceptible to dried-shrink disease are being constructed by the BSA (Bulked Segregation Analysis) methods: one is resistant to disease, another is susceptible to disease. Second, molecular markers linked with resistance to dried-shrink disease will be screened. Total genomic DNA will be extracted from leaf tissue from two gene pools, different molecular markers (e.g. SSR, ISSR, RAPD and AFLP) linked with disease resistance will be screened out. Finally, varieties with high resistance to dried-shrink disease will be bred by MAS. Other, disease-resistant varieties in present cultivated varieties or lines of sea buckthorn will be identified and screened by the molecular markers linking with disease resistance. (2) Map-based cloning for genes controlling resistance to dried-shrink disease, including the construction of a genetic linkage map based on RAPD, AFLP, ISSR and SSR markers, the screening of molecular markers connected with the target gene, the orientation of the target gene, the construction of a large fragment genome library as well as the screening and identification of the target gene. (3) Cloning genes related with resistance to dried shrink disease based on mRNA differential display reverse transcriptional PCR (DDRT-

PCR), including the construction of cDNA libraries of sea buckthorn susceptible and resistant to dried shrink disease, the screening of anti-disease cDNA fragments by fluorescent DDRT-PCR, the gain of genes with resistance to dried-shrink disease, and the gene expression profile of sea buckthorn induced by dried-shrink disease. Currently, parts of these works are being conducted in our lab, and we expect to get fine resistant lines by genetic transformation and MAS in 3-5 years.

In addition, there are still no studied reports on the genetic engineering of sea buckthorn, e.g. transgenic sea buckthorn and secondary metabolite production in bioreactors. This may be because an optimal tissue culture system has still not been developed, which could be used as a base of transgenic studies. Genetic engineering is important for improving the quality and resistance of sea buckthorn to biotic and abiotic factors, and the next step aught to be the establishment of optimal tissue culture and transgenic systems.

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REFERENCES

* in Chinese with English abstract

Agrawala PK, Goel HC (2002) Protective effects of RH-3 with special reference to radiation induced micronuclei in mouse bone marrow cells. *Indian Journal of Experimental Biology* **40**, 525-530

Bartish GI, Jeppsson N, Bartish IV, Nybom H (2001) Assessment of genetic diversity using RAPD analysis in a germplasm collection of sea buckthorn. *Agricultural and Food Science in Finland* **9**, 279-288

Bartish IV, Jeppsson N, Bartish GI, Lu R, Nybom H (2000) Inter- and intraspecific genetic variation in *Hippophae* (Elaeagnaceae) investigated by RAPD markers. *Plant Systematics and Evolution* **225**, 85-101

Bartish IV, Jeppsson N, Nybom H (1999) Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by random amplified polymorphic DNA (RAPD) markers. *Molecular Ecology* **8**, 791-802

Bartish IV, Jeppsson N, Swenson U, Nybom H (2002) Phylogeny of *Hippophae* (Elaeagnaceae) inferred from parsimony analysis of chloroplast DNA and morphology. *Systematic Botany* **27**, 41-54

Burdasov VM, Sviridenko EI (1988) Production of regenerates of sea buckthorn from apical meristems in *in vitro* culture. *Sibirskii Vestnik Sel'skokhozyajstvennoi Nauki* **2**, 106-110

Chen F (2000) *Introduction and Cultivar of Big Fruit Sea Buckthorn*, Shijietushu Press, Beijing, China, pp 48-64

Chen YM, Chen YQ (2003) Mechanism of hydrology and soil and water conservation effect of artificial sea buckthorn forest in Loess Hilly Region. *Acta Botanica Boreali-Occidentalia Sinica* **23**, 1357-1361*

Cheng J, Kondo K, Suzuki Y, Ikeda Y, Meng X, Umemura K (2003) Inhibitory effects of total flavones of *Hippophae rhamnoides* L. on thrombosis in mouse femoral artery and *in vitro* platelet aggregation. *Life Sciences* **72**, 2263-2271

Chowdhur YMA, Jana S, Schroeder WR (2000) Phenotypic diversity in four woody species on the Canadian prairies. *Canadian Journal of Plant Science* **80**, 137-142

Du HJ (2002) Identification and screen of resistance to dried-shrink disease of sea buckthorn cultivars. *Hippophae* **15**, 13-14*

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirement of suspension culture of soybean root cells. *Experimental Cell Research* **9**, 195-198

Ge X, Shi G, Zhang Y (1985) Application of sea buckthorn in medicine. *Shanxi Medicine Research* **2**, 9-14*

