

Propagation of Seabuckthorn (*Hippophae rhamnoides* L.)

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ABSTRACT

One kilogram of air-dried seeds of seabuckthorn (*Hippophae rhamnoides* L.) contains 100,000-130,000 seeds. If the germination rate reaches to 50 percent, one kilogram of seeds can produce 50,000-65,000 seedlings of seabuckthorn. It is better to collect overripe fruits for a higher seed germination percentage. After separation, seeds are stored in paper bags, in a dry room at room temperature. Generally, at room temperature, viability of seeds is quite high (85-100%) for 2 years, but then it declines rapidly. To enhance early sprouting, seeds may be given some treatments like GA3 for 24 hours or IN H²S04 for 2 minutes. The seeds are sown in the drills and covered with 1-2 cm of soil layer.

For propagation from lignified or hardwood cuttings, they may be collected in the autumn, winter or early spring (beginning of March or April). Highest rate of rooting was also observed in the cutting of 0.8-1.5 cm diameter and 10-15 cm length. Before plantation, basal part of the cuttings is dipped quickly into 500 ppm NAA. It was observed that indole butyric acid (IBA) and application of polythene mulch help in promoting rooting in the cuttings. Man made matrix like sand: humic soil: soil under seabuckthorn vegetation: 5:3:1, can ensure proper temperature mixture and aeration of rooting. Basal part of cuttings (2/3 part) is placed in water for 4-5 days, changing the water 3 times. Cuttings are planted at a spacing of 20 cm x 10 cm. Half part of the cuttings remains out of the soil, with 2-3 buds. After plantation, cuttings are well irrigated.

For propagation from softwood cuttings, it was observed that semi-hardwood cuttings, collected in late June, or early July in Altai region of Siberia in Russia were beneficial. Cuttings of 12-15 cm length with a single bud on top were found most useful. Growth hormones like NAA, IAA, indole propionic acid (IPA), indole butyric acid (IBA) and adenosine triphosphate (ATP) have been used by various workers for accelerating root formation in seabuckthorn. The Basal part of cuttings are placed at 2-3 cm depth in solution of growth hormone at room temperature for 14-16 hours, then washed with water and planted. Optimal air temperature for rooting should be 22-27°C. Relative humidity should not be lesser than 90%. Weeds are not allowed to grow. Propagation of seabuckthorn through laying, suckers and grafting is also discussed. Further new methods of propagation are also reported.

Key words: Seabuckthorn, propagation, seeds, hardwood and soft wood cuttings, treatments, management and new methods.

INTRODUCTION

Seabuckthorn is a plant, which has got global attention due to its rich medicinal properties and its role in environmental conservation of degraded mountainous lands. On one hand, seabuckthorn is being planted for raising orchards for commercial purpose in countries like Russia, Germany and China, on other hand, it is being used for afforestation programmes for greening of hilly regions (Lu Rongsen, 1992). In order to meet the requirement of plantation programmes, propagation methods of seabuckthorn needs to be reviewed, as many scientists in several countries have done a lot of work on this aspect of seabuckthorn. Seabuckthorn can be propagated from the seeds, hard or soft word cuttings, grafted seedlings and root turion seedlings. Among them, the most common propagation methods are raising plants from seeds and cuttings from hardwood or softwood.

PROPAGATION FROM SEEDS

Seeds for raising vigorous nurseries should be collected from healthy plants with desirable characteristics. Seed comprises 3-10%, averaging 5% of the total weight of fruit. Raising seabuckthorn plants from seeds is a simple technique. This method produces a greater number of plants and involves lower costs than other methods. Seeds of seabuckthorn are minute (2-3 mm). One kilogram of air-dried seeds contains 1,00,000-1,30,000 seeds. If the germination rate reaches to 50 percent, one kilogram of seeds can produce 50,000-65,000 seedlings of seabuckthorn (In Lu Rongsen, 1992).

Time of collection

Seeds are separated in the stage of full biological maturity. At the beginning of the browning phase of seeds, the majority of seeds are capable of germination. It is better to collect overripe fruits for a higher seed germination percentage. On further fruit ripening, there is a short increase in seed germination capacity, followed by a rapid decline due to the onset of short physiological dormancy, after which the seeds germinate readily (Eliseev and Mishulina, 1972, 1977). According to Eliseev and Mishulina (1972), fruit may be collected when the seeds turn brown. They found an increase in seed germination when collected from 15th to 20th August, whereas most seeds failed to germinate when collected from 20th August to 20th September onwards. However, after 20th September, seeds collected from overripe fruits germinated satisfactorily.

Separation

Traditionally fruits are pressed and juice is separated. The remains with seeds are dried for 2-3 days by spreading them in a thin layer in a shaded room or sun. Seeds after separation from fruit pulp may be washed with water several times and shade dried for a while. Weak, damaged and diseased seeds are discarded. Fruits can be collected in polythene bags, having several pores at the bottom and sides. After the extraction of juice, crushed fruits are soon dried for 3-5 days. Mixer grinder, generally used in home kitchen, easily separates seeds from the sun-dried fruits (Singh, 1997). Russian workers developed a machine for seed separation from the fruits (Polupamev and Dulgov, 1988). The fruits are rubbed by a rasping drum, the pulp is washed away by water jets, and the seeds are removed in a separating drum. By this method, they could collect seeds 5 kg fruits per hour.

Storage

After separation, seeds are stored in paper bags, in a dry room at room temperature. Generally, at room temperature, viability of seeds is quite high (85-100%) for 2 years, but then it declines rapidly. However, Fefelov and Eliseev (1986) estimated 92-94% germination after 3.5 years under laboratory conditions (20-24°C and relative humidity of 50-60%). Storage period under room conditions is known in average to be 3 years. The seed storage at low temperature for long period does not affect the viability, however, storage under high humidity badly affects their germination. X-ray photography is suitable for assessing viability of fresh seeds, but stored seeds should first be stained with tetrazolium chloride (Smirnova and Tikhomirova, 1980).

Seabuckthorn nursery should be raised in a place close to the expected plantation site. There should be enough sunshine, irrigation and transportation facilities. Before the seedbeds are prepared, better before onset of winter, stones are removed from the field, sufficient banyard/FYM manure should be mixed in the soil and deep ploughing (about 20-30 cm) is done (In Lu Rongsen, 1992). Generally the seed bed is 3-10 m long and 1 m wide, which is convenient for irrigation, weeding and other operations.

Treatments

Seabuckthorn seeds have short-term physiological dormancy, therefore they can be sown without any treatment, however even then, they take a long time (15-30 days) in germination under field conditions. To enhance early sprouting, seeds may be given some treatments. Internal seed dormancy can be broken by storing the seeds at 5°C for 3 months (Siabough, 1974). Avanzato et al (1987) have reported better germination, when the seeds were treated with 800 ppm GA₃ for 24 hours or 1N H₂SO₄ for 2 minutes. Eliseev and Mishulina (1972) found that dipping seabuckthorn seeds in 0.02% KI or 0.05% Zn SO₄ solutions increased germination by 12 and 9.1% respectively. Placing in solutions of ammonia molybdate, MoSO₄ or CoSO₄, each at 0.05%, increased germination by 8.6%. Simonov et al. (1983) observed that seed soaking in ammonium molybdate, improved the seed germination, increased chlorophyll content and photosynthesis rate in the seedlings. Mochalova (1998) obtained encouraging results of germination (94%) of dormant seeds by treating them with mutagenic chemical Mg SO₄ at a concentration of 500 mg/litre for 48 hours. Fefelov and Selekhev (2003) suggested that seeds are sterilized with the solution of calcium hypochlorite (10 g/140 ml water) and filtered within 5 minutes and thoroughly washed. Then seeds are mixed with sand in the ratio of 1:3, with a humidity of 70%. This mixture is placed in a vessel. The vessel is kept at low temperature of 2-5°C for 30-40 days. Moisture is constantly maintained during this period, while intermixing the mixture. Another method they suggested, in which vessel with mixture is kept at 10-12°C for 10-13 days. As soon as seeds swell and start germination, vessels are placed in the refrigerator at 0-1 °C for 13-15 days. They also suggested a simple method of dipping seeds in water for 5 days at temperature of 20-25°C. Before sowing in the beds, they are shade dried for one day. For enhancing germination of small number of seeds, they are scarified by mechanical means like scalpel or edge of razor, then sown as dry or made wet (20-25°C) for one day before sowing.

