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SEED PROPAGATION OF *TILIA* SP. – AGROBIOLOGICAL AND TECHNOLOGICAL ASPECTS

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Abstract

Tilia is a widely applied tree for urban and suburban landscaping. The traditional propagation of these species is possible to be carried out through wood and semi wood cuttings as well as shots. One of the poorly studyied methods with high perspective is seed propagation. This method for propagation is related to high difficulties due to a strong dormancy in linden seeds. The main aim of this review is to identify and analyze the main and modern scientific researches concerning the seed propagation of different species of the genus *Tilia*. In this article was included the main aspects of biological behaviours and technological ways about propagation with seeds of a linden tree.

The main problems of seed maturity, the moment of their harvest, the seed development characteristics and the reasons for the difficulties in this way of decomposition are examined. At the same time, questions about technological practices and growing conditions are also included.

Keywords: dormancy, germination, sprouting, harvest, linden.

INTRODUCTION

The Tilia genus includes about 40 species originating in temperate regions of Europe, North America and Asia. Linden is medium to large deciduous trees that are not demanding for the soil. They best develop on soils with moderate humidity and low acidity.

According to Chalupa (1999), the main species are *Tilia cordata* Mill. (small-leaved lime), *Tilia platyphyllos* Scop, and *Tilia americana* L. (American linden, Basswood).

The separate lindens are characterized by different biological requirements both to environmental conditions and to the possibilities for their propagation (Kowalski et al., 2012; Shaykhmatov and Seydafarov, 2013; Seydarfarov, 2013). Difficulties in the propagation of the linden by the seeds way are mostly related to the presence of a very well-defined dormancy. This strongly prevents the normal germination, sprouts and growth of young plants.

It is also of particular importance that the maturity of seeds associated with the time of their harvesting (Berles, 2012 and Frochot et al., 2009)

The main aim of this review is to identify and analyze the main and modern scientific researches about the propagation of different species of the *Tilia* genus.

MATERIAL AND METHODS

The realization of this review article was carried out by studying literary sources from recent years. Scientific publications in specialized issue in the field of ornamental horticulture, dendrology and floriculture have been studied in the following scientific publications:

Dendrobiology,
New Forests,
Dendrobiology,
Horticultural Reviews,
Advances in Horticultural Science, etc.

The articles published in the World Infomation Network (Internet) are included, using databases in the following information sources:

Google scholar
Web of Science,
Scopus,
Ebsco,
Elsiver etc.

The own research papers and researches in the area of linden multiplication also have been used.

The information obtained is arranged according to the main moments of the linden seed propagation, it is analyzed and the opinions of the individual authors are compared.

On this basis, the main problems and contradictory conclusions are outlined in this way of propagation.

Existing and undecided issues are highlighted and the need for further targeted studies is emphasized.

Analysis of the scientific researches and publications

1. Existence of dormancy, the ways to overcome it and term of harvesting of lime seeds

The seeds of *Tilia* are characterized by the presence of deep dormancy that prevents their germination and sprouts even when they are in a suitable environment – optimal temperature, humidity and aeration. To carry out the germination, the seeds and embryo must undergo physiological and morphological changes – scarification and stratification. These changes are only made in a certain combination of temperature, humidity and aeration, and in some cases light or darkness (Stokes, 1995). Most common in lime seeds is both mechanical and deep physiological dormancy (Berles, 2012). According to Frochot et al. (2009), the difference in the occurrence of physiological dormancy in the seeds of large-leaved lime and small-leaved lime is not observed.

Yang et al. (2011) reported that lime seeds are characterized by very high dormancy. This makes it difficult for them to propagate by seedlings. It is relatively difficult to overcome this dormancy, and it is recommended that the *Tilia amurensis* in China conditions propagate through woody cuttings to be applied.

Prolonged and slow germination of lime seeds associated with deep rest for 18 months established Ganchev (1972) and Milev (2004). There is a strong variation in the seed germination of the three widespread lime species ranging from 50-100% and most often up to 80%. (Regulation No. 13, 2008, Regulation No. 21, 2012 and Regulation No. 73, 2012).