Goel HC, Prasad J, Singh S, Sagar RK, Kumar IP, Sinha AK (2002) Radioprotection by herbal preparation of *Hippophae rhamnoides*, RH-3, against whole body lethal irradiation in mice. *Phytomedicine* **9**, 15-25

Gong CW, Chai CG, Zhang JY (2006) The studies on the technique of rapid reproduction of *Hippophae rhamnoides*. *Pratacultural Science* **23**, 65-67*

Guo CH, Xu YX (2000) Tissue culture of stem apex of superior lines of sea buckthorn. *Hippophae* **13**, 26-27*

Gurevich SK (1956) The application of sea buckthorn oil in ophthalmology. *Vesttn Oftalmologii* **2**, 30-33

Hai XY, Niu CS (2006) Study on shoot cutting breeding and forestation of big fruit sea buckthorn. *Journal of Qinghai Agriculture and Forestry Science and Technology* **3**, 13-14, 17*

Heinz C, Barbaza M (1998) Environmental changes during the Late Glacial and Post-Glacial period in the central Pyrenees (France): new charcoal analysis and archaeological data. *Review of Palaeobotany and Palynology* **104**, 1-17

Hou XL, Bai GS, Cao QY (1995) Contrast study on soil infiltration capacity and anti-scourability in *Robinia pseudoacacia*, *Caragana microphylla* and *Hippophae rhamnoides* woodlands. *Journal of Soil and Water Conservation* **9**, 90-95*

Huang DL, Chang XZ, Gui HN, Tian YD, Chen LX, Li ZP, Xing L (1991) Analysis of 156 cases of chronic hepatitis treated with sea buckthorn. *Zhongxiyi Jiehe Zazhi* **11**, 697-698*

Huang Q (2005) The research progress on sea buckthorn breeding in China. *The 2nd International Sea Buckthorn Association Conference*, August 26-29, 2005, Beijing, China, 2

Huang Q, Zhao H, Wang B, Zong D (1991) The development and utilization of sea buckthorn in Mongolia. *Hippophae* **2(3)**, 43-46*

Huang Q, Zhao Y (2004) Selection and breeding of Wucifeng and Shengqiuhong sea buckthorn. *Hippophae* **17(4)**, 7-9*

Huang YQ, Xiao YY (1991) Comparative study on the vitamin E content in Shanxi sea buckthorn. *China Journal of Chinese Materia Medica* **11(9)**, 24-27*

Iirkina MI, Shishkina EE (1976) Sea buckthorn food products. *Sadovodstov* **8**, 29

Jeppsson N, Bartish IV, Persson HA (1999) DNA analysis as a tool in sea buckthorn breeding. In: Janick J (Ed) *Perspectives on New Crops and New Uses*, ASHS Press, Alexandria, VA, USA, pp 338-341

Jiang ZR (1991) The determination of fat-soluble V_E in sea buckthorn oil and its content variance in refined oil. *Chemistry and Industry of Forest Products* **11(3)**, 34-39*

Kahu S (2000) Testatusti Kestävät. *Puutarha and Kauppa* **37**, 6-7 (in Finnish)

Kang B, Zhang GJ, Lu YL, Li WH, Zhang XH, Zhang JX, Cao JJ (2002) Studies on tissue culture of fine variety of *Hippophae rahmnoides* L. from Russia. *Journal of Northwest Sci-Tech University of Agriculture and Forestry (Natural Science Edition)* **30**, 162-166*

Le Thierry d'Ennequin M, Panau O, Toupance B (2000) Assessment of genetic relationships between *Setaria italica* and its wild relative *S. viridis* using AFLP markers. *Theoretical and Applied Genetics* **100**, 1061-1066

Li GP (2002) Experimental on cutting seedling of *Hippophae tibetana*. *Journal of Gansu Forestry Science and Technology* **27(3)**, 49-50*

Li HM (2003) Study on the intraspecies genetic diversity in sea buckthorn. MSc Dissertation, Inner Mongolia Agricultural University, China, 41 pp*

Li M (2004) The function of protection bio-diversity of sea buckthorn in semi-arid region. *Hippophae* **17(4)**, 17-22*

Li SM, Liu CX, Yang HW, Sun WS (2006) Experimental study on the seedling technique of softwood cutting of *Hippophae rhamnoides* ssp. *sinensis* using full illumination spray. *Hippophae* **19(1)**, 14-16*