Lebeda (personal comm.) recommends the water treatment, in which, seeds are placed in 4-5 cm deep water for 3-4 days and water is stirred up for 6-8 times a day. Seeds start germination in 4-5 days. Furrows of 4 cm width and 2 cm depth are prepared at the spacing of 20-25 cm in the nursery beds of 1 m x 3 m or 1 m x 9 m sizes. Seeds are sown in the furrows and covered with 1-2 cm of soil (In Lu Rongsen, 1992). For stratification,

the seeds are mixed with wet coarse sand in the ratio of sand : seeds :: 3 : 1 and placed in freezer at 0-5°C for 30-40 days. Seeds are mixed twice a week. On beginning of germination, seeds are placed on melting ice or under snow for storage before sowing. Lu Rongsen (1992) suggested that a high rate of germination under field conditions can be obtained either by soaking the seeds in cold water for 7 days or soaking the seeds in hot water (60-70°C) for 30 minutes, stirring frequently, then letting the water cool at room temperature for 2 days. After soaking, when the seeds are well expanded, they are air dried for few hours before sowing. This technique is useful when sowing out doors in spring or indoors for green house sowing.

Sowing

Seeds can be sown both before and after winter. Trofimov (1976) after sowing dry seeds during both seasons, found no difference in germination in Moscow region. However to avoid failures, he recommended stratification of seeds. Salatova (1973) found result of autumn sowing better in western Siberia. Plekhanova (1989) considers the vernal sowing better on the heavy soil. Both in Moscow and western Siberian regions, early vernal sowing of stratified seeds had sprouts in 7-10 days, whereas dry seeds germinate after one month (Trofimov, 1976; Ermakov and Faustov, 1983).

Li and Schroeder (1999) sowed the non-stratified seeds in late September at a depth of 1 cm and a rate of 100 seeds/m in rows, 60 cm apart at the PFRA shelterbelt Centre, Saskatchewan, Canada. In the following spring they obtained a high rate of 90% germination. Following the melting of snow in spring (April), soil temperature (15-20°C) at the depth of 5-10 cm is suitable for sowing of seabuckthorn seeds. Lebeda recommends sowing of seeds in a fertile site at a depth of 2 cm in late autumn and 5 cm in early spring. Sowing of seeds by tractor is also carried out, which may use 3.5 g per 1 m of furrow. Seeds are covered by thin (1.0-1.5 cm) layer of mixture of humus: sand: 1:1, while distance between furrows is maintained at 18-20 cm, between seeds at 1.5-2.0 cm. He recommends dry seeds for late autumn sowing, to avoid infection of fungi. During the spring sowing, he recommends wet seeds. Lu Rongsen (1992) recommends sowing in spring, when the temperature of the soil at 5-10 cm depth, rises to 15-20°C. Ditch and drill seeding is generally practiced in northern China. The drill furrow of 4 cm width and 2.5 cm depth are made in the nursery beds. The drill spacing is maintained at 20-25 cm. The seeds are sown in the drills and covered with 1 -2 cm of soil layer.

Management

Seeds should not be sown deeper than 2.5 cm. Seeds sprout within 12-18 days of sowing. In case ground surface becomes dry and hard, the seeds are unable to sprout properly and seedling may curl and suffocate under the ground surface. Strong sunlight may also raise soil temperature. Timely cultivation of soil and breaking up the hard ground surface to avoid the effect of the rising soil temperature on the delicate seedlings is important for their survival (In Lu Rongsen, 1992). Moisture level is maintained above 70-80% in the beds. Seedlings may be covered with a mulch of straw to conserve the moisture or shade for protection from bright sunlight. When the growth is about 3-4 cm, excessive dense seedlings are thinned down and weeds are pulled out. During the appearance of second pair of leaves, distance between seedlings is maintained by thinning to 3 cm and during the appearance of fourth pair of leaves to 8 cm. When the seedlings sprout 3-5 pieces of macrophylla, the seedling is likely to survive. From this stage, weeding is carried out frequently, to alleviate the inhibitory effect of weeds.

Insecticides like gammexame or dylox are used to check the under ground insects. During the rainy season (July-September), the seedlings attain a height of 5-7 cm. If the water accumulates over the seedlings, they may face mortality. In order to avoid their mortality, there should be proper drainage of accumulated water, particularly in rainy days. For a higher growth of seedlings, supplementary chemical fertilizers are applied before or after the rain or irrigation. The seeds of seabuckthorn may be dusted with TMTD at 3 g/kg seeds or the fungicide is applied at 40 g/m² of soil, before sowing.

After depressive effect for 2 years due to toxicity, satisfactory plant growth in the 3rd year may observed (Golubinskaya, 1972). For cultivation of plants under polyhouse, the following conditions have been recommended by Garonovich (2003): fertilizers of Ng0 P150 Km in substrate before sowing, top dressing of one year seedling with N100 P250 K100 liquid top-dressing with ammonia nitrate (20 g/m²), putting microfertilizers, tilling of roots before planting with IAA, IBA, esculetin during 8 hours, non-root tilling of growth points with solutions of microelements and stimulators (IAA-0.05%, K-IAA-0.02%, A-NAA-0.02%, etc.). While growing seedling, one should use herbicides in doses of 30 kg/ha THA and 1-2 kg/ha. 2,4-D twice a season on fallow field and also 1 kg/ha. of simazin in experimental plot. Agronomical methods have favorable effects on seabuckthorn. Autumn sowing is recommended in polyethylene houses. After 2 years of growth under nursery, seedlings obtain a length of 30-50 cm and can be transplanted in the field (In Lu Rongsen, 1992).

Problems with seeds

Seabuckthorn is a dioecious and cross pollinated (by wind) plant. The plants raised from seeds can not maintain the fine biological (height, branching acanaceous, length of fruit stalk, fruit size, shape and colour) and economic (fruit production, bioactive substances) characteristics that are genetically identical to the

selected mother plant. There may be more males than females among the seedlings, and even, it is not possible to distinguish between male and female plants before flowering and fruiting. Further entering to the fruiting period, it requires a lot of reforming work and labour in regulating or changing the ratio of female to male, for ensuring a higher fruiting in female plants. If it is not regulated, male plants may dominate in plantation and it reduces the quality and quantity of fruit, which hardly meet the demands of raw material for the industries. For the selection purpose and afforestation programme, raising of seedlings from seeds may be preference.

VEGETATIVE PROPAGATION

In order to meet the demand of development of adequate seabuckthorn resources with a regulated ratio of male and female plants, propagation from cuttings should be preferred. Plants raised from the stem or branch cuttings maintain the genetic properties of mother plants and female plants can bear fruit earlier than the female plants, raised from the seeds. Therefore propagation from cutting is an important technology for raising artificial plantations and introducing and acclimating new and improved varieties of seabuckthorn.

PROPAGATION FROM HARDWOOD/LIGNIFIED CUTTING

It is easier to collect and transport the hard wood cuttings. Propagation from hardwood cuttings has been widely used by the foresters and horticulturists, for examples in poplar and willow. Studies on seabuckthorn reveal that cuttings take roots easily, but the rate of rooting is uncertain and susceptible to environmental conditions of different regions. Therefore detail studies are required to increase the reliability of this method. During the last decade, several studies have been carried out on propagation from softwood cuttings. Several studies have been carried out in many countries, particularly in former Soviet Union and China on propagation from hardwood cuttings.

