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THE BIOLOGY OF THE PROPAGATION OF SPECIES *SCHISANDRA CHINENSIS* (TURCZ.) BAILL.

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Abstract: The paper presents aspects regarding the possibilities for the propagation of species *Schisandra chinensis* (Turz.) Baill, as well as its reaction in the pedo-climatic conditions of the Republic of Moldova. Situated in the Lianarium of the Botanical Garden (Institute) AŞM since 1975, *Schisandra chinensis* (Turcz.) Baill. grows, develops and fructifies abundantly every year. It is propagated vegetatively and generatively with some difficulty. In the case of generative propagation, in order to obtain a high germination percentage, the seeds are stratified in three phases, at different temperatures and are sown in spring. Germination percentages of 80-90% were obtained. *Schisandra chinensis* is also propagated by greenwood cuttings, semi-hardwood or hardwood cuttings, by layering or by division. The best results were obtained by using semi-hardwood and hardwood cuttings taken in summer, in June-July, from younger plants. The potential for *in vitro* propagation of this species was also tested. The explants consisting of apical meristems inoculated on MS medium + 0.5 mg/l BAP evolved the best.

Key words: propagation, cutting, climber, medicinal plant, *Schisandraceae*

Introduction

The pedoclimatic conditions of the Republic of Moldova are relatively favourable for non-traditional fruit shrubs which, as they easily adapt to the environment they can be successfully introduced into culture. Also, on the market in the republic of Moldova there is an increasing interest for the introduction into culture of some new plant species from the spontaneous flora. One of these species is the Magnolia vine – *Schisandra chinensis* (Turz.) Baill., a perennial climber from Family *Schisandraceae*.

Utilization. This species is utilized as an ornamental and medicinal plant. As an ornamental plant it is a decorative climber used for decorating balconies, terraces and buildings.

The majority of vegetative and generative organs contain many biologically active substances, but the most important one is schizandrine and its derivatives. In the leaves and fruits there are, in a higher amount, the vitamins C and B (580 mg/%), catequins, organic acids, ketones (18%); tannic substances; sugars (15%). The seeds contain 33% oils. The fruits, leaves, bark and seeds are used. The infusion obtained from leaves, shoots and fruits is used for stimulating the vitality of the body as a whole, for stimulating the activity of the heart, for calming the nervous system. The extract, decoction, tincture prepared from fruits and seeds is used for treating tuberculosis, bronchytic asthma, gastritis, hepatitis, kidneys, dysenteria in children and other diseases that cause the weakening of the organism. The juice, the fresh fruits as well as the tincture from the leaves, fruits and shoots is used as an

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immunomodulator of the body, for increasing intellectual as well as physical working capacity, for eyesight and for strengthening the body. The tea from leaves and shoots has a pleasant flavour similar to that of lemon and it has a slightly sour taste.
The essential oils extracted from this plant are used for decreasing the glucide content of the blood. The use of *Schisandra chinensis* fruits in small amounts is recommended for people that suffer from insomnia, who have psychical problems or high arterial blood pressure and dysfunctions of heart activity.

The fruits are also used in the cosmetic industry and food industry (confectionery, marmelades, chocolates), in the production of soap and detergents.

Spread. The Magnolia vine can be found mostly in mixed forests in the Far East, mostly on the river banks in China, Japan, Sachalin Islands, and in the Republic of Moldova in Soroca, Chişinău. It is cultured by some amateurs and it can be found in several botanical gardens worldwide.

Ecological requirements. It withstands low temperatures and shaded places. It grows fast. It can be cultured on sandy soils. On soils rich in humus it develops better. It does not withstand acidic, dense, compact soils.

Morpho-biological peculiarities. *Schisandra chinensis* is a climber that can reach heights of 14-15 m and 1.5-2 cm in thickness. The roots are branched, situated close to soil surface. The shoots, the roots and the fruits are smelling. The stem of this climber is dark brown in colour and can reach 2 cm in thickness and winds around the trellis in clockwise direction. The leaves are altern, ovate, 5-10 cm in length, the petiole is reddish. The flowers are white, aromatic, they have a pinkish nuance towards the end of flowering. The fruits are of an intense red colour, juicy, sour, spherical, 5-10 mm in length, usually with one seed. The buds are mixed, oval in shape, a little lengthened and pointed. Flowering and fruiting takes place on the annual shoots (Fig.1).

