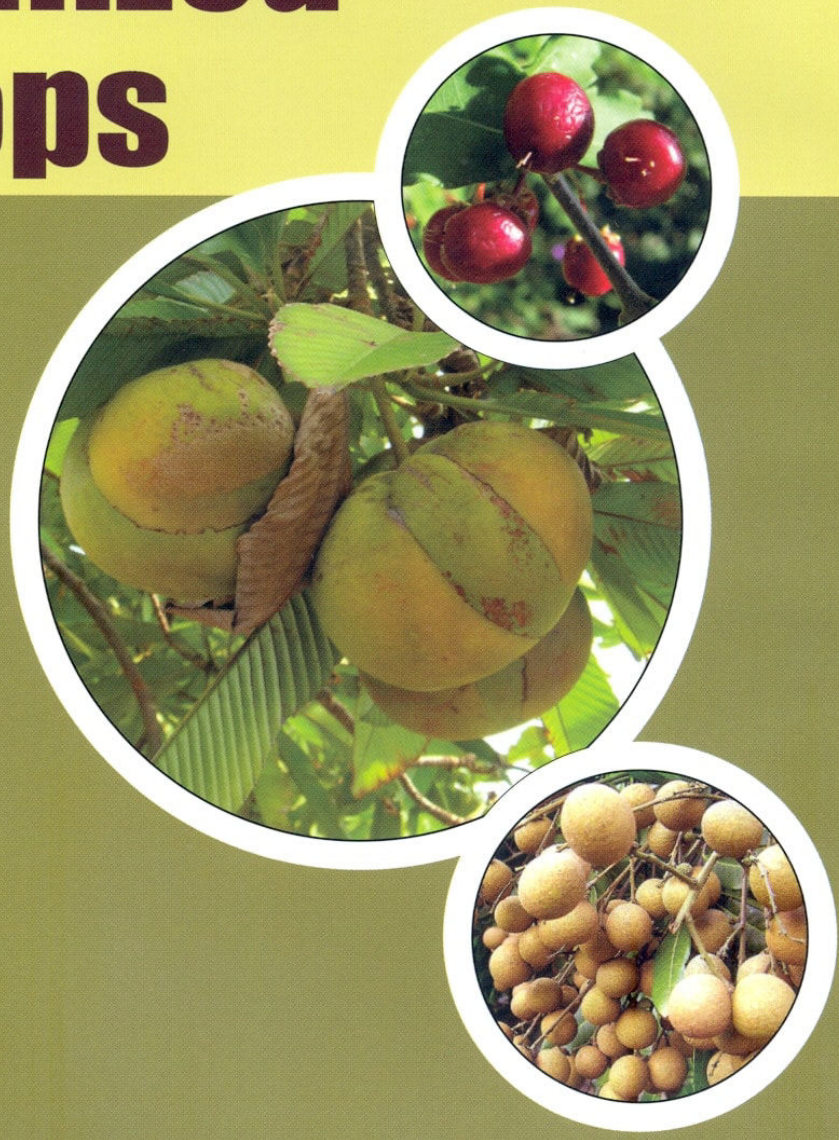
Breeding of Underutilized Fruit Crops

PART-I



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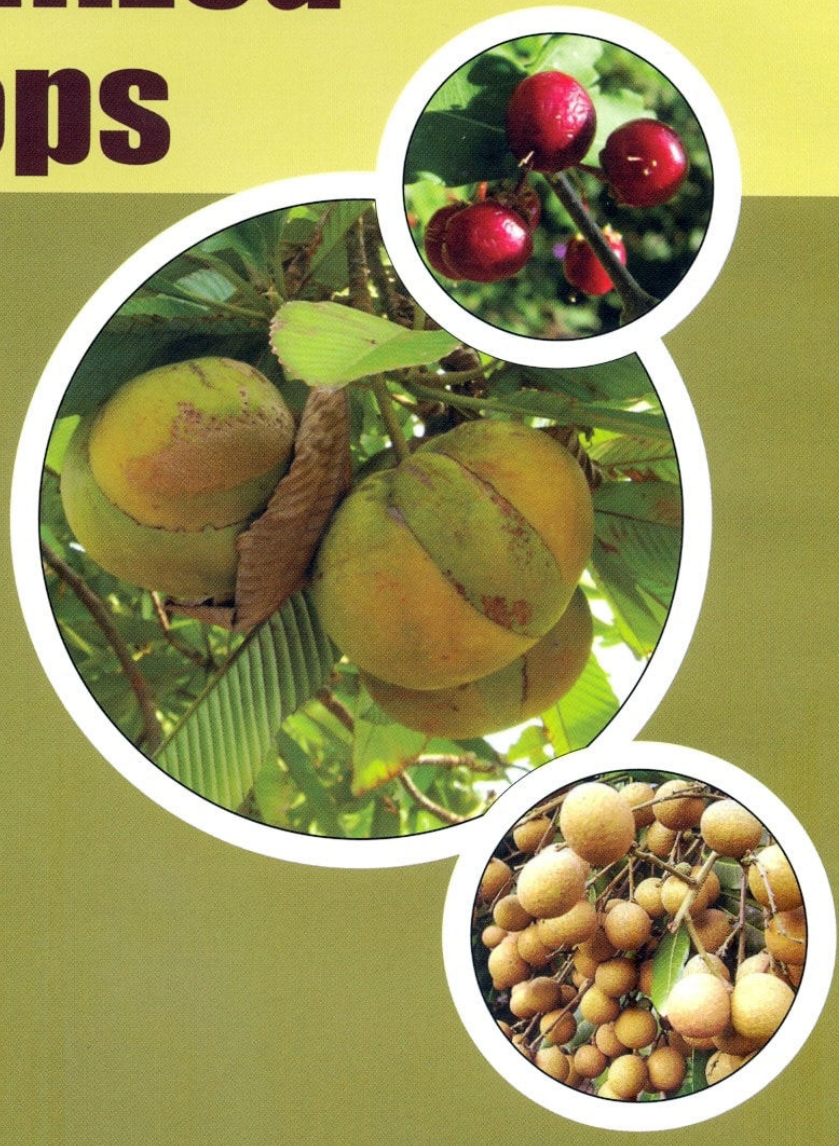
Breeding of Underutilized Fruit Crops

PART-II



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CHAPTER 31

SEABUCKTHORN

(*Hippophae species*)