Time of collection

Cuttings may be collected in the autumn, winter or early spring (beginning of March). Li and Schroeder (1999) obtained 90% rooting of cuttings collected in mid-March. Cuttings were stored in plastic bags at 0°C until May and placed in pots filled with peat in a heated propagation box (18-22°C) indoors under fluorescent light. It is therefore concluded that cuttings can be collected during winter (October-March) and should be stored at low temperature in sand for some period before planting them before their bud break during early spring (April). Author collected cuttings during the early April, gave high rate of survival (89%) after placing their base parts into water for 2 days (Singh, 1995). Shuhua et al. (1995) found that seabuckthorn hardwood cuttings can be collected from October to March. However, those collected before March, should be stored in sand at low temperature (3-5°C).

Size of cuttings

Diameter and length of the cuttings influence the rooting rate. The highest percentage of rooting was observed by Shuhua et al. (1989, 95) in the cuttings of about 0.8 cm diameter and 36% higher than the cutting below 0.5 cm diameter. Highest rate of rooting was also observed in the cutting of 10-15 cm length. Dry mass of roots was 0.16 g, taken from the cuttings between 0.8-1.5 cm in diameter. It was 81% higher than cuttings below 0.5 cm in diameter and 63% higher than cuttings between 0.5-0.8 cm in diameter. Dry weight of roots from 10-15 cm long cuttings was 0.11 g, which was 30-40% higher than that of 5-10 cm or 15-20 cm long cutting. It was therefore obvious that the higher percentage of roots could be obtained from the cuttings of 0.8-1.5 cm diameter and 10-15 cm length. Treatment of basal part of cutting with 300-500 ppm NAA improved the growth of the roots (Shuhua et al., 1989, 95). They are of the view that one-year-old cuttings (lesser than 0.5 cm diameter) lack stored food and are unable to produce roots and are bound to die, whereas 3 years old branch gave best results. Tishchenko (1991) reported a better growth and survival in 22 cm long cuttings than 11 cm long cuttings. Singh (1995) found that cuttings of 1.0-1.5 cm diameter and 15-20 cm length gave high rate of survival (79%) in a cold desert area of Himalayas. R.P. Singh et al. (1997) also found that shoot cuttings of 20 cm length gave best result of survival.

The most adventitious roots of seedlings, are borne above leaf scar in the scattered state on the cuttings of seabuckthorn. Shuhua et al. (1989) measured an average of 3-5 roots formed, which stretched horizontally. The roots grown from the epidermis of stems, also have small callus on the cut surface. However, roots are rarely formed from calli. The observation under the microscope shows that the roots initials originate from vascular rays and cambium. Cuttings collected from the basal part of the branches showed 24% higher rooting rate than the upper part. Average length of root on the cuttings, collected from the base part was 3.6 cm, considerably higher than cuttings of upper portion (1.5 cm). Therefore cuttings can be collected from the base parts of branches for raising a vigorous nursery. Age of the cuttings also affect the rooting rate. One-year-old cutting has low rooting rate and pretend to die. One-year-old cuttings are generally less than 0.5 cm in

diameter and lack adequate stored food material. At the time of sprouting buds begin to grow into leaves and consume a large amount of food, thus little food or no food material is available for root formation. Consequently few roots or no roots are borne on the lower base of the cuttings. Therefore, cutting of 2 or 3 year old branches should be selected for raising nursery.

Canopy technology

A detail study on propagation from hardwood cuttings carried out by Shuhua et al. (1989, 1995), needs special attention. In order to prevent frost damage to young plants, they were covered with grass screens and plastic film canopy. They studied the effects of different collecting seasons, size of cutting, hormone and soil media on the rooting rates of hardwood cuttings in a nursery of Dabuxiang, Zhuolu county, 1300 m asl, in Hebei province. It has the average annual rainfall of 145 mm, extreme highest and lowest temperatures of 39°C and -30°C respectively (average 5°C). Climate of the frost-free period was 114 days, frost period from September to May. Site has bitter cold with little snow in winter, dry and windy in spring. It was rainy and hot during summer. The content of clay (< 0.01 m diameter) was about 41.5% and soil texture was medium loam. In order to improve the environment of planting material (cutting), plastic arch canopy of 33 m long and 10 m wide was established over the nursery beds. Small arch canopies of 4.5 m long, 1.3 m wide and 0.5 m high) are installed inside the plastic canopy, from late March to late April. Small seedlings are covered with grass screens might protect them from being frozen.

Treatments

Experiments have shown that storing of cuttings in sand at low temperature; facilitate root formation, which may be related more or less to the presence of inhibitor in cuttings. Therefore, storing of cuttings in cold sand during winter may destroy the inhibitor present in the cuttings, in February. Since the plants are in a state of deep dormancy, the content of inhibitor is higher, therefore the rooting rate is also lower. Kondrashov and Kuimov (1987) showed that 2-year-old branch, cut before bud break and stored for 10 days in wet sand at 10 to 15°C. gave 100% rooting. Shuhua et al. (1989, 95) studied that base parts of cuttings should be immersed in running water for 24 hours or bucket for 1-3 days, changing its water twice a day. Before plantation, basal part of the cuttings is dipped quickly into 500 ppm NAA. Kuznetsov (1985) recommended collecting cuttings before bud break, soaking in water (18-20°C) for 7 days and planting in field with dark polythene mulch. As temperature rises in late March, dormancy will also gradually be over, the rate of plant growth hormones increase, so the rooting rate is also higher. Excellent result was achieved by Mamadrizukhanav et al. (1989), when black currant and seabuckthorn were treated with Krezatsin (tris-2-Okyethylammonium orthocresokyactate), a synthetic analogue of a natural auxin, or NAA. Rooting percentage in black currents grown in a controlled environment was 100% with sprays of 20 mg Krezatsin with or without added Tur (chlormequat) at 0.1 mg/litre with 100 mg NAA/litre applied as spray or a seed soak.

In a trial conducted at 2210 m alt, cuttings of seabuckthorn showed the best rooting (94%) after spraying with 20 mg krezatsin/litre; soaking the seed in 0.1 mg Tur or spraying with 100 mg NAA/litre each gave 92% rooting. Li and Schroeder (1996) found that giving treatment before plantation with 300-500 ppm NAA, may accelerate root formation in the cuttings. Tsar'kova (1988) studied the effect of different physiologically active substances on the rooting and growth of lignified cuttings of seabuckthorn. He treated the cuttings for 2 days with IBA at 50-200 mg/ litre, DIMG (Daminozidel at 0.5-10.0 g/litre, KANU (NAA-potassium) at 25-50 mg/litre and Hydrel (bis (2-chloroethyl-phosphanate) hydrazinium in ethephon derivative) at 2-7 g/litre. It was observed that IBA at 50 mg/litre increased root numbers per plant by 1.4 times, root length by 1.8 times, compared to control. DIMG at 0.5 and 1.25 g/litre also gave similar results. However, hydrel at 2-7 g/litre proved toxic and even prevented rooting. Therefore, besides NAA, IBA at 50 mg/litre and DIMG at 0.5 and 1.25 g/litre can be used to promote root and plant growth of seabuckthorn under nursery conditions.

Kuznetsov (1985) observed that indole butyric acid (IBA) and application of polythene mulch help in promoting rooting in the cuttings. He collected, before bud burst, hardwood cuttings of improved varieties, Vitaminnaya and Novost Altaya of *H. rhamnoides* ssp. *mongolica*. The cuttings were soaked in water at 18-20°C between 30 April and 6 May and some cuttings were treated with IBA at 200 mg/litre. All cuttings were planted on 8-9 May and some were mulched with dark or transparent polythenes. In the cuttings of Vitaminnaya, soaking in water and treatment with IBA had no appreciable effect on rooting but in Novost Altaya, it increased rooting by 10-25%. Dark polythene had a beneficial effect on rooting, use of transparent polythene improved the growth of transplanted plants.