Li SW, Fan XF, Lu DP (2001) Studies on callus inducement and plant regeneration of *Hippophae rhamnoides* L. *Acta Botanica Boreali-Occidentalia Sinica* **21**, 262-266*

Lian XB (1994) Effects of ABT powder for striking root in raising seedlings of cottage with *Hippophae* hardwood cutting on dryland. *Hippophae* **7(1)**, 28-31*

Lian YS, Lu SG, Xie XK, Chen XL (2000) Biology and chemical in *Hippophe*. Gansu Science Press, Gansu, China, pp 80-115 (in Chinese)

Liu B, Wu Z, Liu W (1980) Preliminary observation on curing effects of sea buckthorn fruit juice for high blood cholesterol and coronary heart disease. *Acta Academiae Medicinae Sichuan* **11(3)**, 178-182*

Lloyd G, McCown B (1981) Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. *Combined Proceedings of the International Plant Propagators' Society* **30**, 421- 427

Lu RS (1988) Study on the sea buckthorn resources in Sichuan. *Hippophae* **1(2)**, 10-14*

Lu SG, Jin ZP, Wen XF, Gu YK, Jin SY (2005) Study on breeding of eco-economical sea buckthorn types – study on selection standard and variation of F_1 progeny female plant of "Mongolia × Chinese sea buckthorn". *The Globe Sea Buckthorn Research and Development* **3**, 14-19*

Lu YL, Zhang GJ, Kang B (2002) A study on quick reproduction by excised culture of *Hippophae rhamnoides* L. *Journal of Hunan Agricultural University (Natural Sciences Edition)* **28**, 405-407*

Lummerding P (2001) Micropropagation protocol development for sea buckthorn (*Hippophae rhamnoides*) selection for commercial orchard production. Agri-Food Innovation Fund Project # 19980162 Final Report, October 2001. www.agr.gov.sk.ca/afif/Projects/19980162.pdf

Luo HM, Wang ZG, Liu MH, Huang Q (2000) Hardwood cuttings of sea buckthorn in arid desert region. *Hippophae* **13(2)**, 11-12*

Ma Z, Cui Y (1989) Studies on the fruit character and biochemical composition of some forms within the Chinese sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*) in Shanxi, China. *Proceedings of International Symposium of Sea Buckthorn*, Xian, China, 106-112

Ma ZB (1988) Oil content and its chemical components in *Hippiphae rhamnoides* growing in Shanxi. *Hippophae* **1(2)**, 24-27*

Miahahoba H (1998) Breeding achievement of sea buckthorn in Breyat, Russia. *Hippophae* **11**, 23-25*