D.P. Sharma, H.P. Sankhyan and Mirza Musayev

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1. INTRODUCTION

Seabuckthorn (*Hippophae* species) also called sallow thorn belonging to the family Elaeagnaceae is a deciduous shrub with yellow or orange fruits (Li and Schroeder, 1996). It rapidly develops an extensive root system and is therefore an ideal plant for preventing soil erosion (Yao and Tigerstedt, 1994). Seabuckthorn has also been used in land reclamation (Schroeder and Yao, 1995) for its ability to fix atmospheric nitrogen and conserve other essential nutrients (Akkermans *et al.*, 1983). It can withstand temperatures from -43° to 40°C (Lu, 1992). Although, it is considered to be drought resistant (Heinze and Fiedler, 1981), irrigation is needed in regions receiving less than 400 mm of rainfall per year for better growth (Lu, 1992). Legends about Seabuckthorn tell us how the ancient Greeks used it in a diet for race horse; hence, its botanical name *Hippophae* (Shiny horse) was derived. Accordingly to another legend, Seabuckthorn leaves were the preferable food for flying horse-Pegasus. In ancient Greece, sea-buckthorn was known as a remedy for horses. Leaves and young branches were added to the fodder. This resulted in rapid weight gain and a shiny coat for the horse. This, in fact, gave the name to the plant in Latin 'Hippo' - horse, 'phaos' - to shine. One more legend describes *Hippophae* as meaning giving light to a horse of supposed power to cure equine blindness. The name Seabuckthorn might be related to the

fact, that in England the spiny shrubs and trees (it can grow either way, depending on the soil and climate) of Seabuckthorn used to grow in sand dunes along the sea beaches. Similarly the German name Sanddorn for Seabuckthorn may be translated as Sand spine. In a natural seabuckthorn forest, fruits can remain on the branches until the following spring. During this period (usually cold winter), the fruit gradually shrink but do not fall. Therefore they become the favourite food of animals, especially birds.

In China Seabuckthorn industry is estimated to be worth more than Rs. 3000 Crore and global trade based on this single plant is almost Rs. 17000 Crore. China has more than 300 industries, producing a whole range of health foods, cosmetics and medicines based on this plant. At present, the largest producers and consumers of sea-buckthorn products are China, Russia, and Mongolia. They all have large scale processing facilities. Processed products include: oil, juice, alcoholic beverages, candies, ice-cream, tea, jam, biscuits, vitamin C tablets, food colors, medicines, cosmetics and shampoos.

Seabuckthorn was not known commercially in India till the year 2001, i.e., till the emergence of Leh Berry on the national scene and India's contribution from Seabuckthorn in the international market is almost nil till date. Sea-buckthorn fruits are edible, of sour-sweet taste and consumed in fresh and processed form. They are used to prepare compotes, comfitures, jams, juices, jelly bean, nectar, butter, puree, jelly, and teas.

Sea buckthorn fruit is rich in carbohydrates, protein, organic acids, amino acids and vitamins. Fruits contain 16-28 mg carotenoids per 100g fruit which can be used as food additives. Flavonoid content in leaves and fruit ranges from 310-2100mg/100 g air dried leaf and 120-1000mg/100 g fresh fruit, respectively. Total volatile oil from the fruit is 36mg/kg, and essential oil extracted from seeds ranged from 8-12% w/w.

The vitamin C (ascorbic acid) concentration ranges to 360 mg/100 g fruit in European species. Thus, concentration of this vitamin in fruit of Seabuckthorn is higher than in strawberry (64 mg), kiwi (100-470 mg), orange (50 mg), tomato (12 mg), carrot (8 mg) and hawthorn (100-150 mg) as reported by (Lu, 1992). It also contains vitamin B_1 (0.04 mg /100g), vitamin B_2 (0.56 mg/100g) and organic acid (2-4%). As reported by Lu (1992), the vitamin E content in Seabuckthorn (202.9 mg/100g fruit) is also higher than that in wheat embryo (144.5 mg), safflower (3.3 mg), maize (34 mg) and soybean (7.5 mg).

Table 1. Biochemical composition of different Seabuckthorn species.

Species	Hundred fruit weight (g)	Juice extract rate (% of fresh fruit)	Total sugar contents (g/ 100 ml)	Vitamin C (mg/100 ml)	Free amino acids (mg/ 100 ml)	Total acids (g/100 ml)	Vitamin C contents (mg/100 g)
H. rhamnoides	18.3	79.1	6.8	1289.0	83.6	6.2	297.2-1117.8
H. salicifolia	19.0	76.6	10.3	1729.0	264.0	8.3	307.1-1635.1
H. tibetana	40.0	82.5	8.9	159.0	76.4	3.8	530.9-1320.9
H. neurocarpa	4.5	Less	2.1	3.5	666.6	1.6	640.2-1201.2
H. gyantsensis	6.5	33.5	3.7	23.4	65.7	2.2	806.4-1907.2

Seabuckthorn is also high in protein, especially globulins and albumins (Soloneko and Shishkina, 1983) and fatty acids such as linoleic and linolenic (Lu, 1992). It is a good source of various free amino acids and microelements such as iron, potassium, calcium, phosphorous, magnesium, sodium, cobalt, selection, molybdenum, etc.

Medicinal uses of sea buckthorn are well documented in Asia and Europe. Sea buckthorn oil is approved for clinical use in hospitals in Russia. In China, it was formally listed in the "Pharmacopoeia" in 1977. More than ten different drugs have been developed from sea buckthorn in these countries and are available in different forms (e.g., liquids, powders, plasters, films, pastes, pills, liniments, suppositories, aerosols, etc. The most important pharmacological functions of sea buckthorn oil can be summarized as diminishing inflammation, disinfecting bacteria, relieving pain, and promoting regeneration of tissues. It also can be used for skin grafting, cosmetology, and treatment of conceal wounds.

2. DOMESTICATION

The use of sea-buckthorn has a very long history, especially on the Tibetan Plateau. In the 8th century, the medical use of sea-buckthorn was mentioned in the Tibetan medical classic the 'rGyud bzi' – the four parts of pharmacopoeia, written by Yu Tuo Yuan Dan Kong Bu and completed during the Chinese Tang Dynasty (618 to 907 AD). The 'rGyud bzi' gives 84 different set prescriptions for the preparation of sea-buckthorn medicines. During the 13th century, the 'rGyud bzi' was disseminated through Mongolia and sea-buckthorn began to be used in traditional Mongolian medicines. In the Qing Dynasty (1821 to 1850), the Mongolian scholar, Losan Quepei, wrote a 120-chapter book – A Selection of Traditional Mongolian Medicine, 13 chapters of which document the properties of sea-buckthorn and its effects in clinical cases. There are 37 different set preparations based on sea-buckthorn.