Propagation. The Magnolia vine is propagated vegetatively and generatively with great difficulty. It is propagated by cuttings, layering, division and by seeds [GLUKHOV & al. 2000; KHROMOVA, 1980; TITLYANOV, 1969]. The seeds are sown quickly in the autumn after harvest or in spring after stratification.

There are few scientific papers regarding the micropropagation of Schisandra sinesnsis. Micropropagation has been done by using nodal explants [STANIENĖ & STANYS, 2007], cotiledonary nodes from seeds germinated in vitro [HONG & al. 2004], somatic embryogenesis starting from zygotic embryos [CHEN & al. 2010, SMÍŠKOVÁ & al. 2005] and embryogenesis starting from unopened flower buds [YANG & al. 2011].

Material and methods

The plant material used in the experiments was in the Lianarium of the Botanical Garden (Institute) AŞM, where *Schisandra chinensis* (Turcz.) Baill. grows, develops and fruits abundantly every year, after its introduction into the collections in 1975 and also from the experimental field of the Laboratory of Embriology and Biotechnology.

Experiments were carried out regarding the possibility for the propagation of this species by seeds, by cuttings and by micropropagation.

Observations were done regarding the growth and development of the species in the conditions of the Republic of Moldova as well as experiments regarding the possibilities for the propagation of this species.

For propagation by seeds, before sowing the seeds were stratified in 3 phases: the first phase for 30 days at t = 18-20 °C, then 30 days at t = 3-5 °C and the third phase, for two months at t = 8 - 10 °C.

For vegetative propagation the collecting and making of cuttings was carried out in summer, at the end of June and a the beginning of July when the shoots of the mother plant start to lignify. Lignified as well as semi-hardwood cuttings were made using well sharpened instruments, and the time for harvesting the shoots was in the morning. The cuttings had 12-15 cm in length, with 2-3 nodes.

For propagation by hardwood cuttings the cuttings were made in February and March before the beginning of the vegetation period. The cuttings were kept in sand and sawdust until the danger of frost at ground level passed and then they were planted into cold frames.

The cuttings were subjected to treatment with a weak (pink in colour) solution of $KMnO_4$ and with growth regulators, with IBA at 0.005% for a period of 16 hours or with IAA at 0.01% for a period of 5 hours [HROMOVA, 1980]. They were planted into cold frames into two substrates: sand or sand + peat in a ratio of 1:1 and minimum 24% artificial mist. After one year the cuttings were planted into containers or in the open field.

Having in view that by generative propagation some qualities specific to the mother plant appear or disappear and vegetative propagation presents some difficulty, the initiation of *in vitro* cultures was tested for this species.

The plant material consisted in various explant types: apical meristems, lateral meristems of the 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} degree, fragments of juvenile leaves, shoot fragments from the apical part, shoot fragments with lateral meristem, Fragments of young leaves with veins, ovules, ovary with a fragment from the stem, stem fragment with the apical meristem. For culture initiation nine experimental variants were tested, with MS (1962) basal medium and various concentrations of plant hormones (Tab. 1).

The operations were carried out according to the standard laboratory procedures.

Results and discussions

The research and observations carried out in a period of several years in various ecological conditions show that *Schisandra chinensis* develops differently according to the zone. It was found that *Schisandra* prefers rich, humid, loose soil and zones of shadow. In the sunny places in the Republic of moldova growth is inhibited. It does not withstand drought and high air temperatures in the period of vegetation. It is resistant to frost. It withstands temperatures even as low as -45 ^{o}C. In the drought-stricken years, irrigation and soil loosening is necessary. Fertilization or the addition of chernozem to the roots of the plants is recommended. Growth per decade is about 20 cm.

The root system is superficial and can reach to depths of 25 cm. The young shoots are greenish-grey and, as they mature, they become reddish-brown. The plant is monoecious, the flowers are monosexuate. In the conditions of the Republic of Moldova it flowers in May-June. Since the beginning of bud development until their sprouting there is a period of 11-17 days. The flowering period is of 15-19 days. The fruits fully mature in 14-21 days. The flowers that have strong flavour are small, up to 1.5 cm, grouped 3-5 at the axils of the leaves, on flexible, thin peduncles 1-4 cm in length. The flowers are white, with pink nuances and they develop on the annual shoots. The flower formula is $\male^{*}P_{3+3+3}A\infty$[4],

or ♀♂ *P $^{Co}_{6-9}$ $A_{(3-7)}$ $G_{\underline{30-40}}$ [5]. The Magnolia vine sets fruit starting from the age of 4-5 years.