Mironov VA (1989) Chemical composition of *Hippophae rhamnoides* of dif-

ferent populations of the USSR. *Proceedings of International Symposium of Sea Buckthorn*, Xian, China, pp 67-69
Montpetit D, Lalonde M (1988) *In vitro* propagation and subsequent nodulation of the actinorhizal *Hippophae rhamnoides* L. *Plant Cell, Tissue and Organ Culture* **15**, 189-199
Morzewski S, Bakowska E (1960) The use of the fruit of the sea buckthorn in food production. *Przemysl spozywczy* **12**, 23-25
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497
Niu M (1991) Developing *Hippophae* on a large scale for sharing the sorrows with the country and serving the well-being of the people. *Hippophae* **1(1)**, 1-7*
Pan R, Zhang Z, Ma Y, Sun Z, Deng B (1989) The distribution characters of sea buckthorn (*H. rhamnoides* L.) and its research progress in China. *Proceedings of International Symposium of Sea Buckthorn*, Xian, China, pp 1-16
Pashina L (1993) *Plants and Cosmetics*, Minsk, Byelorussia, 130 pp
Pukhalsskaia EC (1958) The effect of extracts of *Hippophae rhamnoides* upon the development of tumor transplants in animals. *Bulletin of Experimental Biology and Medicine* **45**, 363-366
Qi DF, Ma MC, Li J (2007) Influence of auxine on survival rate of the big fruit *Hippophae rhamnoides* greenhouse layer cottage. *Chinese Agricultural Science Bulletin* **23**, 234-236*
Qu DM, Wang S, Han SH (1998) Nitrogen fixing root nodules and its frankia endophyte of *Hippophae rhamnoides* L. *Acta Botanica Boreali-Occidentalia Sinica* **18**, 60-65*
Quirin KW, Gerard D (1993) Sea buckthorn pulp and kernel oils: valuable lipids for skin care. Unpublished Report, Flavex Natuuextrakte Co., 8 pp
Rousi A, Aulin H (1977) Ascorbic acid content in relation to ripeness in fruit of six *Hippophae rhamnoides* clones from Pyhäranta, SW Finland. *Annales Agricultural Fennnici* **16**, 80-87
Roy PS, Porwal MC, Sharma L (2001) Mapping of *Hippophae rhamnoides* Linn. in the adjoining areas of Kaza in Lahul and Spiti using remote sensing and GIS. *Current Science* **80**, 1107-1111
Ruan CJ (2006) Genetic relationships among some sea buckthorn cultivars from China, Russia and Mongolia using AFLP markers. *Journal of Horticultural Science and Biotechnology* **81**, 409-414
Ruan CJ, Li D (2002) Community characteristics of *Hippophae rhamnoides* forest and water and nutrient condition of the woodland in Loess Hilly Region. *Chinese Journal of Applied Ecology* **13**, 1061-1064*
Ruan CJ, Li DQ (2005) AFLP Fingerprinting analysis of some cultivated varieties of sea buckthorn (*Hippophae rhamnoides*). *Journal of Genetics* **83**, 311-316
Ruan CJ, Li DQ (2000) Review on oil content and bio-chemical components of different species of sea buckthorn. *Shaanxi Forest Science and Technology* **(1)**, 59-63*
Ruan CJ, Qin P, Zheng JW, He ZX (2004) Genetic relationships among some cultivars of sea buckthorn from China, Russia and Mongolia based on RAPD analysis. *Scientia Horticulturae* **101**, 417-426
Ruan CJ, Xie QL, Li DQ (2000) Function and benefit of sea buckthorn improving eco-environment of Loess Plateau. *Environmental Protection* **5**, 30-31*
Sadek IA, Abou-Gabal A (1999) β-carotene lock the effect of acrylamide on liver in the Egyptian toad. *Pakistan Journal of Biological Sciences* **2**, 1100-1101
Schapiro DC (1989) Biochemical studies on some hopeful forms and species of sea buckthorn in USSR. *Proceedings of the International Symposium of Sea buckthorn* (*H. rhamnoides* L.), Xian, China, pp 64-66
Shan SL (2006) Micropropagation technology of softwood cutting of big fruit sea buckthorn. J*ournal of Gansu Forestry Science and Technology* **31(2)**, 51-53*
Sheng LX (2004) The evaluation on main characters and the studies on sex markers of sea buckthorn. MSc Dissertation, Jilin Agricultural University, China, 47 pp*
Sokoloff BK, Funaoka M, Fujisawa CC, Saelhof E, Raniguchi LB, Miller C (1961) An oncostatic factor present in the bark of *Hippophae rhamnoides*. *Growth* **25(5)**, 401-409
Solonenko LP, Shishkina EE (1989) Electrophoretic and amino acid analysis of proteins in the sea buckthorn fruit. *Proceedings of the International Symposium of Sea Buckthorn*, Xian, China, pp 91-95
Suleyman H, Demirezer LO, Buyukokuroglu ME, Akcay MF, Gepdiremen A, Banoglu ZN, Gocer F (2001) Antiulcerogenic effect of *Hippophae rhamnoides* L. *Phytotherapy Research* **15**, 625-627
Sun K, Chen XL, Ma RJ, Li CB, Wang Q, Ge S (2002) Molecular phylogenetics of *Hippophae* L. (Elaeagnaceae) based on the internal transcribed spacer (ITS) sequences of nrDNA. *Plant Systematics and Evolution* **235**, 121-134
Sun LY (2005) A study on sea buckthorn tissue culture and plant regeneration. *The Globe Sea Buckthorn Research and Development* **3**, 28-30*
Swenson U, Bartish IV (2002) Taxonomic synopsis of *Hippophae* (Elaeagnaceae). *Nordic Journal of Botany* **22**, 369-374
Tang XR, Tigerstedt PMA (2001) Variation of physical and chemical characters within an elite sea buckthorn (*Hippophae rhamnoides* L.) breeding population. *Scientia Horticulturae* **88**, 203-214