Vanstone (1982) examined the humidity of lime seeds during harvesting. He points out that it is most appropriate for lime seeds to be harvested in the early autumn with humidity and recommended by 16%. During maturing, the seeds lose up to 2% of their humidity daily, which requires very strict seed monitoring before harvesting.

The colouring of the fruit pericarp can serve as a good indicator of humidity and its relation to the germination of lime seeds (Bradley et al, 2005) at this point the pericarp changes the colouration from green to grey-brown and at the same time becomes hard and tough. In this case, the seeds require considerable effort to carry out scarification.

In the experiments conducted at the Horticulture Department of Agricultural University of Plovdiv, Bulgaria, I found that the best seed harvest time is 75 or 90 days after flowering. Previously harvested seeds at 30, 45 and 60 days did not germinate at all (Panchev, 2018).

The significance of maturity of lime seeds is confirmed by the research conducted by Vanstone (1982). It records that ripening can vary within a few weeks, but only individual trees can mature more simultaneity and uniformity. If the seed is more difficult to pick up, the harvesting must be postponed until full maturation. The easier harvesting of the Indian seeds, Brinkman (1974) attains in their collection after the period of stronger frosts and when the branches are shaken, their release is relatively faster and in greater quantity.

Overcoming the seeds dormancy, according to Chawla (2004), can be successfully achieved by applying embryo cultures. The author pointed out that the causes of the dormancy are from a different character, such as the presence of endogenous inhibitors, specific requirements to light, poor maturation of the seeds and the embryo. In some species of linden, such as American, very often the seed dormancy is associated with non-pericardial failure, i.e. of the fruit coat. Thus, so-called mechanical resistance is present, and this determines the significant difficulties in seed germination. But by removing the embryo, normal sprouting has been achieved. It is recommended that this technique can be used for propagation by seeds of typical vegetative propagated species.

Rose (1991) reported that, in the above-mentioned species, *Tilia americana*, the dry seed sown in the soil prior to the winter, has relatively low germination. A major limiting factor for this is the limited and difficult absorption of water from the seeds. In order for germination to take place, it is recommended to place them in a humid substrate under laboratory conditions. It has been established that the fruit and seed coat of the linden is not a cause of dormancy, but it significantly contributes to prolonging its duration as it occurs both in the embryo and in the endosperm, and often in both structures. In order to overcome, it is necessary to remove the seed (fruit) coat after ripening and to place the seeds at low temperatures and to sow just before the freezing of the soil. The author finds that additional ripening occurs at 0-2°C, but there is still no germination. These seeds germinate best at only 10-12°C, but significantly stronger than in laboratory conditions. The temperature of 4–6°C is very suitable for additional maturity and sprouting, but this process is best at 12°C. In this additional ripening, the concentration of water ions increases, the bound water and the activity of the oxidase enzymes, such as catalase are also higher. The lime seeds contain significant amounts of fat and phytosterols, and the oxidase activity in these seeds is low and the peroxidase is higher in germinated seeds. When germinated, the amount of free acids in the seeds is higher and the additional spraying with water leads to an increase in these acids and in still untreated seeds, but they have passed over. Thus, lipase activity increases. However, in order to ensure high germination, this author finds it appropriate to collect the lime seed at a humidity lower than that stated by Vanstone (1982), namely about 10% or less, and not to be stored under warm and dry conditions for a prolonged period.

Lime seeds are characterized by specific anatomy and morphological structure, which determine strong rest and significantly impede their germination. The Werker (2013) studies indicated a strong non-permeability of the seed coat to water and oxygen and significant mechanical hardness that prevents embryo root protraction. The inability to absorb water is associated with watertightness, as the individual layers of the seed coat as well as the hylum and the chalaza. These layers usually consist of tightly spaced cells consisting of various hydrophobic compounds, most commonly containing cellulose. The presence of waterproofing prevents the entry of oxygen. This may be due to longer diffusion and swallowing of various compounds in the seed coat as well as to the specific permeability of the cells and thus to the fact that oxygen can not reach the embryo. The main difficulty for the exit of the embry root in lime seeds, according to the author, is due to mechanical barriers.