Domestication of sea buckthorn started in Siberia in the 1930s (Kalinina and Panteleyeva, 1987). Local germplasm (ssp. *mongolica*) from the Altai Mountains was used in the onset of the breeding. Breeding projects have, later on, been initiated also in other countries such as Germany (Albrecht, 1990), Finland (Yao and Tigerstedt, 1994), China (Huang, 1995), and Canada (Li and Schroeder, 1996). At SLU-Balsgård, Sweden, breeding of sea buckthorn started in 1986. Conventional breeding methods, including germplasm evaluation, hybridization and selection, are used (Trajkovski and Jeppsson, 1999). Recently use the PCR-based method RAPD (Random Amplified Polymorphic DNA) to study population structure in the ssp. *rhamnoides* and to find a marker linked to gender determination.

First, in the world factory on production of sea-buckthorn oil was built in 1949 in the Altai (Biysk city). But the limited stocks of sea-buckthorn and capacity of the factory could not satisfy increasing requirements for medical products from seabuckthorn fruits. For solving the problem of raw material supply the need in finding new stands of this species, extension of existing populations' area through planting and establishment of

plantations, need for studies on various forms, introduction in culture and development of varieties were felt. Broad researches on studying and selection of economic and valuable forms of the sea-buckthorn, growing in various botany-geographical areas were conducted in the Research Institute of Horticulture of Siberia named after M. A. Lisavenko. The first sowings of the seeds, collected from promising forms of sea-buckthorn, have been made in 1934-1935 by M. A. Lisavenko. Numerous forms of wild-growing sea-buckthorn have been studied by Z. I. Gatin. Later in result of breeding works numerous sea-buckthorn varieties were developed.

With the gradually worsening quality of the environment and the overriding poverty in many countries today, sea-buckthorn has attracted a great deal of attention from scientists and engineers all over the world because of its concentrated ecological and socio-economical benefits.

3. TAXONOMY

Hippophae L. belongs to family *Elaeagnaceae*, a small family with three genera, the others being *Elaeagnus* L. with approximately 40 species and *Shepherdia* L. with only three species. The family has a wide distribution in Eurasia, Northeast Australia, and North America. All genera of *Elaeagnaceae* have nitrogen-fixing bacteria in their root nodules.

Taxonomically there are three major *Hippophae* species namely *H. rhamnoides* Linn., *H. salicifolia* D. Don and *H. tibetana* Schlecht (Rousi, 1971). A fourth species, *H. neurocarpa* was later on described in 1978 (Liu and He, 1978).

The genus *Hippophae* is systematically well defined and differs clearly from other genera of family *Elaeagnaceae,* viz., *Hippophae rhamnoides, Hippophae salicifolia* D. Don, *Hippophae tibetana* Schlechtendal and *Hippophae neurocarpa,*. Seabuckthorn belongs to family Elaeagnaceae, with chromosome no x = 12 and 2n = 24 with diploid chromosome number in *Hippophae*. Till now, only four species and nine sub species of *Hippophae rhamnoides* are reported so far form the world over as listed below:

- *Hippophae rhamnoides* sub species *sinensis*
- *Hippophae rhamnoides* sub species *turkestanica*
- *Hippophae rhamnoides* sub species *yunnanesis*
- *Hippophae rhamnoides* sub species *mongolica*
- *Hippophae rhamnoides* sub species *caucasia*
- *Hippophae rhamnoides* sub species *carpatica*
- *Hippophae rhamnoides* sub species *fluviatillis*
- *Hippophae rhamnoides* sub species *rhamnoides*
- *Hippophae rhamnoides* sub species *gyantsensis*

(The sub species has recently got the status of species based on taxonomic evolutionary relationship).

4. CENTRE OF ORIGIN/DIVERSITY

The genus *Hippophae* L. originated in the Himalayan mountain regions and then spread to southwest, northwest and northern China and eastern Inner Mongolia, as well as to the northwest regions of Eurasia where one route progressed west to reach the Alps via the Caspian and Black seas before finally arriving at the northwest shore of the Scandinavian peninsula and another route progressed northwest to reach northwestern Mongolia and southern Siberia in the Russian Federation via India, Nepal, Pakistan, Afghanistan and several Central Asian and Caucasus countries of the former Soviet Union.

It occurs at least in many countries including Afghanistan, Azerbaijan, Belarus, Bhutan, Britain, Bulgaria, Canada, China, Czech Republic, Denmark, Estonia, Finland, France, Germany, India, Iran, Italy, Kyrgyzstan, Kazakhstan, Latvia, Lithuania, Moldova, Mongolia, Nepal, Netherlands, Hungary, Norway, Pakistan, Poland, Portugal, Romania, Russia, Slovakia, Sweden, Switzerland, Turkey, Ukraine and Uzbekistan.

In Western Europe, it is largely confined to sea coasts where salt spray off the sea prevents other larger plants from outcompeting it, but in central Asia, it is more widespread in dry semi desert sites where other plants cannot survive the dry conditions. In central Europe and Asia, it also occurs as a subalpine shrub above tree line in mountains, and other sunny areas such as river banks. They are tolerant of salt in the air and soil, but demand full sunlight for good growth and do not tolerate shady conditions near larger trees. They typically grow in dry, sandy areas. More than 90% or about 1,500,000 ha (5,800 sq mi) of the world's natural sea-buckthorn habitat is found in China, Russia, northern Europe and Canada where the plant is used for soil, water and wildlife conservation purposes and for consumer products.

Sea buckthorn grows mostly wild throughout a few of India's cold, dry regions. Areas in which the fruit naturally thrives are the Hindu Kush range along the far northern border of Pakistan and India, Ladakh, Kumaon-Garwal in Uttaranchal, Lauhual-Spiti and Kinnaur in Himachal Pradesh, and the sacred forests of Sikkim and Arunachal Pradesh. Sea buckthorn encompasses a decent amount of landmass in some areas for instance 30,000 hectares in Ladakh alone, but the country's commercialization efforts are nascent. After seeing China's growing interest in the fruit, a few of India's agricultural departments are now taking note and pledging to cultivate one million hectares of sea buckthorn by 2020 as part of the Green India Mission.

Hippophae salicifolia (willow-leaved sea-buckthorn) is restricted to the Himalayas, to the south of the common sea-buckthorn, growing at high altitudes in dry valleys; it differs from *H. rhamnoides* in having broader (to 10 millimetres (0.39 in)) and greener (less silvery) leaves, and yellow berries. A wild variant occurs in the same area, but at even higher altitudes in the alpine zone. It is a low shrub not growing taller than 1 metre (3.3 ft) with small leaves 1–3 centimetres (0.39–1.18 in) long.