Tian C, Nan P, Shi S, Chen J, Zhong Y (2004b) Molecular genetic variation in Chinese populations of three subspecies of *Hippophae rhamnoides*. *Biochemical Genetics* **42**, 259-267
Tian CJ, Lei YD, Shi SH, Nan P, Chen JK, Zhong Y (2004a) Genetic diversity of sea buckthorn (*Hippophae rhamnoides*) populations in northeastern and northwestern China as revealed by ISSR markers. *New Forests* **27**, 229-237
Tong J, Zhang C, Zhao Z, Yang Y, Tian K (1989) The determination of the physical-chemical constants and sixteen mineral elements in raw sea buckthorn juice. *Proceedings of the International Symposium of Sea Buckthorn*, Xian, China, pp 132-137
Tsydendambaev VD, Vereshchagin AG (2003) Changes in triacylglycerol composition during ripening of sea buckthorn (*Hippophae rhamnoides* L.) seeds. *Journal of Agricultural and Food Chemistry* **51**, 1278-1283
Vikberg V, Itamies J (1999) Buckthorn (*Hippophae rhamnoides* L., Elaeagnaceae), a new important food plant for the larva of *Tenthredo vespa* Retzius in Finland (Hymenoptera, Tenthredinidae). *Entomologica Fennica* **10**, 201-208
Wahlberg K, Jeppsson N (1992) Development of cultivars and growing techniques for sea buckthorn, black chokeberry, honeysuckle and rowan. Verksamhetsberättelse 1990-1991 report, pp 86-100 (in Swedish with English abstract)
Wang L, Yu J (2006) Experimental study of hard pieces of cutting influence of wild sea buckthorn of Xinjiang of different factors. *Northern Horticulture* **5**, 43-44*
Wang ZK, Guo BP, Yan LY, Zhou HB, Zhai YM (1997) Research of sea buckthorn nodules in fixing nitrogen. *Soil and Water Conservation in China* **3**, 46-48*
Wei TX, Yu XX, Zhu JZ (1998) Litter interception of forests in southwestern Shanxi province. *Journal of Beijing Forestry University* **20**, 1-6*
Wolf D, Wegert F (1993) Experience gained in the cultivation, harvesting and utilization of sea buckthorn. *Cultivation and Utilization of Wild Fruit Crops*, Bernhard Thalacker Verlag GmbH & Co., pp 23-29 (in German)
Wu F (1991) A series of *Hippophae* drugs in the USSR. *Hippophae* **2(2)**, 38-41*
Wu QF, Tian CJ, He XY, Zhou YZ, Han GY (2002) Study on the symbiotic association of *Hippophae rhamnoides* L. (non-leguminous nitrogen-fixing trees) and microbes in pure artificial culture. *Chinese Journal of Ecology* **2**, 22-23*
Xiao Z, Peng W, Zhu B, Wang Z (2003) The inhibitory effect of total flavonoids of Hippophae on the activation of NF-κβ by stretching cultured cardiac myocytes. *Journal of Sichuan University* (*Medical Science Edit*) **34**, 283-285*
Xing J, Yang B, Dong Y, Wang B, Wang J, Kallio H (2002) Effects of sea buckthorn (*Hippophae rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. *Fitoterapia* **73**, 644-650
Xu H, Liang ZS (2001) Studies of tissue culture techniques of *Hippophae* L. *Acta Botanica Boreali-Occidentalia Sinica* **21**, 267-272*
Xu M (1994) The medical research and exploitation of sea buckthorn. *Hippophae* **7(2)**, 32-41*
Yang B, Kallio H (2002) Supercritical CO_2-extracted sea buckthorn (*Hippophae rhamnoides*) oils as new food ingredients for cardiovascular health. *Proceedings of Health Ingredients Europe*, September 17-19, 2002, Paris, France, 7
Yang C (1995) A clinical study of reducing fat and anti-oxidation of dried *Hippophae* emulsion. *Hippophae* **8(3)**, 33-35*
Yang H, Wang S, Su X (1988) A study on the dynamic variation of ascorbic acid in *Hippophae*. *Hippophae* **4(1)**, 41-44*
Yang LP, Zhang HL, Zhao XM (2004) Rapid propagation of sea buckthorn (*Hippophae rhamnoides* L.). *The Globe Sea Buckthorn Research and Development* **2**, 12-16*
Yang RH, Wang YP, Duan XC, Liu SY, Zhang SM (2004) A study on cutting and seedling sea buckthorn with big fruit. *Journal of Northwest Forestry University* **19(3)**, 28-30*
Yao Y, Tigerstedt PMA (1993) Isozyme studies of genetic diversity and evolution in *Hippophae*. *Genetic Resources and Crop Evolution* **40**, 153-164
Yao Y, Tigerstedt PMA (1994) Genetic diversity in *Hippophae* L. and its use in plant breeding. *Euphytica* **77**, 165-169
Yao Y, Tigerstedt PMA, Joy P (1992) Variation of vitamin C concentration and character correlation between and within natural sea buckthorn (*Hippophae rhamnoides* L.) populations. *Acta Agriculturae Scandinavica* **42**, 12-17
Yao YM (1995) Micropropagation of sea buckthorn (*Hippophae rhamnoides* L.). *Agricultural Science in Finland* **4**, 503-512
Yu L (1993) Effects of *Hippophae rhamnoides* juice on immunologic and antitumor functions. *Acta Nutrimenta Sinica* **15**, 280-283*
Zadernowski R, Naczk M, Nowak-Polakowsk AH, Nesterowicz J (2002) Effect of sea buckthorn (*Hippophae rhamnoides* L.) berry extracts on the activity of lipase and lipoxygenase. *Journal of Food Lipids* **9**, 249-258
Zeb A (2004) Important therapeutic uses of sea buckthorn (*Hippophae*): A review. *Journal of Biological Sciences* **4**, 687-693
Zhang H (2005) Homoploid hybrid origin of *Hippophae goniocarpa* subsp. *goniocarpa* – evidence from chloroplast genome and nuclear genome. MSc Dissertation, Northwest Normal University, China, 63 pp*
Zhang JG, Luo HM, Huang Q, Shan JY, Wang CY (2005) A comparative study on berry characteristics of large berry cultivars of sea buckthorn. *Forest*