The hard pericarp of this linden, small-leaved lime, impermeability of the seed coat and embryo status determine the significant dormancy that requires scarification or stratification. In the present investigations, scarification lasting from 10 to 50 minutes at 23°C to 27°C has proven to be the most appropriate. Subjecting them to lower temperatures requires a longer period than with their acid treatment. It has been observed that soaking in water for 1-2 days and subsequent treatment with acid for 20 to 50 minutes also gives good results. Mechanical scarification, treatment with sodium hypochlorite, ethanol and low temperatures from -80°C to -185°C were also suitable. It is very useful to have the stratification carried out at a temperature of 1 to 3°C for a period of 3 months in a mixture of peat and sand in a ratio of 1: 1 and humidity of 30%.

Processing at a temperature of 4°C increases evenly the enzymatic activity, the low protein content and the amino acids in the lime seeds. Without this treatment, untreated seeds may not germinate for more than 5 years. However, the large-leaved lime requires stratification initially at high temperature, followed by seed placement for 3-5 months at low temperatures, but this is not a guarantee of their normal sprouting. It is also appropriate to apply seeds in a wooden container in wet sand under external conditions and sowing ate the following autumn. Strong differences in the pericarp and seed coat are observed between the seeds of different batches. In Europe, scarification with acid and thermal and subsequent stratification is more often used. In order to achieve complete imbition (water absorption) of seeds, it is necessary to storage them for 4 months at a temperature of 20-25°C. The same result can be achieved by scraping with concentrated sulfuric acid for 12 minutes. They are then subjected to stratification for 14-18 weeks at 3°C. Stratification finish when the first seeds germinate. The duration of this process is about 8-9 months (Bradley, 2005). Ritchie et al. (2010) have achieved an increase of germination to 74-80% in mature or stored silver-linden seeds by subjecting them to a 5-6 month stratification.

Long-term storage of fruit, respectively, of the seeds of large-leaved lime, with humidity of 10%, is achieved in closed containers at a temperature of 3°C for a period of 16 years. Similar to Bradley (2005), Tylkowski (2006) recommends a combined seed treatment of lime for accelerating sprouting. In order to overcome the dormancy, it is necessary to carry out chemical treatment i. e. scarification, the seeds separated from the fruit coat with concentrated sulfuric acid for 10 minutes. Stratification is then carried out without pre-cooling, at 3°C for 20-24 weeks or until the first seeds germinate. From a subsequent test of these seeds at temperatures in the range of 3 to 15°C with a duration of action of 16 hours per day, the germination rates have reached to approximately 90%, but for a period of several weeks, have been demonstrated. Studies conducted in Poznań, Poland, manifested that is better to be sown at the beginning of spring in containers under a plastic tunnel to ensure better development of these stratified and scarified seeds. Sowing outdoors, even in the early spring, in these more northerly conditions, does not produce good results. It is pointed out that this method is suitable for sprouting hard *Tilia* seed.

2. Agro-climatic condition for linden seed development.

One of the main conditions for the normal development of lime seeds is the presence of a suitable temperature. In studies conducted by Pigott (1981) in Finnish conditions with small-leaved linden seeds, collected in September, it is found that many of them have poorly developed embryos and have not yet reached normal maturity. Despite the availability of suitable temperatures for pollination and fertilization during the previous months of seed development, the sharp decline of the temperature during the months of September - October does not allow the complete development of the embryo and endosperm.

The moisture content of small-leaved linden (*Tilia cordata* Mill.) seeds is of particular importance with regard to germination. In the experiments with further drying with liquid nitrogen (-196°C), Chmielarz (2002) monitors the drying time over 24 hours of seed at 11 different humidity levels of 3.1% to 22.8%. All the variants studied were subjected to scarification with concentrated sulfuric acid both before and after freezing with liquid nitrogen. The pre-treated seeds, prior to germination at 3-15°C for 6-8 hours, were subjected to cold stratification at 3°C without placing them in a substrate. The low seed humidity of 3.1% significantly reduced the germination, compared to those with a humidity of 5.2-20.9%. Thereby, the seeds of small-leaved lime in this condition can be considered as a standard seed. High germination of 79-87% is retained after freezing in liquid nitrogen and seed dried to 9.0-17.4% if the scarification is carried out after freezing. The highest percentage of developed seeds - 65-75% is obtained after the seed has been led up to a moisture content of 11.1-20.1% as a result of its drying by freezing in liquid nitrogen and previously scarified, as well as seeds dried up to 7.3-17.8%, where this percentage is within 53–71%, but scarified after freezing. Particularly important for the flowering and the development of lime seeds are light, moisture and low temperatures. In this sense, Piggo (1975) reports that if the temperatures fall below 12°C the pollen tube growth is inhibited. Most seeds fall out of the trees in autumn, but some remain on the tree and fall off in the snow. It has been noticed that the seeds that have remained on the tree branches have different physiological properties than those that have fallen in the autumn.