Three species, namely *Hippophae tibetana*, two sub-species of *H. rhamnoides* namely *turkestanica* and *sinensis* and *H. salicifolia* occur naturally between 1500 to 5500 m in cold deserts of Himachal Pradesh. Among the three species, *H. rhamnoides* ssp. *turkestanica* has the widest distribution (2500 to 4000 m), followed by *H. salicifolia. H. rhamnoides* constitutes nearly 70 per cent of the total population, where as *Hippophae tibetana is* confined only to higher reaches (>4000 m) and constitutes less than 10 per cent of the natural population in Himachal Pradesh. *Hippophae* species occupy more than 1000 ha in Lahaul and Spiti district. Besides this, it is also found growing in districts of Kinnaur (Baspa valley), Chamba (Pangi area), Kullu (Shange and Parvati valleys), Kangra (Dhauladhar ranges) and Shimla (Dodrakwar).

5. OBJECTIVE OF IMPROVEMENT

i. Development of superior varieties of different species of Seabuckthorn adapted to various agro climatic zones for health food.

ii. Development of high yielding cultivars having better fruit size and quality.

iii. Development of thornless cultivars is considered to be one of the main breeding objectives in Seabuckthorn improvement.

iv. Development of cultivars of early maturity and having growth habit that facilitate the mechanical harvesting

v. Development of cultivars having more nitrogen-fixing ability.

vi. It was established that pollinator have a significant impact on the amount of harvest, fruit set, their size and biochemical composition. Therefore, it is necessary breeding male forms of sea buckthorn having pollen fertility of 94-96%.

6. CYTOGENETIC

The systematic division of the genus *Hippophae* into species and sub species is presented and the taxonomic status of the different entities is discussed herein. The C-banded karyotype gives further support to the view that 2n = 24 is a diploid chromosome number in *Hippophae*. A distinct difference in the C-banded karyotype emphasizes the evolutionary differentiation between European and Asiatic subspecies of *H. Rhamnoides* has been observed. A set of morphological descriptors for the genus *Hippophae* was developed after thorough study of the germplasm obtained from explorations in different parts of India. The pattern of variability in morphological characteristics associated with habitat, plant growth habit, stem, leaf, fruit, flower, seed and biochemical parameters were taken into consideration. The suitability of these parameters in the characterization of *Hippophae* germplasm was demonstrated successfully. It was observed that *H. rhamnoides, H. salicifolia* and *H. tibetana* formed separate clusters in a dendrogram based on unweighted hierarchical cluster analysis. *H. rhamnoides* and *H. salicifolia* were found to be genetically closer than with *H. tibetana*. Genetic variation within the species *H. rhamnoides* was also estimated.

DNA-based markers have proved to be efficient in bringing out exact genetic distances among and within the species in *Hippophae* and were reported to be superior to morphological methods (Bartish *et al.*, 2000). The major reason for this is attributed to the lack of a precise analytical procedure devoid of numerical weighting. Analyses, such as for phylogenetic studies, demanding highly precise conclusions, require competent methodologies, such as chloroplast DNA analysis, and in seabuckthorn, even for these, an accurate morphological assessment will enhance the quality of the result (Bartish *et al.*, 2002). However, a careful study of the morphological traits coupled with unweighted hierarchical clustering has the potential to provide results on par with RAPD analysis (Mathew, 2003), making it possible to avoid expensive and tedious procedures. Thus the present descriptor list was proved to be efficient in identifying inter- and intraspecific distances in *Hippophae* by considering a maximum number of qualitative and quantitative characters simultaneously.

Seabuckthorn has a chromosome number of 2n = 24, base number X = 12. The karyo type constitution is 2n = 2 × 24 = 10 m + 6m (SAT) + 8 SM. *i.e.*, there are 8 pairs of centromere chromosome (including 3 pairs of sat chromosome with satellite on shortarm) and 4 pairs of sub centromere chromosome . The chromosome length varies from 1.7 to 4.4 microns. Seabuckthorn has a karyotype of asymmetrical 2B. Phyto phenological morphological features and genesis of seedling form the base for inheritance pattern. The estimation by way of paternal progeny characters or by indirect - correlation seems to be more advisable.

7. INHERITANCE PATTERN/ LINKAGE OF CHARACTERS OF ASSOCIATED

Morphological, biological and biochemical characteristics complex varies in nature and plantation. These characteristics are:

- Size indices of vegetative organs – plant height, leave size, leaf size etc.
- Fruit size/ mass, shape and colour
- Seed size/ mass, shape, colour and viability
- Correlation of male and female plants /seedlings
- Biochemical composition of fruit hypanthium
  - Organic acid contents
  - Oil content
  - Vitamin C content
  - Carotene content
  - Amino acid content
  - Dry substance content

Valuable features of seabuckthorn change within variety and their genetic makeup testifies that it is very important and perspective to choose the initial natural variety for selection. Variability of different indication is variable within the variety. Indications that are under strict gene control have low factors of variety and those that have less determined signs are under the influence of environment and their qualitative signs are changeable.

Bio morphological peculiarities of natural varieties of seabuckthorn and its use in selection varies its growing areas along river beds, flood lands sea shores, lakes, waterlogged areas and also in types of stands i.e. pure or mixed and different ecological niches.

Every characteristic of the species has variation in regard to variation coefficients, nutrient organs and propagative organs. The variation of most characteristics changes with the trees shapes upto 10^{th} year and their variation trend will have a remarkable change in term of physiological situations and shape of all major characteristics in fruit, thorn quality, leaf, seed and branch. Thus in this species, it is possible to have a lot of combinations of different characters, with a great variation frequency. The combination of trees with main trunk, bearing fruits of orange-yellow colour, oblate shape and middle size can be regarded as the highest frequency.

8. PROBLEM IN BREEDING

There are more than 20 seabuckthorn forms from different geographical seed sources in China and from Russia, using genecology selection, there are 5 eco-economical forms and two economical forms. They have characterized by fast growth, high canopy density, bigger fruit size (fruit diameter is 0.8-0.9 cm; weight of per 100 fruit being 20-31 g) and higher vitamin C and oil contents. Male and female association and ratio along with pollination behaviour are the main problems in breeding. It is essential to consider the effects of the topography and microclimate on pollination when determining pollen dispersing distance. When raising plantation, male plants should be planted in surrounding areas to form a favorable pollination condition. Relatively centralized male and female plants could supply convenience for improvement and identification of seabuckthorn seedlings. When the plantation has natural pollen sources up and down slope and its width is less than 100 m, pollination will be no problem. This will increase land productivity and reduce labour input for the management.

Second breeding problem in seabuckthorn demonstrates that apomixis is diplospory with change in meiosis II, so apomixis considered as a source of recombinogenesis with simultaneous homozygocity, it ensure pure lines of material that can hardly be obtained by conventional methods of breeding. However the essential obstacle is the high frequency of apomictics, ovaries elimination and low vital capacities of seed. Apomixis associated with embryo culture allows us to obtain homozygous plants of seabuckthorn.