Schisandra chinensis (Turcz.) Baill. is characterized by rapid shoot growth in june-July and towards the end of August growth diminishes. Its leaves fall down right from the beginning of September. In the meteorological conditions of the Republic of Moldova the species has a period of profound dormancy, which confers resistance to low temperatures during winter and and to late frosts in spring. In late spring the plant enters the vegetation period, which has a duration of 175-190 days depending upon the meteorological conditions of the year (sum of temperatures and amount of precipitations). Another factor that favourizes the development of this species is the presence of phreatic water at shallow depths.

Propagation by seeds. 1000 seeds weigh 25 g. Seed stratification in the three phases at different temperatures ensures a germination percentage of 80-90%. The plants obtained in this way have well developed roots and will set fruit at the age of 4-5 years (Fig. 2). 2-3 year old seedlings need protection during winter. But in *Schisandra chinensis* (Turcz.) Baill. as well as in other fruit shrubs obtained by generative propagation some specific qualities of the mother plant may appear or disappear.

In order to keep the characteristics and qualities of the cultivar, vegetative propagation by cuttings is recommended (Fig. 3). The advantage of propagation by cuttings is that the cuttings are selected from healthy, vigorous and productive plants and plant material with the same characteristics is obtained.

The rooting capacity of the cuttings also depends on the biological features of each species, soil conditions and the special interventions for stimulating the cuttings. Such, in the process of root formation, some hormones like auxin stimulate growth, which can also be achieved artificially by treating the cuttings with stimulating substances, for example heteroauxin. The success of the culture of cuttings depends upon the amount of nutritive substances that they contain and the conditions offereed to them when planting – a well prepared, loosened, fertile, well aerated soil and sufficient humidity.

The roots of the cuttings result from the root rudiments, which are groups of meristematic cells localized in the contact point of the medullary rays with the cambium. The root rudiments are formed long the axis of the shoot, with higher densities at the base of the shoot, close to the axillary buds.

As a result of the cut made by making the cutting from the harvested shoot, a primary parenchimatic tissue named callus is formed, which has the role of cicatrizing the wound and which, later, forms a cambium with adventitious buds from which the roots develop. Until the formation of roots, the cuttings consumes the nutritive substances from its own reserves.

The cuttings are made from the sunny part of the plant, from the lower and midle part of the vine. The leaf lamina is shortened with 1/3-1/2 in order to decrease transpiration. A higher percentage (with 10-12%, as compared to the untreated ones) of rooting was obtained in the cuttings treated for 24 hours with 0.01% heteroauxin and stuck into the substrate to a depth of 2-3 cm. In the first 3-4 weeks the cuttings should be watered 2-3 times a day, and then once a day, then 1-2 times a week. The rooting percentage of the cuttings was of 40-50%. It was found that the rooting percentage of the cuttings taken from young plants was of 57-60 %, whereas from older plants 45-50%.

Propagation by hardwood cuttings (winter cuttings) in cold frames needs a well processed substrate, loosened to the depth of 40 cm, with rich aeration and humidity. The

cuttings were harvested in February and March before the beginning of the vegetation period. The cuttings were kept in sand and sawdust until the danger of frost at ground level passed, then they were planted in cold frames. During the insertion of the cuttings into the substrate, the cutting has to adhere well to the particles of the substrate and for this purpose the substrate has to be well prepared and loosened so as to prevent the bruising and wounding of the cutting.

Burying the cuttings into the substrate is done vertically, with the buds upwards and at 1-2 cm below substrate level, so as to prevent the drying out of the tips of the cuttings. Harvesting the shoots for making the cuttings is done from special cultures of mother plants. One year old shoots are harvested, from which the cuttings are made with a well-sharpened knife. The cuttings should be straight, well formed, they should have at least 2 buds, without mechanical lesions. The length of the cuttings was of minimum 15-20 cm and the thickness 8-20 mm at the upper end. For making the cuttings, the upper part of the shoots, which is not sufficiently lignified, should not be used. The cuts should be smooth, perpendicular on the axis of the shoot, without cracks and bark exfoliations. The cut at the upper end should be at 1-2 cm above the bud.

