Green Grassy Shoots Cuttings of Seabuckthorn for Propagation of Prospective Varieties of Plants for Tannins Production

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ABSTRACT

Seabuckthorn *(Hippophae rhamnoides* L.) varieties (wild forms and cultivars) nowadays is in the center of close attention of different specialists (experts in chemistry, agrobiology and technology) as the source of biologically active compounds for production of various medicaments at their basis. The plants are widely used in traditional and officinal medicines of Russia and all over the world for treatment of wounds, ulcers, different damages of skin and mucous membranes. It is also used in culinary for preparation of various domestic products (cakes, jams, juices etc). This plant is prospective for production of various compounds: vitamins, essential polyunsaturated fatty acids, polyphenols (tannins, flavonoids and catechins), amino-acids, and polyprenoids. Biologically active principals from berry juice, fruit fatty oil, and extracts possess reparation, capillary strengthening and anti-tumor activity. In All-Russian Research Institute of Medicinal and Aromatic Plants there has been worked out a new antiviral drug Hiporamin from seabuckthorn leaves at a basis of the purified fraction of monomeric hydrolyzing gallo- and ellagi-tannins. Green herbaceous shoots cuttings are proposed for propagation of seabuckthorn plants and foundation of plantation for commercial production of cultivars using as the alternative plant source for the drug.

Keywords: Seabuckthorn leaves, green herbaceous shoots cuttings, propagation, tannins content and seasonal accumulation.

INTRODUCTION

Seabuckthorn fruits are used in Russian traditional and officinal medicines for production of fatty oil, which is rich of essential polyunsaturated fatty acids – vitamin F (over 63%) and vitamins A, E, K and steroids, and of various pharmaceutical and cosmetic products at their basis. Juice from fruits is also rich of aqueous soluble vitamins used for the preparation of various drinks, dietetic and culinary products (Bogacheva *et al.,* 2001; Budantsev, 1996; Budantsev and Lesnovskaya, 2001; Lebeda, 2003). Seabuckthorn leaves contain various biologically active compounds: tannins, flavonoids and catechins (Sheichenko *et al.,* 1987; Mamedov, 1984, 1986). Thus polypyrenols from the ether extracts of leaves and from the waste products of the postharvest treatment of seabuckthorn, containing up to 5% berries, possess antihypertensive, antiulcer action and interferon induction activity (Kukina and Raldugin, 1991). Polyphenols of seabuckthorn are active in the therapy of the stomach ulcers and the skin burns (Pomerantseva *et al.,* 1986). Seabuckthorn leaves are the novel source which used in Russia for commercial production of the antiviral phytochemical drug Hiporamin (Bykov *et al.,* 2008. Sheichenko *et al.,* 2014a,b). The drug possesses a wide spectrum of antiviral activity against pathogenic human, animal and plant viruses (Shipulina *et al.,* 2005; Redin, 1999; Tsubera, 1998). Available methods of the plant leaves purchase are limited and dependent on the peculiarities of the plant variety. There are known manual methods of seabuckthorn leaves purchase in the natural regions of their growth or their production from the

plantation of cultivars (Morozov *et al.* 2004; Morozov, 2007). Comparative study of seabuckthorn leaves purchase methods were carried out for their commercial scale production. Today the wild species of the plant source are preferably used commercially in the pharmaceutical industry. There were shown that the manual methods of purchase are costly and labor-consuming. Drawback of the manual methods is also connected with the peculiarities of the plant possessing of thorny branches. Therefore thornless forms are preferable for exploitation. Machine purchase of leaves led to plant damages for majority of cultivars and as a result to their mortality, depending in a considerable extent to the strength of leaves attach to stems in the period of maximal accumulation of active principals. Therefore an efficient method elaboration of seabuckthorn leaves production is of a high importance.

Thus creation of the seabuckthorn plantation in the Moscow region for the plant source production of Hiporamin is of great importance (Morozov *et al.,* 2004; Morozov, 2007). This problem was earlier discussed in our work (Bykov *et al.,* 2006, 2008), while some new approaches (plants reproduction and rational methods of seabuckthorn purchase) are developed in this publication.

PROPAGATION

Historic information on seabuckthorn, systematics, origins, spreading areas, its morphological and biological peculiarities, its basic economical signs, biochemical composition of fruits constituents, and physico-chemical indexes of fruit oil, biological activity of fatty oil and its fractions, application of fatty oil, drugs at its basis and the cosmetic products, food-additives, agricultural significance of the plant and some other problems are discussed in the book of Koshelev and Ageeva (2004) and of Eidelnant (2006). Demidova has presented a fundamental review on seabuckthorn study in Russian Federation (2007): agrobiologic subjects (plant varieties, distribution, botanical, morphologic aspects, genetics, cultivation, ecologic factors, propagation and breeding, biochemical and utilization of the plant products).