Cooling treatment

The experiment shows that cooling of upper part of hardwood cuttings improves considerably, the propagation coefficient and regeneration. Therefore under extreme conditions of cold, to make a better climate, Kilchevaniye can be used for propagation particularly, where snow is easily available for cooling purpose. Ermakov (1993) developed a new technique for cooling of the upper part of hard wood cuttings before plantation. This technique also known as Kilchevaniye, has been recommended as an alternate to soft wood propagation method, where expensive sheltered ground is used during summer to raise plants from green

seabuckthorn cuttings. In this method developed, by a Russian scientist, seabuckthorn sprout cut during autumn or winter are stored in snow clamps. Cuttings are made from the sprout at the end of March or beginning of April and placed in formed bed, full of snow, which is 70 cm in depth. Snow layer thickness is 65 cm. Bundles of 20-25 cuttings are prepared and placed with upper parts in downwards position, making bed with ramming snow layer. For acumination of solar heat, bundles are placed closely with some humid manure straw powder (0.05 cm) covering them. The whole structure is covered with a polythene sheet. During the cooling of upper part of cuttings, the inner-side of sheet is covered by condensator, a higher temperature is created in basal part of cuttings, and lower temperature is upper part, since it is located on the snow. Cooling of upper part is stopped when root tubercles appear on the basal part. At this stage, however, leave buds do not expand. After cooling, cuttings are planted into the nursery soil. They form secondary roots in 4-5 days. Roots of the first order are formed in 3-4 numbers. Roots of second and third orders are also formed during the first year of growth.

Laser treatment

Budagovskii et al. (1993) applied laser technology for enhancing the rooting rate in seabuckthorn. They achieved as high as 89-93% rooting rate, in the cuttings of seabuckthorn and other horticultural plants as compared with 14-66% in their controls. In the sites, where temperature falls considerably during early spring, protective measures during night like polythene sheet cover, also help the cuttings to survive frost or extreme drop in the temperature of the site.

Soil media

Adequate aeration and water permeability of soil are required for vigorous rooting in hard wood cuttings. Man made matrix like sand: humic soil: soil under seabuckthorn vegetation: 5:3:1, can ensure proper temperature mixture and aeration of rooting (Shuhua et al., 1989). In case, cuttings are planted in open in a nursery, soil must be loose and with proper drainage. As the natural distribution of seabuckthorn is found on sandy river beds and sides, it shows that sand being porous (higher oxygen), is an important medium for raising seabuckthorn nurseries. Shuhua et al. (1989) found that rooting rate was maximum, when cuttings were planted in pure sand as compared to sand and humic soil mixture of 1:1 ratio. For a better growth of plants, they recommended a soil mixture of sand humic soil and soil under the seabuckthorn vegetation in the ratio 5:3:1 (rooting rate 89%) beside mixing soil in small amount collected from the soil under seabuckthorn vegetation. Adequate area and water permeability of soil are suitable particularly for root formation in the hardwood cutting. Under nursery conditions a soil mixture of soil: sand: FYM with a ratio of 5:3:1 along with a small amount of soil collected from the seabuckthorn vegetation (for Frankia bacteria) provide better soil conditions for raising a healthy and vigorous nursery of seabuckthorn for a massive afforestation programme (Singh, unpub.). Rooting in the hard wood cuttings is sensitive to environmental changes, for raising big nurseries, particularly in these regions where there is sudden increase in temperature during early spring.

Plantation of cuttings

Lebeda suggests that during the early spring, when the soil at the depth of 15-20 cm, warm up to 5°C, cuttings are planted in the open soil. Basal part of cuttings (2/3 part) is placed in water for 4-5 days, changing the water 3 times. Cuttings are planted at a spacing of 20 cm x 10 cm. Half part of the cuttings remains out of the soil, with 2-3 buds. After plantation, cuttings are well irrigated. In cold regions like Dabu of China, cuttings are planted about 30-40 days earlier than out side, in mid canopies, inside the plastic arch canopy. Covering small canopies with grass screens at night protects the cuttings, maintaining the mixture. Cutting start producing roots on the 8th day after plantation, when the cumulated temperature equals or above 10°C at 5 cm in soil depth, is up to 121.6°C. They produce several roots in 15-20 days, when the cumulated temperature is 312.5-317.0°C. When complete and semi woody root system is developed with lateral roots and root nodules, then the cuttings can be transplanted into nursery. After that, the second batch of cuttings can be planted. The cuttings of the second batch are also selected at the same time when selecting the first batch and stored in wet sand at low temperature before plantation (Shuhua et al., 1995).

Large cuttings

Kondrashov (1994) used a new technology of planting of long cuttings in the orchards. He prepared the cuttings of 40-50 cm length for improved variety Novost Altaya and other horticultural crops and planted them vertically or at an angle of 60° into 35-40 cm deep holes, leaving 3-6 cm above the soil surface. Survival for seabuckthorn and other plants ranged from 73 to 100%. However this methods needs to be tried under different soil and climates conditions before adoption for raising orchards or plantations.

Pot method

Seabuckthorn can also be propagated under pot conditions. Bruvelis (1991) found that in no case, it was possible to observe any essential influence of the size of pot, rooting medium, length of cutting or vertical gradient of temperature on the rooting rate in the hardwood cuttings. Pot size not less than 20 cm³, was found to be suitable, for raising plants. In the pot, loose and air- penetrable rooting media with long-fibral peat,

without fertilizers should be used. Cuttings length, although, has a positive correction with growth of new shoots, but only in the beginning. After transplanting to open ground difference decreases. Therefore the length of cuttings can be shortened initially to 5-7 cm to save plant material. Diameter of cuttings with well developed buds, should be over 6 mm. Unfavorable influence of the vertical gradient of temperature should be avoided, if possible by using under heating. For the same, cutting should be planted at least 3 cm above the bottom of the pot. Before sticking, all buds except the two terminal ones should be removed from male cuttings, whereas in female cuttings, buds should be removed only from the terminal part. Economic viability of this pot method developed by Bruvelis (1991), is not known.

Management

Adequate care may be taken for protection of cuttings by canopies for shading, irrigation, weeding and tillage of soil. With the formation of leaves and roots, shelter from sun is removed and irrigation may be lowered. Shlyapnikova (1985) observed that rooted cuttings of seabuckthorn had a poorly developed root and shoot system. When he applied Simazine and Ienacil at 1-3 and 1.5-4 kg/ha, respectively, it increased seedling height, growth, numbers of shoots and root development compared to hand weeded control treatments. Application of herbicides in 2 split dressings 7-10 days after planting and 4-6 weeks, later enhanced the root establishment and seedling growth. Gardner et al. (1984) found that use of appropriate Frankia bacteria and mycorrhiza in seabuckthorn mycorrhizal roots, shows significant improvement in plant growth, phosphate uptake and nitrogenase activity in mycorrhizal/nodulated plants in comparison with nodulated-only and mycorrhizal-only plants, when grown in a medium poor in combined nitrogen and soluble phosphate. During winter, cuttings are covered with thin layer of pine needles or fallen leaves. During next spring, they are transplanted in the field or allowed for more growth.

Comparison with soft wood cuttings

Garanovich (1984) compared data on the rooting of green cuttings and lignified cuttings of varieties with superior fruit quality in various types of substrate, with and without chemical stimulants, in greenhouses under artificial mist. Rooting success was 20% lower with lignified cuttings than with green cuttings, but plants from lignified cuttings attained heights of 90 cm by the end of the first growing season and could be planted out the next spring, whereas plants from green cuttings needed to be grown carefully under special conditions with periodic watering for 1 or 2 years. Avdeev (1984) compared the rooting capacity of soft- and hardwood cuttings of pomegranate, fig, seabuckthorn, mulberry, rose, currant, persimmon and pear. He found that for good transplant production, pomegranate, fig, seabuckthorn and black currants should be propagated by hardwood cuttings. Softwood cuttings of pomegranate and fig also gave good results when watered by hand.

PROPAGATION FROM SOFTWOOD CUTTINGS

Propagation from softwood cuttings is widely practiced in Europe and China. Massive nurseries can be raised from the green cuttings under controlled temperature and humidity conditions in mist chambers. Therefore unlike hardwood cuttings, they are not prone to environmental changes, which ensure vigorous root formation and growth in the cuttings. Softwood cutting is a more effective propagation method than hardwood cuttings. The method needs artificial mist sprays and a plastic film house with other equipments.