After being cut, the cuttings should be put into $KMnO_4$ solution and then immediately fixed into sand in vertical or slightly bent position, leaving the upper end at 1 cm above the sand, placed into the greenhouse or cold frame, where humid air should be provided to them, as well as the free access of oxygen to their basal end and an optimal regime of heat and light. These conditions can be ensured by the correct construction of the cold frame, by inserting the cuttings at 0.5-1.0 cm into the sand, by moderate and gradual watering of the sand and by providing a constant temperature of 20-26 °C.

For constructing the cold frame, one should take care that between the surface of the sand and the window of the cold frame there should be 12-15 cm of space. Before inserting the cuttings the sand should be watered abundantly with boiling water and with $KMnO_4$ solution. The cuttings should be put at distances of 6-10 cm between the rows and 4-5 cm in the row. Immediately after inserting the cuttings, fine spraying is applied and the frame is covered completely.

The cold frames should be shaded in such a way that only diffuse light should enter (sparsely knitted mats or staves are used, which should cover 1/3 of the surface of the frame). The cuttings should be sprayed 3-5 times in the sunny days and 1-2 times in the cloudy days and in the evening the weeds and the blackened cuttings should be pulled out.

After rooting, the frames should be opened gradually, so that the plantlets get accustomed to the outer environment and then kept open permanently and watering should be done until October. The cuttings which have grown good roots until October should be taken out and transferred into the nursery or into containers and they should be watered and shaded during warm days. If they do not have well developed roots in October, the cuttings should be kept in the cold frame, covered with sawdust, until spring.

The root system of the cuttings is relatively poorly developed and one should keep in mind that the roots reach to a depth of just 15-20 cm under ground level.

The length of the first-order root system in the plants obtained from semi-hardwood cuttings reach to 2-5 cm in length from summer till next spring.

In the conditions of the Botanical Gardens, propagation by softwood cuttings did not give good results, only 1% of the softwood cuttings rooted. In the case of propagation by root cuttings, the resulting plants are poorly developed.

In vitro propagation. In Tab. 1 the nine variants of nutritive media are presented, all of them with 100% MS as basal medium [MURASHIGE & SKOOG, 1962].

In Tab. 2 are presented the results of testing various explants under the influence of auxins and cytokinins present in the nutritive media. The explants consisting of apical meristems had the best reaction on the variants MS -100% with 0.5 mg/l BAP, resulting 44.40% viable plantlets and MS-100% + 0.5 mg/l BAP, 0.1 mg/l NAA, resulting 36.30% viable plantlets (Fig. 4).

The following explant types also reacted positively: shoot fragment from the apical part, shoot fragment with apical meristem. The reaction of all the explant types on the nine variants of media is presented in Tab. 2.

Conclusions

Schisandra chinensis (Turcz.) Baill. is a species that has adapted to the pedoclimatic conditions in the republic of Moldova.

It can be propagated generatively or vegetatively.

In the case of propagation by seed it is recommended to stratify the seeds in three phases: I - for 30 days at t = 18-20 °C, II- 30 days at t = 3-5 °C and III- 60 days at t = 8-10°C and then the seeds should be sown, such ensuring 80-90% germination.

As a result of the process of propagation by hardwood and semi-hardwood cuttings, uniform genetic material is obtained, healthy, vigorous plantlets that posess the features and qualities of the mother plant. The rooting of the cuttings depends on several factors: the quality of the cuttings and of the substrate, The conditions for the growth and development of the mother plants, respecting the optimal timeframes for propagation by cuttings and correctly applying the technology of propagation by cuttings, the density of the cuttings in the cold frame etc. For obtaining a higher rooting percentage of *Schisandra chinensis* (Turcz.) Baill. It is necessary to select the shoots from young plants, which were also obtained by vegetative propagation, in the 20th of June-10th of July, which coincides with the end of flowering and the beginning of fruit set.

The optimal substrate for the vegetative propagation of *Schisandra chinensis* (Turcz.) Baill. is made up of sand + peat 1:1.

Among the rhyzogenesis stimulators used for rooting the semi-hardwood *Schizandara chinensis* (Turcz.) Baill. cuttings, it was established that the stimulators IBA and IAA-0,01% plus sucrose at 10 g/l concentration stimulate rooting.

The plants obtained from cuttings as well as the ones obtained from seeds are very sensitive to low temperatures in the spring in the first years, hence the plants must be protected by leaves.

Among the 10 types of explants tested for *in vitro* culture initiation in *Schisandra chinensis* (Turcz.) Baill, the apical meristems reacted the best, especially on the variant with MS medium + 0.5mg/l BAP (44.44% viable plantlets obtained). The explants consisting of shoot fragments from the apical part and shoot fragments with apical meristem also had positive reaction.