Site specific timings of bud break, small size of flower, flowering and fruit development are also the linked problems in breeding. Lack of key techniques of introduction and

breeding, garden for gathering tassels and seeds; high production and quality seabuckthorn garden for intensive management and breeding base of different forms are the chief barriers. Hardwood cuttings, short growing season, long freezing winter are also the factors affecting breeding programmes, considered as eco- physiological reasons for improved/ moderate genetic gains besides, conventional breeding methods for genetic improvement to create variability for further selection and not generally practiced in quality seed production.

9. FLORAL BIOLOGY

9.1. Flowering

The flower buds are borne every year during active growth season of summer or autumn, along with vegetative buds and open in spring during the next year. Male buds are larger than the female buds, with 6-8 covering scales. The smaller female buds are more elongated and have 2 covering scales. Male buds open for a few days earlier the female buds. Male and female buds open at the about 7 days before leaf buds. Flowering generally occurs in May-June and ripening sets in by late August and September. The time taken from flowering to fruit maturation is 12 to 15 weeks.

9.2. Flower

Seabuckthorn bushes are either male or female i.e. dioecious. Generally, the male floral bud consists of four to six flowers. The males produce pollen and have flowers without petals. Each flower contains four stamens. The female floral bud consists of one flower and rarely two or three. The female produce fruit and seed and have flowers, also without petals. Each flower contains one ovary and an ovule.

Very small yellowish pistillate flowers appear, usually before the leaves, in early March to mid May, depending on the geographical features of the place of growth. Both male and female flowers are very small. The female flower is without petals and it consists of a pistil, a hypanthium and 2-lobed perianth and found in small racemes in the leaf axils. The male flower has longer oblong perianth leaves and contains four stamens of same length and it also has no petals.

The sex of a young seabuckthorn plant cannot be judged until the first flower bud appears. In precocious plants this may be in the third year, whereas in slow plants it may happen in the fifth or sixth year. This makes it difficult to identify and give the right position to the male plant in a plantation, or to root out the unwanted male and inferior females when they are at the nursery stage.

9.3. Pollination

When the atmospheric temperature ranges from 6°C-10°C, the anthers split and the pollen is blown out by the wind in large quantities. The female flower depends almost entirely

on the wind for pollination. Neither the male nor female flower has nectar, so they can not attract bees or insects to collect nectar. Honey bees and a variety of other insects often visit male flowers for forage pollen for protein, but the rarely visit the female flowers.

Recommendations for male and female ratio vary from 1:6 to 1:8. Reports from the Siberian Institute of Horticulture in Russia indicated that one male: female mixed row for every two rows of female plants is sufficient, and in the mixed row every fifth plant is male. This design gave significantly higher total yield than other designs.

9.4. Fruit

Seabuckthorn bears a special fruit that is different from other common fruit or berries. Morphologically it develops from an ovary and a calyx tube which is closely connected to the ovary. Young fruits are hard and greenish, but turn soft and orange to orange-red as they mature. Actually the fruit is a combination of an unsplit, fleshy, expanded calyx tube and an ovary. Seabuckthorn bushes or trees hold the mature fruit for several months; this gives ample time to harvest them. In other words, the expanded, juicy calyx tube is the important part with economic value.

Sea-buckthorn fruits often less than 1 cm in diameter, varying in weight of 100 fruits from 15 to 25 g of wild samples. Some cultivars are of higher weight (40-90 g/100). Fruits are of attractive colours of red, reddish-orange or yellow, therefore this species was planted as a garden plant for beautification in many countries. Fruit shape varies to ovate, spherical, cylindrical, elliptic etc. Fruits of sea-buckthorn are made from unsplit, fleshy, expanded calyx tube and an ovary. The period from flowering to fruit maturation is 3 to 7 months, depending on the geographical conditions of growth and the biological characteristics of the samples. Most of the fruits are borne on 2-year-old branch. The young fruit is green and hard, which become soft on maturation in early July or August, depending on the timing of ripening varieties. Mature fruits may remain attached to the female plants for several months, unless they fall by wind or snow or eaten by birds. They are generally harvested from late August to early October. Later in winter they become over mature and shrink and often eaten by wild animals especially by birds. One female plant generally bears 0.2 kg from young plant to 3 kg in a mature plant of 10 years or so. One can harvest 2-5 tons of fresh fruits from wild forest of sea-buckthorn. Improved varieties have been reported to produce 10-25 kg of fruits per plant or 10-30 tons per ha. Female plants bear fruits after 4-5 years in plants grown from seeds and 3-4 years in plants grown from stem cuttings.

9.4.1. Seeds

Each fruit has a single seed surrounded by a fleshy hypanthium (edible fleshy pulp). Seeds make 2.7-17.0 percent of fruit weight and they are of 3-7 mm in length and 2-4 mm in

width and light to dark brown in colour. The seed is surrounded by a parchment like ovarian wall. Weight of 1000 sea-buckthorn seeds is about 14-27 grams.The skin of the seed is greyish-brown or dark brown, leathery, and lustrous.

10. DIFFERENT SPECIES

10.1. *Hippophae rhamnoides*

The common sea-buckthorn, is by far the most widespread of the species in the genus. The Seabuckthorn plants are spine scent shrub or small tree up to 10 m in height with rough brown bark. Leaves small, linear lanceolate, alternate or opposite, covered on very small, greenish or yellowish, appear with new leaves, male flower in axillary clusters, female flowers solitary with a diameter of 6 mm, orange yellow or scarlet in colour and sour to highly acidic in taste. Seed is single, oblong with shiny testa. The root system makes it suitable to grow even on fragile slopes. A five year old plant can develop a tap root of about 5 m deep, with horizontal roots spreading 6 to 10 m. The shrubs reach 0.5 to 6.0 m tall, rarely up to 10 m in Central Asia.

10.2. *Hippophae salicifolia* (Willow-Leaved Sea-Buckthorn)

It is restricted to the Himalayas, to the south of the common sea-buckthorn, growing at high altitudes in dry valleys. *Hippophae salicifolia* differs from *H. rhamnoides* in having broader (10 mm) and greener (less silvery) leaves with yellow berries. A wild variant occurs in the same area, but at even higher altitudes in the alpine zone it is a low shrub not growing taller than 1 m with small leaves 1 to 3 cm.

Its trees are medium to tall in height (4-7 m). It has a willow like appearance. Leaf's margin revolute, adaxial surface densely covered with stellate hairs. Mid rib is clear. It is mild thorny. It is also found in Xizang (Cuona, Yadong, Jilong), Pakistan and Nepal. In India, is distributed in Lahaul, Kinnaur and upper areas of Uttrakhand in Himalayas. The altitude of distribution is 1500-3200 m.