Time of collection

Timing of collection of cuttings affects the root formation. Avdeev (1976) reported a higher rooting rate of 96-100% in the softwood cuttings collected during the beginning of growth of mother plants (early spring). Cuttings collected during intensive growth of mother plants gave low rooting percentage, but again at the termination of active growth gave 86-100% rooting. Osipov and Morozova (1983) found 65-70% rooting in the cuttings collected during early June, which is the time of vigorous shoot growth. Buonous et al (1992) found better rooting in cuttings when they were collected in late June. Potapov (1978) observed that semi-hardwood cuttings, collected in late June or early July in Altai region of Siberia in Russia was beneficial. The time of day, when cuttings were collected did not affect the rooting. Kniga (1989) reported in Kiev region of Ukraine, the optimum time of cutting collection was late of May. Kondrashov and Kuimov (1987) observed that cuttings taken in late June from severely pruned branches (pruning was conducted in early spring before bud break) successfully rooted (95-98%) in the green house under a mist. In Hebei province of China, the favorable time is from the middle of June to the beginning of August (Zhixiang et al. personal comm.). In Balgaird, Sweden, cuttings are collected during the end of June or beginning of July (Wahlberg, 1992-94). Shuhua et al. (1995) are of the view that the green (semi-wood) cuttings should be taken from the healthy plants (without pest) during middle of July to the beginning of August.

Collecting of cuttings

The cuttings from small or thin young shoot lack the ability to form the roots. Cuttings from highly lignified branches also have a poor capacity to form roots. Studies have shown that the shoot with medium growing

power, in the same layer of the crown, of the same age, exposed to sunshine, are most suitable for raising the nurseries of seabuckthorn. Cuttings are generally collected during end of June to beginning of July, when there is a beginning of lignification of branches. Cuttings are collected by sharp knife or by the blade of safety razor on 3-4 mm below bud (Lebeda, personal comm.). Cuttings should be collected during the early morning, when the leaves are covered with dew. Papp (1982) found the cuttings of 10-12 cm length are best suited for propagation, whereas Pletneva (1983) recommended 15-20 cm length of the cuttings. Garonovich (2003) used cuttings of 12-15 cm length with a single bud on top. Shuhua et al. (1995) observed the best results of rooting in 7-12 cm long cuttings treated with NAA. In an experiment of pots (7 x 7 x 8 cm³), Wahlberg (1992-94) observed that the cuttings of 10-12 cm collected from the top part of a branch gave better results after treatment with 50 ppm IBA for 24 hours, than the cuttings collected from the middle and base parts of branches. Cuttings of 15-20 cm length are collected from the branches and immediately put into the plastic film bags to protect them from wind drying and withering. These cuttings can remain fresh for 3 days in this condition or 7 days in water without losing their rooting capacity (In Rongsen, 1992).

The rooting rate of cuttings with top bud is much higher (90%) than without top bud. It is presumed that the top bud produces hormones to stimulate rooting (Shuhua et al., 1995). Rooting rate of cuttings from 1 year old branches is higher (100%) than 2 year old (90%) and 3 years old branches (73%). Cuttings collected from the upper part of the same plant had higher rooting (80.0%) than the lower part (63.3%) (Shuhua et al., 1995). Green or soft cuttings of seabuckthorn have stronger ability to produce several adventitious roots within a short period of plantation. Roots are borne out at the part of 0.5-2.5 cm above the cut surface of the cuttings, belonging to the type of epidermal rooting. Roots are always many and concentrated and generally arranged in the form of brush on the crevice of epidermis (Shuhua et al., 1995). They recommended that cuttings of 7-12 cm length with top bud and 2-3 pairs of leaves, must be selected from the lower part of maternal plants. Lower part should be treated with NAA for 10 seconds and planted in the soil matrix of sand: saw dust: soil under seabuckthorn vegetation with a ratio of 10:3:1 or 10:7:0, which should be sterilized with 0.5% KMnO₄ solution before putting into containers. Seedlings can be transplanted to nursery, from containers, after 25-30 days in beds and 7 days in shed for training.

Hormone treatment

In case, we want to avoid hormone treatment, basal part of cuttings is placed into running water. However, hormone promotes root formation and healthy growth of plants. Growth hormones like NAA, IAA, indole propionic acid (IPA), indole butyric acid (IBA) and adenosine triphosphate (ATP) have been used by various workers for accelerating root formation in seabuckthorn. The basal part of cuttings are placed at 2-3 cm depth in solution of growth hormone at room temperature for 14-16 hours, then washed with water and planted. Avdeev (1976) treated softwood cuttings of seabuckthorn with 50 mg/l IBA and planted in peat: sand: 2:1 mixture. Treated and untreated cuttings started rooting on 9-11th and 11-13th day after plantation. Potapov (1978) observed that IAA at 200-400 mg/litre or IBA at 100-200 mg/litre improved rooting in the cuttings. Spraying the leaves with hormone required less material and was simpler to apply than treating the base of the cuttings. Super phosphate applied to the cutting bed enlarged the root system.

Papp (1982) observed that the cut end can be dipped in any of the solutions of NAA, IAA and IBA. Ivanicka (1988) compared the effect of IBA on the rootings of cuttings in 6 species including seabuckthorn. Semi lignified cuttings of various cultivars, collected during early July, were treated with IBA at 0.1-0.3% and planted in a substrate of peat, polystyrene granule and sand (1-2:1:0.5) under mist in a plastic house. Untreated seabuckthorn, *Arunica melanocarpa*, *Sambucus nigra* rooted readily, but *Cornus mas*, *Corylus avellana* and *Rosa villosa* cuttings rooted better with IBA treatment. Salikhov (1986) found IBA favorably affected rooting in softwood cuttings taken from the base of the shoots but had no marked effect on the apical cuttings. He further observed that presence of the apical points in different types of cuttings improved rooting and their removal reduced rooting and subsequently growth. Cuttings with some wood or with a heel rooted and developed well. Using cuttings with a heel, increased labor productivity and gave up to 88-92% rooting under commercial conditions. Avdeev (1976) observed that soft wood cutting treated with IBA at 50 mg/l, which were planted in 2:1 peat/sand substrate, started rooting on the 9-13th day. Garonovich (2003) recommended treatment of cuttings with IBA (50 mg/litre), IAA (100 mg/litre), NAA (50 mg/litre) for 16 hours.

2,4-D at low concentrations was used by Polikarpova et al. (1988) to increase the rooting of softwood cuttings in easy- and difficult to root sour cherry cultivar Malinovka of seabuckthorn and the apple rootstock M-26, but at the concentration tested (not specified), 2,4-D reduced the percentage of rooting in cuttings of readily-rooting *V. rhamnoides* and land current cultivars. The stimulation of root development in M-26 by 2,4-D was comparable to the stimulation by IBA.

Bounous et al. (1992) collected and treated seabuckthorn cuttings in late June from mountain area of Italy, with 3000 ppm IBA. Balabushka (1990) compared the effect of IAA, IBA and chlorophenoxyacetic acid on rooting of seabuckthorn and found that IAA showed better results as compared to other hormones. Wahlberg (1992-94) found better result of propagation, when the cuttings of 10-12 cm length, selected from the top branches and

treated with 50 ppm IBA for 24 hours. Lebeda suggests the application of IAA at 10-25 mg/l for cuttings collected from upper part of branch, 25-50 mg at middle part and 50-100 mg at basal part of branch for rooting.

Lu Rongsen (personal comm.) suggested that before the treatment with hormone, cuttings are placed in water to keep them fresh. Then these cuttings are taken out of the water and bundles of 40-50 cuttings, kept together by a rubber band, are placed into a box filled with solution of 50 ppm IBA at a depth of 3-4 mm. All the cuttings soaked in the IBA solution be covered with a plastic film to maintain a temperature of 20-25°C for 16 hrs. Before planting, these cuttings should be taken out of the IBA solution and washed to remove the hormone. Shuhua et al. (1995) concluded that a cutting dipped in NAA solution at 300-1000 ppm for 10 seconds accelerated root formation up to 80-100%. It was observed that for European seabuckthorn, IBA has been a preference for cutting treatment, whereas in China, NAA has been widely used.