Schisandra chinensis (Turcz.) Baill. can be recommended in the range of species used for setting up green areas, as it is a robust climber, with decorative value during the whole year and also used as a medicinal plant with multiple active and stimulating substances, with a wide range of applicability.

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a b

Fig. 1. *Schisandra chinensis* (Turcz.) Baill. a) The flowering phase; b) The fruiting phase

Fig. 2. Propagation by seeds

Fig. 3. Propagation by cuttings

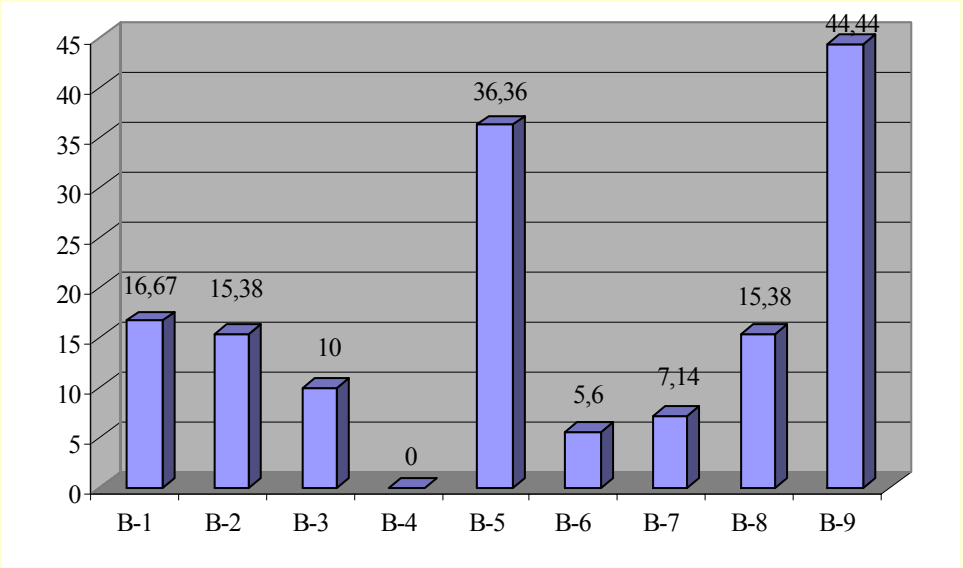

Fig. 4. The reaction of the apical meristems on the nine variants of media (%)

Tab. 1. The composition of the nutritive media for the microclonal propagation of species *Schisandra chinensis* (Turcz.) Baill.

Variants	Basal medium	Supplementary additives
B-1	MS-100%	Kin - 0.5 mg/l NAA- 0.1mg
B-2	MS-100%	Kin -1.0 mg/l IAA – 0.5 mg/l
B-3	MS-100%	Kin – 0.5 mg/l IAA – 0.1 mg/l
B-4	MS-100%	Kin – 2.0 mg/l IAA– 0.5 mg/l
B-5	MS-100%	BAP – 0.5 mg/l NAA -0.1 mg/l
B-6	MS-100%	BAP – 0.5 mg/l GA^3 – 0.5 mg/l
B-7	MS-100%	BAP – 0.5 mg/l IAA – 0.1 mg/l
B-8	MS-100%	BAP – 0.5 mg/l IAA – 0.5 mg/l
B-9	MS-100%	BAP – 0.5 mg/l

Tab. 2. The initiation and *in vitro* reaction of various types of explants in *Schisandra chinensis*

Explant Medium	Apical meristem	Lateral meristems of the 2nd, 3rd, and 4th degree	Lateral meristems of the 5th and 6th degree	Fragments of juvenile leaves	Shoot fragments from the apical part	Shoot fragments with lateral meristem	Fragments of young leaves with veins	Ovules	Ovary with a fragment from the stem	Stem fragment with the apical meristem
1	2	3	4	5	6	7	8	9	10	11
B-1	+			~				~	~	
B-2	+	~	~	~	+	+	+	~		
B-3	+	~	~	~	-	+		~		
B-4	-	-	-	+	+					
B-5	+	~	~		+	+		~		+
B-6	+									
B-7	+	~			+	+	~	~		
B-8	+		~	~	+	+	~	+	+	+
B-9	+	~	~	~	~	~	~	~		

\+ progress; - necroses; ~ without changes