Research **18**, 643-650*

Zhang P, Ding X, Mao L, Li D, Li L (1989) Anti-tumor effects of fruit juice and seed oil of *Hippophae rhamnoides* and their influences on immune function. *Proceedings of the International Symposium of Sea Buckthorn*, Xian, China, pp 373-381

Zhang RJ, Chang YZ, Yang J, Duan ZQ (2001) Influence of cutting to seedling of hardwood cottage of sea buckthorn. *Hippophae* **14(4)**, 9-11*

Zhang W, Yan J, Duo J, Ren B, Guo J (1989) Preliminary study of biochemical constituents of sea buckthorn berries growing in Shanxi Province and their changing trend. *Proceedings of the International Symposium of Sea Buckthorn*, Xian, China, pp 96-105

Zhang W, Zhang Z, Fan J, Yang J, Li M, Deng Z, Wang G, Zhang F (1988) The preliminary results of experimental observation and clinical application of acute radiodermatis treated with sea buckthorn oil. *Hippophae* **1(1)**, 27-30*

Zhao GL, Liu JL, Zhu B (1989) Tissue culture and plantlet regeneration of *Hippophae rhamnoides*. *Plant Physiology Communications* **25**, 42*

Zhao H, Zhu C, Gao C, Li H, Liu Z, Sun W (1991) Geographic variation of fruit traits of the Chinese sea buckthorn and selection of provenances for fruit use. *Hippophae* **4(3)**, 15-18*

Zhao HZ (1996) Advanced in genetic improvement of *Hippophae* varieties in China. *Hippophae* **9(3)**, 1-4*

Zhao Y (1994) Clinical effects of *Hippophae* seed oil in the treatment of 32 burn cases. *Hippophae* **7(1)**, 36-37*

Zheng Q, Ruan CJ (2002) Review on oil content and bio-chemical components of different provenances of sea buckthorn. *Shaanxi Forest Science and Technology* **(1)**, 73-77*

Zhou DS (2005) Study on the genetic diversity of *Hippophae rhamnoides* subsp. *sinensis* and *Hippophae rhamnoides* subsp. *yunnanensis*. MSc Dissertation, Chinese Academy of Forestry, China, 59 pp*

Zhou J, Yue DM, Chen G, Zhang LJ (2005) *In vitro* propagation of *Hippophae rhamnoides*. *Journal of Anhui Agricultural Science* **33**, 236-237*

Zhou SK, Song XD, Zhang ZQ, Zhai QR, Zhang Y, Zhou FL (2006) A study on tissue culture of fine variety "Shiyou 1" of *Hippophae rhamnoides*. *Journal of Northwest Forestry University* **21(3)**, 67-71*

Zhou ZZ (2005) Over-winter technique of softwood seedling of sea buckthorn. *Hippophae* **18(2)**, 32*

Zubarev YA (2005) The main directions of sea buckthorn breeding program in Siberia. *The 2nd International Sea Buckthorn Association Conference*, August 26-29, 2005, Beijing, China, p 1