Treatments of minerals

Simonav et al. (1986) observed that growth substances (humic acid and Agrostilin) and minor elements (Mn and Zn) markedly improved the planting material produced during the first year and this reduced the cost of growing-on of non-standard plants during the second year in the nursery. Treatment of softwood cuttings with humic acid at 150 g/10 litre water and application of Mn and Zn to the rooting substrate have been recommended. Studies were carried out by Krylova (1991) on the softwood cuttings of sour cherries, plus, seabuckthorn, black currants, gooseberries and apple clonal rootstocks. The cuttings were treated with IBA at 25-50 mg/litre and planted in an unheated peat/sand substrate under mist. In apples, application of P at 0.4 g/m² of substrate on the day of insertion markedly stimulated rooting. Data are tabulated on the differential response of apple, sour cherry, plum, sea buckthorn and black currant cuttings to 4 different fertilizers (with varying N: P: K ratios) during rooting, and shoot and root biomass formation. Generally, all species benefited from N application, except sea buckthorn, which fixed atmospheric N, but responded readily to high K rates.

Magnetized water treatment

Magnetized water has also been tried by some workers to affect root formation in the softwood cuttings of seabuckthorn. Tsarkova and Polikarpova (1980) dipped seabuckthorn cuttings in magnetized water at 18-20°C for 18 hours. The magnetized water was obtained by passing it through a magnetic field (2400 oersted at 0.33 m/sec.). Dipping of cuttings had no beneficial effect on rooting but propagation under MV mist gave up to 100% rooting. Subsequent shoot growth was 33-40% greater than in the control (ordinary mist).

Radiation treatment

Some scientists have tried gamma radiation for higher rooting in the cuttings (Potapov and Borodachev, 1979). Cuttings taken from the shoot apices and irradiated at 500 rad showed the best rooting rate (97%) and those taken from shoot bases showed only 76% rooting, as compared to 70-71% rooting in controls respectively. Higher irradiation does decreased rooting percentages, especially in cuttings taken from the shoot bases. The 2000 rad dose was found lethal. Laser technique has also been found useful in propagation of seabuckthorn. Budagovskii et al. (1993) treated softwood and hardwood cuttings of seabuckthorn, apple, black current and red currents before plantations. In this method, 20,000-150,000 cuttings could be treated with laser rays per hour. Laser treatment stimulated rooting in all the species studied, which ranged from 89 to 93% as compared with 14 to 66% in the control.

Growth chamber

Cultivation of softwood cuttings is carried out in the plastic film house equipped with an artificial mist sprayers and drainage system. The seedbed is prepared flat and the medium of the seed bed should be sand and soil mixture in a ratio of 3:1. Shuhua et al. (1995) observed that the rooting rate and the root biomass are the maximum, when the matrix of sand: saw dust (and soil under seabuckthorn vegetation is prepared with a ratio of 10:7). It should be sterilized with 0.5% KMnO₄ solution. The seedbed is watered to keep the medium wet enough. Before planting the cuttings, a marker is used to rule small shallow furrows in the seedbed. The distance between the furrows should be 7 cm and cuttings 3 cm. Then the cuttings are inserted into the soil mixture at a depth of 1.5-2.0 cm. Placing of cuttings closely may be spoiled by mould. After the planting of cuttings, seed bed is again watered to make the medium around the cuttings settled down (In Lu Rongsen, 1992). Further improvements have been made in propagation by the soft wood.

There are two types of plants raising equipments; one is a green house, which contains an automatic intermittent mist spray controlled by an electric leaf. Another way involves a small plastic film canopy equipped with an artificial mist sprayer and shaded by foliage. The latest model devised by the Beijing Forestry University can be described here (Shuhua et al., 1995). This methods use a 33 m long and 10 m wide plastic canopy, filled with spraying tubes inside. From the middle or late March to late April, small arch canopies (4.5 m long, 1.3 m wide and 0.5 m high) are installed inside the plastic canopies. To protect the young plants/cuttings from being frozen, the small ones are covered with grass screens and plastic film. The electronic leaf is installed in the cutting bed. By using the humidity auto-regulating instrument (SWK-1) to

control the electronic-magnetic value (DFI-2), the surface of cuttings, leaves can always keep a layer of water and the relative humidity of air around the cuttings is maintained more than 90%.

The light permeability ratio of the shed is maintained at 50-60%, 2 m above the ground. The small-enclosed arch plastic canopies (5 m long, 1.7 m wide and 0.5 m high) are set on the cutting bed under the shed canopy. There is a tube suspended to the top of small canopy on which there are several spraying heads 50 cm apart. The temperature and humidity of air and matrix under the small canopy is regulated by changing the spraying frequencies and time. After planting of cuttings, spraying for 20-30 seconds and 2-3 times a day is practiced during the first week and then 1-2 times a day is followed. Soil mixture of sand, saw dust, humid soil and soil under the seabuckthorn vegetation (for Frankia) is used as matrix. It is sterilized with 0.5% KMnO₄ solution or in the sun before being put into the containers. The length of bed is dependent on the topography. It may be 1.5 m wide and 40 cm deep. Cobbles are spread at the bottom about 8 cm thick, then the sand is about 10 cm thick.

As Lebeda suggests, the delves are dug up to a depth of 40 cm for the making the arrangement of glass beds, plastic film glass house or covered beds. The bottom parts are filled with layers of gravel or crushed bricks (15 cm) and layer of coarse-grained sand (5 cm), then layer of humus: sand: 1:1 soil (15 cm). Upper most layer of substrate of pure coarse-grained sand or mixture of sand with peat with a ratio of 3:1 (5 cm). Solovyeva (1998) used two layered substrate, which consisted of well composed manure, sand and soil in equal proportions, spread on the floor of the green house, a sand layer made on the top of the substrate.

Management

Aftercare improves the rooting rate and survival of cuttings and plants. Garonovich (2003) is of the view that optimal air temperature for rooting should be 22-27 °C. Relative humidity should not be lesser than 90%. During the first few days, beds are irrigated frequently (3-5 times a day), but it should not be too much, by using water can or pipe with automated nozzle. During hot sunny days, cuttings are shaded by glass or film or pulling gauze over them. Weeds are not allowed to grow. After 15 days, watering is reduced to 1-2 times a day, but it should be adequate. The rooting of cuttings depends upon the temperature and moisture in the matrix (soil and air around the cuttings). At the time of planting of cuttings, the temperature of the soil should be higher by 1-3°C than the air. Generally, when the daily average temperature of open land is up to 18.5°C, the average temperature of the soil is up to 19°C and the relative humidity of the air is 40-50 percent. However, within the plastic film, the average temperature of day and night should be 19°C, the average temperature of the soil should be 24°C and the relative humidity of the air should be maintained around 88-100%, by using spray-mist at times, if needed. This high level of humidity is indicated by the appearance of water layer on the leaf surface of the cuttings. In case, the water layer disappear, it is necessary to spray mist until the leaf surface retains the water layer again. After the cuttings have already produced sufficient roots, the humidity should be gradually decreased and the film house should be well ventilated. With the onset of autumn, the rooted cuttings should be gradually hardened. First, the plastic film is removed for a short period every day and this is increased day by day. After 15-20 days, the plastic film can be removed permanently.

After the onset of winter, it is necessary to cover the place, where the roots have been developed with a layer (5-6 mm) of taller leaves in order to protect them from cold damage (Ermakov, 1985, In Lu Rongsen, 1992). During growth period, shoots may be trained to prepare them for winter. For the same, careful aeration of whole structure is carried out (but not for long, open up plastic film cover or slightly raise hotbed panes). In mid of August, plastic film covers and panes are removed completely. Garonovich (2003) recommended fertilizers of N80, P150 K100 in soil media before sowing, top dressing of one year old seedlings with N100 P250 K100, liquid top dressing with ammonium nitrate (20g/m²), putting microfertilizers, tilling of roots before planting after treatment with IAA, IBA, esculetin for 8 hours, besides non-root tilling of growth points. For over wintering and to save them from frost and snow damage, mulches of 5-6 cm thick layer of needles, leaves, dry humus or peat are spread over the beds. Under the mulches, poisoning baits are placed against the mice. Before the initiation of bud burst in next spring, rooted cuttings are dug out and transplanted in another site.