10.3. *H. rhamnoides*

10.3.1. *Subsp. sinensis*

It is a small tree or shrub, 2-18 m high. It is found in hills, on mountain slopes, valley bottoms, river banks or river beds of dried up, between 400-3100 (3700) m in China. It is very common in the Loess Plateau. It is the main resource for sea-buckthorn development in China. It is found in Sichuan, Qinghai, Gansu, Shaanxi, Ningxia, Inner Mongolia, Shanxi, Hebei, Beijing and Liaoning. The current year branches are stiff. Leaves are opposite or subopposite. There are scale-hairs on the lower surface, white or rarely rusty-red. Most of fruits are tangerine. Seed surface in this type is shining.

10.3.2. *Subsp. yunnanensis*

This subspecies is found in valley bottoms, river banks and woodlands in foothills at an altitude of 2200-3700 m in China (Yunnan, Xizang, Sichuan, Qinghai (Nangqian alt. 3700 m). It is a. tree or small tree, upto 20 m high. The current year branchless is soft. Its most leaves are alternate, the midrib on the above impressed and up to top appearing as deeper sulcus, with most rusty-red scale-hairs on the lower surface. Its fruits are yellow; sometimes the carpodermis is hard to be separated from seed coat. Its seed are usually flat.

10.3.3. *Subsp. turkestanica*

It is a hardy shrub or small tree 3-7 (15) m in height. It is found in India (Himachal Pradesh, Ladakh), China (Xizang, Xinjiang, Gansu), Afghanistan, Tajikistan, Kirghizia, Kazakhstan and branched thorns. Leaves are alternate, narrower, 2-4 (5) mm wide, both surface silvery. The length of most fruits greater than breadth. Fruits are reddish-orange in colour.

10.3.4. *Subsp. mongoiica*

It is a 2-6 m high shrub. It is found in river terrace or flood land, at 1200-1800 m in China (Altai mountains), Mongolia, Russia and Eastern Kazakhstan. Branches are brown, with less and no branched thorns. Leaves are alternate, the widest usually above the middle, 5-8 mm wide, above green, adaxial surface silvery, acuminate at apices. It is commercially cultivated in Russia.

10.3.5. *Subsp. Caucasia*

It is a tree, usually up to 10 m high. It grows along river banks and in flood lands, between 1000- 2500 (3000) m in Azerbaijan, Georgia, Armenia, Russia, Turkey and Iran. Branches are brown, with less and no branched thorns. Leaves are alternate, the widest commonly below the middle, 3-7 mm wide, abaxial usually green, adaxial surface silvery, acuminate at apices.

10.3.6. *Subsp. carpatica*

It is. a shrub or small tree. It is found in valleys of Alps, Carpathian mountains, Danube and the shore of the black Sea, from sea level to 380 m. It is found in Romania, Ukraine, Hungary, Austria and Germany. Branches are straight. Leaves are alternate, 5-7 mm wide, adaxial surface is usually mixed with most rusty-red scales. Fruits are 6-8 mm long, 5-7 mm wide.

10.3.7. *Subsp. rhamnoides*

It is a shrub or small tree. It is found growing on sea shores and valley bottoms, from sea level to 1100 m in Poland, Germany, Denmark, Sweden, Finland, Russia, Norway, Netherlands, Belgium, France and Britain. Its branches are more or less curved. Leaves are alternate, mostly 5-8 mm wide. Fruits are 8-11 mm long, 5-7 mm wide. Seeds are compressed.

10.3.8. *Subsp. fluviatilis*

It is a shrub or small tree. It grows in open land, river bottoms, riverbanks and hillsides, between 100-1900m in Austria, Italy, Switzerland, France and Spain. Leaves are alternate, 3-6mm wide, adaxial surface is usually mixed with most rusty-red scale-hairs. Fruits are 5-6mm long, 4-6mm wide. Its seeds are ovate.

10.4. Goniocarpa

10.4.1. *Subsp. litangensis*

It grows in valley terraces, hills, at 3700 m altitude in China (Sichuan, Litang).This subspecies differs from the typical subspecies by the young branches and the lower surface of leaves densely covered with stellate hairs, the leaf margin usually manifestly revolute, the midrib on the above side impressed and up to top appearing as deeper sulcus; the fruits tangerine or dark tangerine, 6.0-7.6 mm long, 4.5-5.3 mm wide, 1.4 times longer than wide.

10.4.2. *Subsp. goniocarpa*

It is a small tree, 5-8 m high. It grows on mountain slopes, river banks, flood lands and valley terrace, at elevations of 2650-3650 m in Sichuan (Sunpan, Ruoagai, Hongyuan, alt. 3500-3650 m), Qinghai (Qilian, alt. 2500-3200). Branches are soft, branchlets of the current year red-brown or dark brown. Leaves adaxial surface are densely covered with scale-hairs. Buds of male flower are cruciform, of female flower near cruciform (bifid ovate, the second pair of the bud scales is clearly visible). Fruits are trite, juicy, apricot-colored or straw-yellow, 6-10 mm long, 4-5.9 mm wide, 1.45-2.1 times longer than width. Seeds are slightly flat, with 3-5 inconspicuous longitudinal angles.

10.5. *H. gyantsensis*

It is a small tree 5-8 m high. It grows in river banks, flood land and valley terrace, between 2600-5000 m in China (Xizang) and Sikkim in India. Its branches are soft, branches of the current year brown-yellow. Leaves abaxial surface are scattered stellate hairs and scale-hairs, adaxial surface is densely covered scale-hairs. Flower buds are ovate bifid. Fruits are yellow, longitudinal angles almost developed in towing-shaped.

10.6. *H. neurocarpa*

10.6.1. *Subsp. stellatopilosa*

It grows on river banks, flood land and river terrace, between 3400-4400 m in Sichuan (Daocheng, Litang, alt. 3700-4000 m); Xizang (Jomda,river banks of Yuqu Riverin Basu, Zuogong, Mangkang, Leiwuqi, Lhasa, alt. 3400-4400 m); Qinghai (Nangqian, Yushu) in China. This subspecies differs from the typical subspecies by the young branchless, and the adaxial surface of leaves densely covered with stellate hairs. The leaf margin usually manifestly revolutes, the midrib on the above impressed and up to top appearing as deeper sulcus; the fruits faintly orange-coloured or yellow-brown, 5.6-6.5 mm long, 2.5-3.1 mm wide, 2.1 times longer than wide.