Wild seabuckthorn plants are mainly reproduced via vegetative cloning by root shoots or by seeds. But these methods of propagation are slow, possess a number disadvantages and insufficiently suitable for practical use in foundation of plantation of standard cultivars for technologic purpose at a commercial scale. On the other hand much time demand for practical use and commercial scale production of standard plants (disease resistant varieties with the high contents of the active principals). Cultivars are completely satisfied to these requirements, produced as results of breeding process. There are two main methods of their production: seed propagation and the green herbaceous shoots cuttings production for the following bedding into the field. There was preferably used an intensive vegetative propagation method of green herbaceous shoots cuttings to produce clone-type cultivars and the tent controlled mist and fog system (Isaev, 1999, 2000; Isaev *et al.,* 1997; Tsarkova, 2006; Tsarkova *et al.,* 1995, 2001, 2003, 2006; Sheichenko *et al.,* 2000a,b). Optimal conditions of the plant reproduction were found. Seed propagation was shown to be slow method and do not allowed to get standard material. Therefore there was extensively studied the second method of green herbaceous shoot cuttings production as the plant source. This method was also described in recent publication of V. Singh *et al.* (2014).

There was studied the plant reaction on the green herbaceous shoots cuttings of different seabuckthorn cultivars on the yield of the vegetative mass during their preparation for production of the drug. Rejuvenation cuttings of seabuckthorn were earlier widely used for production of green herbaceous shoots in monoclonal propagation of the plant (Stepanov, 1986). The method of green herbaceous shoots production has allowed getting green mass without mechanical damaging plants.

There were shown that the known methods of leaves purchase included branches cutting with the following stems and leaves separation have a number disadvantages. Proposed method of the plant reproduction via the green grassy shoots cuttings with the following root age of the clones have been shown to be preferred as the basic method in this work. There have been studied the plant reaction

on various height of the plant cutting for the vegetative mass production of different quality. There were found correlation between length of the branches, periods of plant vegetation and their age, on the yield of the average mass of the crude drug and on contents of polyphenolic substances. There were proposed recommendations for the safe source purchase for the drug production. Creation of special plantations constructed at the principle of maternal for green herbaceous shoots cuttings production. The plants are used in the phase after purchase of fruits or fruitless samples in this case. The conditions of the seabuckthorn green herbaceous shoots cuttings are presented in the works (Tsarkova *et al.,* 2006; Isaev *et al.,* 1999; Isaev, 2000).

There was shown with the physico-chemical methods that the polyphenol fraction from green herbaceous shoots of seabuckthorn is similar to that of leaves in the tannins composition distinguishing only in the total tannin content and the ratio of the corresponding constituents in the natural composition. The plant source produced (green herbaceous shoots) may be separated to produce leaves and leafless material as the source for commercial production of the drug or to use for chemical enrichment of the polyphenol fraction with the key tannins. Complex arrangements have been carried out to produce standard crude drug and the substance from it (Bykov *et al.,* 2005, 2006, 2008). Some distinction in the tannin composition was found to be dependent on the biosynthetic diversities at different phase of purchase. There were shown dynamics of tannin accumulation in green shots during vegetation periods (Sheichenko *et al.,* 1997, 2000). Thus the biosynthetic chain is the following: strictinin - isostrictinin - casuarinin etc.

SEABUCKTHORN PURCHASE

There was also studied alternative method of seabuckthorn leaves purchase with application defoliants using for food cultures and desiccants with the following mechanical treatment of the plants with the shaking type mechanisms. This method has allowed to produce leaves in 69-93% yield per bush (Sheichenko *et al.,* 2006). Mechanical damage of the plants does not take place in this case**.** Experiments were carried out with cultivars "Dar Catuni" and "Zolotoi pochatok". An analysis of the herbal source produced has got standard tannin contents. Residual amounts of defoliants in leaves and in the drug were shown to not exceed their permissible amounts. Yields of tannins in leaves and their contents in drug produced by this technology were satisfied the standard quality.

Chemical study of green shoots cuts has shown that the latter contain similar chemical composition as that in leaves differing only in their ratio (Sheichenko et al., 1997a). Thus this material was shown to be suitable for production of pure leaves after the separation of the shoot axis from leaves.

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