Preparation of site

New site is prepared in autumn by ploughing the soil and mixing fertilizers (10 kg/m² humus, 30 g/m² double super phosphate, 300-500 g/m² wood ash). Weeding and tillage operations are carried out timely, where as moisture in the soil is maintained at 75-80%. After one year of growth, they are transplanted into the plantation field.

LAYING AND SUCKERS

In Russia, air layering has also been found useful in raising nurseries of seabuckthorn (Vorobyev, 1978). In a study on 4-year old plants of seabuckthorn, Dar Katuni variety's leaves and current year's shoots were removed from 10-20 cm of shoot base and the shoot was ringed. The 2.5- 3.0 cm wide ring was treated with IBA (17 mg/g) or IAA (20 mg/g) paste and the area was covered with Sphagnum moss and polyethylene. After

25 days, some 49 roots were found on treated shoots and only 2.8 on the control shoots (defoliated and ringed only). Root cutting has also been used as an effective propagation method for seabuckthorn (A. Bruvelis in Li and Schroeder, 1996). Root cuttings were planted in pots and placed in greenhouse for 6 weeks before being transplanted to the field at a spacing of 8 x 20 cm. Before planting, cuttings need to be acclimated to field conditions by placing pots in a shady area for 7 days. The best results were obtained in sandy loam at pH 6 to 6.5 with medium humus content. Seabuckthorn easily produces suckers within a few years of planting; therefore, it can be a good source of propagation (Kondrashov and Kuimov, 1987). The invasion of surrounding areas by seabuckthorn is quite possible. Therefore routine management and herbicide application are the best control measures for this potential wildness characteristic of seabuckthorn.

GRAFTING

Danilin and Grebennik (1977) successfully tried grafting in the male and female plants of seabuckthorn. Some of the scions, used, were treated with a solution of heteroauxin. Male rootstocks of seabuckthorn gave better scion survival and also better scion growth in the first year. The mean increment of scions treated with heteroauxin was greater than that of controls. The cuttings of 7-9 mm diameter are prepared during autumn and stored at low temperature or snow, to prevent them from drying. Cuttings can also be prepared during early spring. Grafting is carried out in one-year-old branch. The cuttings must carry unbrust buds. The place of grafting is tied with a piece of polythene sheet. After the 40-60 days, polythene sheet is relaxed or removed (Lebeda, personal comm.).

REFERENCES

1. Avdeev, V.I. 1976. Propagation of *Hippophae rhamnoides* by softwood cuttings under mist. *Kratkie tezisы Dokl 2-i Vses Konf Molodykh Uchenykh po Sadovodstvu* p. 12-1A (in Russian)
2. Avdeev, V.I. 1984. Propagation of fruit crops by cuttings in upper Tadjikistan Intensivnye. *Sposoby Vyrashchivaniya Posadochnogo Materiala Sadovykh Kul'tur*, Moscow, USSR. p. 51-56. (In Russian)
3. Avanzato, D., Magherini, R. & Lodoli, E. 1987. Studies on the germination potential of seeds and the rooting ability of cuttings of *Hippophae rhamnoides* L. *Atti Convegno sulla Coltivazione delle Piante Olficinali*, Trento 9 10 ottobre 1986 p. 411-419. (In Italian and English)
4. Balabushka, V.K. 1990. Results of the trials of growth regulators on summer cuttings of introduced woody plants. *Byulletin Glavnogo Botanicheskogo Sada* 156: 65-67.
5. Bounous, G., Bullano, F. & Peano, C. 1992. Softwood cuttings of *Amelanchier canadensis*, *Comus mas*, *Elaeagnus umbellata* and *Hippophae rhamnoides*. *Monti-e-Boschi* 43 (4): 51-57 (In Italian)
6. Bruvelis, A. 1991. Propagation of seabuckthorn (*Hippophae rhamnoides*) by hardwood cuttings. *Verksamhetsberaettelse Balsgaard* (Sweden), p. 194-199.
7. Budagovskii, A.V., Budagovskaya, O.N., Gudi, G.A., Mokrousova, G.I., & Gul'shina, E.V. 1993. Laser technology in horticulture. *Sadovodstvo-I-Vinogradarstvo* 3: 6-7. (In Russian)
8. Danilin, M.A. and Grebennik, A.V. 1977. Grafting on (dioecious) trees considering the sex of *Populus tremula*, *Populus balsamifera* and *Hippophae rhamnoides*. *Lesnoe Khozyaistvo* 2: 56-57. (In Russian)
9. Ermakov, B.S. 1993. Cooling (Kilchevaniye) of the upper part of seabuckthorn wood cuttings. In: *Proceedings of the 2nd International Symposium on Seabuckthorn*, p. 327-329. (In Russian)
10. Ermakov, B.S. and Faustov, V.V. 1983. Technology for the Cultivation of Seabuckthorn. 61 p. (In Russian)
11. Ermakov, B.S. 1985. Introduction of low-growing seabuckthorn into the Moscow area. *Biologicheskie Aspekty Introduktsii, Seleksii i Agrotekhniki Oblepikhi*, p. 58-63 (In Russian and English)
12. Eliseev, I.R and Mishulina, I.A. 1972. New data on the biology of germination of *Hippophae rhamnoides* seeds. *Trudy Gor 'kovskogo Sel'skokhozyaistvennogo Instituta* 38: 107-109. (In Russian)
13. Eliseev, I.R and Mishulina, I.A. 1972. The effect of the minor elements on the increase in seabuckthorn (*Hippophae rhamnoides*) seed germination. *Trudy Gor 'kovskogo SeVskokhozyaistvennogo Instituta* 38: 110-111. (In Russian)
14. Eliseev, I.R and Mishulina, I.A. 1977. Changes in biological and biochemical properties of *Hippophae rhamnoides* seeds during ripening. *Trudy Gor 'kovskogo Sel'skokhozyaistvennogo Instituta* 105: 15-22. (In Russian)