10.6.2. *Subsp. neurocarpa*

It grows on river banks, flood land and river terrace, between 2700-3900 m in China (Sichuan, Qinghai, Gansu). Plants are 1-3.5 m high; the crown cover appearing is as platform in the adult plants. Branches are stiff, branches of the current year are gray-white. Leaves adaxial surface are densely covered with scale-hairs. Flower buds are ovate or ovate bifid. Fruits are black-brown, curved prism, with little or very little juice, one end thinner, with 5-7 longitudinal angles, 7.8-8.4 mm long, 2.8-3.3 mm wide, 2.5 times longer than wide.

10.7. *H. tibetana*

Plants are dwarf, 7-60 (80) cm high. It grows on river bottoms, on river banks and steppes on higher mountains, at the elevations of 2700-5300 m in China (Sichuan, Xizang, Qinghai, Gansu), Nepal, India (Himachal Pradesh, Ladakh, Sikkim). Branches are pointing upwards, usually broom-like. Leaves are whorled, linear. Flower buds are ovate or ovate bifid. Fruits are dark tangerine, with 5-9 brown-black stellate ornamentation at apices, fruits and seeds.

11. CROP IMPROVEMENT METHODS

Russians as early as 60 years back were first to start the breeding work, which started with surveys and selections of promising forms of sea-buckthorn , as wild forms were highly thorny and low yielding (1-2 tons/ha). M.A. Lisavenko and his team, who had started work as early as 1930s, started pioneering work. Many thornless, high yielding, rich in fruit oil and vitamins etc. with large fruit size and high productivity (15-25 tons/ha) were released over the last two decades, through selection, hybridization and even mutagenesis.

11.1. Introduction and Selection

There is need to introduce thorn less varieties of seabuckthorn from Russia. Seed and cuttings of *H. salicifolia* can be collected from Shego (Spiti), Sissu (Lahaul) and Kuppa (Kinnaur), while *H. rhamnoides*, material can be collected from Tabo (Spiti valley),Sissu (Lahaul valley) and Chango (Kinnaur) in the cold deserts of the Himachal Pradesh for production of genetically superior planting stock. Major gene pool areas in the cold desert of Himachal Pradesh and ladakh have been identified and seed and cuttings must be collected from plus seabuckthorn stand.

Presently, China enjoys the most abundant Seabuckthorn resources in the world and the germplasm rich regions are on the Qinghai-Tibet Plateau. The sub species of the Chinese Seabuckthorn rich regions are in the adjacent provinces and autonomous regions in the upper and middle reaches of the Yellow river. China is also the valuable gene bank of Seabuckthorn germplasm in the world.

11.1.1. *Analytical breeding (Selection)*

The first phase of breeding of seabucthorn is- a selection of natural populations of wild of sea-buckthorn ready forms-plants with valuable economic and biological properties.

Analytical breeding of sea-buckthorn should be conducted as follows:

i. Selection of the best forms of wild populations by phenotypic characteristics and properties, which was formed on the basis of the interaction of genotype with specific environmental conditions. Selected forms are propagated by cuttings, root suckers, and then studied in breeding field.

ii. The sowing seeds of the best forms collected in the field of natural habitat of sea-buckthorn. According to genetic concepts reflected in the phenotype only part of hereditary information stored in the genotype. Another part may be manifested in the phenotypes of future generations in the process of seed reproduction. Accordingly, at sowing seeds with natural populations in the breeding nursery can reveal new phenotypes, often with valuable economically recessive trait.

iii. Selection of the best seedlings obtained from seeds of varieties and highly productive forms of open pollination. For this purpose, use existing varieties and elite form. Seeds for breeding purposes to procure better collection of sea-buckthorn sites where open pollination was done by pollen of selected male plants belonging to different populations and ecological and geographical forms. Due to spontaneous combinative variability under different weather conditions over several generations can gain valuable source material for the selection and development of new varieties and forms.

11.2. Hybridization (Synthetic Breeding)

Based on the use of source material, hybridization may results different varieties and male forms. Hybrid offspring produced from artificial interbreeding, is the synthesis of hereditary traits of two or more of the parental forms. Of special importance in synthetic plant breeding (and of sea-buckthorn inclusive) has ecological and geographical interbreeding is more effective method of producing a genetically enriched source material for selection. At present synthetic breeding of sea-buckthorn more have become widespread in many countries of the world.

11.2.1. *Technique of artificial crossing and variety release procedure*

Features of sea-buckthorn hybridization techniques are due dioecious and wind-pollinated. The insulators sew from a tightly woven size of 50 x 25 cm. The isolation of female flowers spends 3-4 days before the mass flowering. Flowering branches of male samples is placed under isolators. For best pollination in the next 2 - 3 days isolators with branches periodically is shaken. Take off them after flowering male plants. The fruits of hybrid seeds are collected in a phase at full maturity. Seeds are cleaned from the pulp and membranous pericarp and stored in paper bags under laboratory conditions. Hybrid seeds of sea-buckthorn should be sown in early spring, after a preliminary of stratification for 30-40 days at a temperature of 4-5 C. Seeding depth during sowing of seeds should be 1-1.5 cm. The seedlings are grown in the nursery for 2-3 years, after which they are planted in the garden. On the basis of 2-3-year study of seedlings in the breeding garden, on a range of valuable economic and biological characteristics, elite and promising ones are vegetatively propagated and planted at the site of the primary Cultivar Investigation Centers. Observing the expression of economical traits the best elite seedlings are further propagated and transferred to the state variety trials.

Sea-buckthorn plant start bearing fruit, a berry, after 3 years of plantation and with application of better management techniques, it remain productive for next 25-30 years, producing an average of 15 tons of fruits per ha per year.

11.3. Induced Mutagenesis and Polyploidy

By using ionizing radiation and chemical mutagens may in the short term based on the best existing varieties and elite forms to get a new genetically modified source material for breeding sea buckthorn. The sea-buckthorn seeds and cuttings are used in aqueous solutions of chemical mutagens for induced mutation. For obtaining induced mutations by using the ionizing radiation, ã-irradiation is recommended at a dose 15-30 cr for seeds and 0.75-1.1 cr for rooted cuttings.

Genetic changes at the plant associated with the number of chromosomes, is now widely used in many experimental breeding field, vegetable and fruit plants. In combination with hybridization, polyploidy, allows creating a new, genetically-rich sea-buckthorn raw material, from which we can more effectively conduct breeding work in different directions.