15. Fefelov, V.A. and Eliseev, I.R. 1986. Biology of germination and emergence of seabuckthorn seeds of different ecological and geographical origin. *Biologiya, khimiya, introduktsiya i selektsiya oblepikhi* p. 110-115. (In Russian)
16. Fefelov, V.A. & Selekhev, V.V. 2003. Propagation of seabuckthorn from seeds. In: *Seabuckthorn—A Multipurpose Plant* (Eds. V. Singh et al.) (In press).
17. Gardner, I.C., Clelland, D.M. & Scott, A. 1984. Mycorrhizal improvement in non-leguminous nitrogen fixing associations with particular reference to *Hippophae rhamnoides* L. *Plant and Soil* 78: 189-199.
18. Garonovich, I.M. 2003. Introduction of *Hippophae* L. in Belarus. In: *Seabuckthorn - A Multipurpose Plant* (Eds. V. Singh et al.). (In press).
19. Golubinskaya, N.S. 1972. Some data on raising seabuckthorn seedlings using fertilizers and the fungicide TMTD. *Voprosy Lesopark, Khozyaistva i Ozeleneniya Novosibirskogo Nauchnogo Tsentra*, p. 152-160.
20. Ivanicka, J. 1988. Propagation of unusual fruit crops from softwood cuttings under mist. *Vedecke-Prace-Vyskumncho-Ustavu-Ovocnych-a-Okrasnych-Drevin-v-Bojniciach* 7: 163-170.
21. Li, T.S.C. & Schroeder, W.R. 1999. *Seabuckthorn Production Guide*. (In press)
22. Lu-Rongsen 1992. *Seabuckthorn - A Multipurpose Plant Species for Fragile Mountains*. ICIMOD, Kathmandu, 62p.
23. Krylova, I.I. 1991. Mineral nutrition of softwood cuttings of top and soft fruit crops. *Sadovodstvo-i-Vinogradarstvo* 4: 16-18.
24. Kondrashov, V.T. 1994. New technology of creating stands of seabuckthorn and other horticultural crops. *Russian Agricultural Sciences* 11: 24-28
25. Kondrashov, V.T. & Kuimov, V.N. 1987. Vegetative propagation of *Hippophae rhamnoides*. *Sadovodstvo* 6: 13- 16. (In Russian)
26. Kuznetsov, P.A. 1985. Effect of pre-planting treatment and plastic mulch on rooting of seabuckthorn hardwood cuttings and transplant quality. *Biologicheskie Aspekty Introduktsii, Seleksii i Agrotekhniki Oblepikhi* p. 159- 163. (In Russian)
27. Kniga, N.M. 1989. Characteristics of rooting softwood cuttings of top and small fruit species in relation to natural photoperiod. *Fiziologiya i Biokhimiya Kulturnykh Rastenii* 21: 403-409. (In Russian)
28. Mamadrizukhonov, A.M., Felaliev, A., Musoev, S. & Oshurmamadov, A. 1989. Effect of growth regulators on the rooting of black currant and seabuckthorn cuttings under conditions of the Western Pamir. *Izvestiya-Akademii-Nauk-Tadzhikskai-SSR,-Biologicheskikh-Nauk* 4: 64-67 . (In Russian)
29. Mochalova, O.V. 1998. Results of treatment by chemical agents of stratified and dormant seeds in *Hippophae* and *Shepherdia*. In: *Proceedings of International Symposium on Seabuckthorn (Hippophae rhamnoides L.)*, p. 134-136, Russia, p. 248.
30. Osipov, Y.V. & Morozova, G.M. 1983. Propagation of seabuckthorn by cuttings. *Sadovodstvo* 12: 20-21. (In Russian)
31. Papp, L. 1982. Importance and propagation of seabuckthorn. *Erdo* 31 (7): 309-312. (In Russian)
32. Plekhanova, M.N. 1989. Seabuckthorn. *Sorta plodovykh lyagodnykh kul'tur nechernozem'ya*, p. 161-167. (In Russian)
33. Pletneva, T.M. 1983. Intensification of seabuckthorn nurseries. *Agrotekhnika i Seleksiya Sadovykh Kul'tur*, p. 149-154. (In Russian)
34. Poluparnev, Yu. I. & Dulgov, N.P. 1988. A seed separating machine for small juicy fruits. *Lesnoe-Khozyaistvo* 1: 50-51.
35. Polikarpova, F.Ya., Protchev, A., Kov, A.V. & Tikhomirov, I.A. 1988. The effectiveness of applying 2,4-dichlorophenoktyacetic acid for root development in softwood cuttings. *Plodovodstvo V Nechernozemnoi Polose, Moscow, USSR*, p: 13-20..
36. Potapov, F.F. 1978. New methods of seabuckthorn propagation from green cuttings. *Rastitel'nye Resursy* 14 (2): 215-220. (In Russian)
37. Potapov, S.P. & Borodachev, M.N. 1979. Treatment of rose and seabuckthorn softwood cuttings with gamma irradiation 6 OCD. *Subtropicheskie-kul'tury* 3: 103-105. (In Russian)
38. Salatova, N.G. 1973. Conservation of growth of *Hippophae rhamnoides* in mountain regions of Siberia. *Okhrana Gomykh Landshaftov Sibiri*, p. 165-167 (In Russian).

39. Salikhov, M.M. 1986. Rooting capacity of different types of Hippophae softwood cuttings. *Sovershenstvovanie Vyrashchivaniya Plodovykh Kul'tur v Nechernozemnoi Polose*, p. 61-68. (In Russian)
40. Saranovich, I.M. 1984. Features of the vegetative propagation of Hippophae rhamnoides for introduction in Belorussia. *Lesnoe-Khozyaistvo* 2: 27-29. (In Russian)
41. Shlyapnikova, A.S. 1985. Efficiency of herbicide application in planting rooted green cuttings of blackcurrants and common sea buckthorn. *Izvestiya Timiryazevskoi-Sel'skokhozyaistvennoi Akademii* 5: 125-129.
42. Shuhua, H., Zhixiang, Z. & Xin, Z. 1989. Studies on the culture technique of hardwood cuttings of seabuckthorn. In: *Proceedings of International Symposium on Seabuckthorn*, p. 217-229, Xian, China.
43. Siabough, P.E. 1974. Hippophae rhamnoides-Common seabuckthorn. In: *Seeds of Woody Plants in the United States*. C.S.Chopmeyer Tech.Co-Ord.USDA-FS Agric.Hdbk. No. 450: 446-447.
44. Smirnova, N.G. and Tikhomirova, N.I. 1980. Combined use of x-ray photography and the tetrazolium method for assessing seed viability. *Byulleten'-Glavnugo-Botanicheskogo-Sada* 117: 81-85. (In Russian)
45. Simonov, I.N., Dudkin, G.I. & Matkarimova, R.K. 1983. Effect of ammonium molybdate on seabuckthorn seed germination and other biological properties. *Vestnik, Karakalpakskii Filial Akademii Nauk Uzbekskoi SSR* 4: 22- 24.
46. Simonav, I.N., Poludennyi, L.V., Baikalov, P.A. & Volkova, T.V. 1986. Effect of manganese, zinc and growth regulators on the growth of Hippophae rhamnoides transplants. *Pitanie-Plodovykh-Rastenii*, p. 105-109.
47. Singh, R.P. Vaneet, J., Nege, D.V. & Jishtu, V. 1997. Studies on nursery techniques of Hippophae rhamnoides Linn, in cold desert of Himachal Pradesh, India. *Annals-of-Forestry* 5:1, 35-38.
48. Singh, V. 1995. Rooting rates of hardwood cuttings of seabuckthorn (*Hippophae rhamnoides ssp.turkestanica* Rousi). *Journal of Tree Sciences* 14 (2): 87-88.
49. Singh, V. 1997. Scientific methods of growing of seabuckthorn. *Parvitya Kheti-Badi* 4-6:17-19. (In Hindi)
50. Singh, V. Studies on the effect of mulches, hormone and soil media on the rooting rates of hardwood cuttings of seabuckthorn. *Indian Forester*. (Submitted)
51. Solovyeva, A.E. 1998. Features of propagation of new seabuckthorn varieties. In: *Proceedings of International Symposium on Seabuckthorn (Hippophae rhamnoides L.)*, p. 187-188, Russia, p. 248.
52. Shuhua, Hou, Xin, Z. & Zhixiang, Z. 1995. Studies on cutting propagation of seabuckthorn. In: *Proceedings of International Workshop on Seabuckthorn*, p. 118-132, Beijing, China, p. 206
53. Tsarkova, T.F. & Polikarpova-F-Ya 1980. Effect of magnetized water on the development of seabuckthorn softwood cuttings. *Lesnoe-Khozyaistvo* 2: 53-54.
54. Trofimov, T.T. 1976. *Hippophae Under Cultivation*. 160 p. (In Russian)
55. Tishchenko-v-ya 1991. Propagation of Hippophae rhamnoides from lignified cuttings. *Lesnoe-Khozyaistvo* 11:37- 38.
56. Tsar'kova, T.F. 1988. The effect of physiologically active substances on the rooting and growth of lignified cuttings of Hippophae rhamnoides. *Agrotehnika Selektivna i Mekhanizatsiya v Yagodovodstve Nechernozem'ya* p. 41-46. (In Russian)
57. Vorobyev, I.S. 1978. Propagation of Hippophae rhamnoides by air layering. *Novye-Pishch.-Rast.-dlya-Sibiri*, p. 196-198. (In Russian)
58. Wahlberg, K. 1992-94. Development of cultivars and growing techniques for seabuckthorn. *Verksamhetsberaettelse Balsgaard* (Sweden), p. 68-74.