12. CULTIVARS DEVELOPED

12.1. Zafarani (Female Variety)

The variety was realized by the Genetic Resources Institute of the Azerbaijan in 1994. This variety was got from hybrid population by selection. It was obtained from hybridization of population of local male variety with "Novost Altaya" introduced from Siberia. Variety is weak thorny, high productive, fruits ripen in August. An average productivity of one plant is 22-31 kg, maximum is 58 kq. Average oil content is 4.75%. "Zafarani" variety is resistant to diseases and pests. The colour of the fruits is yellow, yellow-orange, orange, weight of 100 fruits ranges between 55-60 g. It tastes sour and sweet. The stalk is short, about 1.5-2 mm. Fruit peel is thin, the flesh is not soft. Seeds are small, brown or dark brown colour. Seeds make up 2.7 % of the fruit. 1000 seed weight is 14 g. Fruit's shape is attractive. Dimensions of fruits are: 1.4-11.0 x 8.8-10.2 mm. Consistence of flesh and its juiciness are juicy & of mid-density. Transportation ability of the fruits is low. Main purpose of the variety is universal tasting value mark:

a) In fresh condition - 4.5, b) juice - 4.5.

12.2. Tozlayan (Male Variety)

The variety was realized by the Genetic Resources Institute of the Azerbaijan in 1994. It was obtained by the selection from the natural sea-buckthorn population spread in the area of Shaky of Azerbaijan Republic. It is mid-thorny, resistant to diseases and pests. As a result of investigations it was defined that male individuals of the sea-buckthorn is not less polymorph than female ones. Different male forms on the result of a strong polymorphism not only have got distinct pollen productivity, they also effect to the productivity and fruit quality of female plants. Concerning to these, selection of perspective sea-buckthorn male varieties pollinators were recommended. The experiments showed that "Tozlayan" variety distinguishes mainly according to pollens fecundity degree, and this index is 96,4 %. As pollens of this variety have very strong survive ability they are very useful for farming, because weak survive ability of male plants leads to much location of male individuals in sea-buckthorn gardens, which in turn reduces productivity. As a rule in the sea-buckthorn gardens per each 5-6 female plant one male plant is enough, whereas due to very high survive ability of the "Tozlayan" variety's pollens it is required to grow one male plant ("Tozlayan" variety) per each 8-10 female plant in the gardens.

12.3. Shafa

The variety was realized in the Azerbaijan Republic in 1991 year. The variety was obtained by selecting from the natural sea-buckthorn brushwood grown in Shinchay basin of Shaki region of Azerbaijan. The variety is thornless, late ripening and productive. Productivity of a tree or bush is 16-23 kg. It is resistant to pests and diseases. It is a universal variety. The oil content ranges between 3.75-4.2 % depending on the ecological

condition of the cultivated site. The fruit colour is usually orange or orange-yellow; weight of 100 fruits is 50-52 g. It tastes sour-sweet. Peel of the fruits is thin, flesh and is not soft. It is long and is brown color. Seed is elongate, dark-brown or black color. The seed makes up 3,4- 3,6 % of the fruit, 1000 seed weight is 17 g. Fruit shape is attractive. Dimensions of fruits are: 10-13.2 x 7.4-9.6 mm. Consistence and juiciness of the plump is juicy and dense. It is resistant to diseases and pests. Transportation ability of the fruits is low. Main purpose of the variety is universal character: can be used as fresh fruits, to prepare jam, juice, liqueur, stewed fruit and much more. Tasting value mark: a) in fresh condition - 4.0, b) juice - 4.0.

12.4. Russian Varieties of Sea-Buckthorn

12.4.1. *Novost Altaya*

The fruits are round form and bright orange colour. The average weight of 100 fruits to 50.0 g, the length of the peduncle 3.0-4.0 mm. Fruits ripen in late August. They contain: sugar - up to 5.49%, acids - up to 1.67%, vitamin C - up to 50.0 mg% carotene - up to 4.3 mg%, oil - up to 5,5-8,2% vitamin E - to 8.3 mg%, B vitamins - to 0.54 mg%, vitamin K - up to 0.84 mg% of tannins - up to 0.048%. Winter resistance is high. Yields of 6-7 years of age 13,0-21,5 t / ha (with scheme of allocation of 4 x 2 m). Fruiting is annually. Novost Altaya variety for technical purpose is used to produce sea buckthorn oil, juice, jam.

12.4.2. *Maslicnaya*

Fruits are ovate form and red colour. Weight of 100 fruits is 37.0 g, the peduncle long (4.0-5.0 mm). Fruits contain: sugars - up to 4.0% acid - up to 1.45% of vitamin C - up to 64.0 mg% carotene - up to 7.6 mg% of oil - 4,7-5,8% vitamin E - 14.6 mg%, B vitamins - up to 0.2 mg%, vitamin K1 - up to 1.0 mg% of tannins - 0.05%. Ripen in late August. Winter resistance is high. Yield from 7-year-old orchard is about 19.6 t / ha (at planting scheme 4 x 2 m). Maslicnaya variety is used to produce sea buckthorn oil, juice, jam.

12.4.3. *Oranjevaya*

Fruits are oval form and orange colour. Weight of 100 fruits to 60.0 g, the taste is sour. They contain: sugars - up to 5.4% acid - up to 1.27% of vitamin C - up to 330.0 mg% carotene - up to 4.3 mg%, oil - up to 6.0% of vitamin E - to 10.4 mg%, B vitamins - to 0.66 mg%. Ripen in mid-September. Winter hardiness is high. Yield is annually, high, from 7-year-old orchard 27.6 t/ha (at planting scheme 4 x 2 m). Plant begins to bear fruit in the 4th year after planting. 'Oranlevaya' variety is used to produce sea buckthorn oil, juice, jam.

From a recent hybridization between forms of different origin (Siberia x Azerbaijan) three varieties of sea-buckthorn with early maturation (ultra scope matured) fruits (July) & oil content of 7.1 % were obtained. More perspective and threatened forms of natural populations of sea-buckthorn spread in Azerbaijan Republic were not yet evaluated and included to the collection. Therefore conservation of plant genetic diversity of sea-buckthorn existed in Azerbaijan Republic, selection of productive samples, evaluation and protection are one of the most important task in modern time.

13. FUTURE RESEARCH THRUST

- Characterization, collection, evaluation and documentation of indigenous and exotic species of Seabuckthorn in different regions of the world
- Development of superior varieties of different species of Seabuckthorn adapted to various agro climatic zones for health food
- Development of superior agro techniques for faster propagation, production and harvesting
- Standardization of post harvest technologies for optimum utilization
- Studies for the advancement of appropriate technologies and processes for various health food, pharmaceutical and cosmetic products and their evaluation for health care
- National policy be evolved for afforestration of utilization of waste, barren and marginal lands in cold desert and other regions
- There is a need to create general awareness about this multipurpose plant by using different means available
- International collaborative programme, involving public and private organizations for research and development of Seabuckthorn for food, pharmaceutical , cosmetics, fuel, fodder and environmental conservation with centres in Asia, Europe and North America

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