3. Technological aspects in linden seed propagation.

Pre-treatment of lime seeds has a positive effect on their germination and rooting of the seedlings. Magherini and Nin (1994) to the improvement of germination applied: removal of the pericarp, cooling of moistened seeds, surface sterilization, acid scarification and treatment with gibberellins acid and thiram fungicide. In this study, it is also found that the difficult seed germination of lime is due to the extremely hard pericarp of the fruit and by its removal and the application of fungicides, and surface sterilization causes much more rapid acceleration of germination processes. Scarification with concentrated sulphuric acid (52% and 56%, respectively) of seeds of *T. platyphyllos* and *T. tomentosa* improves germination while soaking in GA_3 solution does not affect this process. By the tested three linden species, the germination rate at *T. cordata* was the lowest - a maximum of 17.4%.

Dirr and Henser (1987) also pointed out that a very good result was obtained by scarification of lime seeds with concentrated sulfuric acid for 12 minutes and subsequent stratification at 3°C for 3.5-4.5 weeks. The process of stratification is terminated when the first seeds are opened his coat.

Rowe and Blazich (2008) found that small-leaved lime seeds require 10-month scarification, with the first five months at a higher temperature and the next five months at 1–5°C. Bonlet-Gerconrt (1997) recommends a different pre-treatment regime involving initial soaking of the seeds in clean water and storage in a 20-25°C room for a period of 4 months.

Appropriate treatment of broadleaf lime seed is offered by Gosling (2007). In the beginning, the seeds are stratified for 12 months, alternating half the period (6 months) under cold conditions and the other part by warming. In contrast, Prada et al. (2008) establish satisfactory germination of seed at 3-5 months cold stratification regime.

The production of lime planting material for the Bulgarian conditions is suitable to be carried out in nurseries, in the lowlands or in the sub-Balkan regions with the presence of deep and fertile soils (Alexandrov, 2000). Significance for the good development of seedlings of different types of linden is the density. Milev et al. (2001) recommend that in the first year of a linear meter there should be 20-25 seedlings and in the second year to reduce their number to 17-18. The depth of sowing of lime seeds has been the subject of a survey by Milev et al. (2001a), and they concluded that the most appropriate, for good development, was that of 1.5-3.0 cm. In their opinion, the sowing rate should be 1.2 g or 30 numbers of linear meters. Similar recommendations are made in Regulation 75 (2007) where the appropriate sowing rate for *Tilia tomentosa* is set at 2.7 g or 30 seeds per linear meter, and the seeding depth is 3-4 cm. A similar assertion, for the depth of sowing of 3-4 cm, is also reported by Hartman et al. (2002).

It is appropriate to sow the lime seed immediately after harvesting or in October to early November, collected in physiological maturity and stored until sowing in wet sand in a 20 cm thick layer. Physiological maturity occurs when the colour of the fruit (walnut) changes from green to yellowish, and the pericarp is hard and tough. At the break, inside the seed coat (BGS 3126, 1982).

Alexandrov (2000) finds that this colour change begins with darkening in a single spot or dot.

The development of seedlings is directly related to the nutrient regime under which new plants are grown. From the application of mineral fertilizers, according to Yordanova et al. (1985) depends on the accumulation of total vegetative mass, the development of the length and the branches of the root system. Pretzsch (1995) also found the effect of mineral fertilization on the number and size of the formed leaves. Applying appropriate water and the nutrient regime is a very good prerequisite for the production of standard saplings in all three types of lime - silver, small-leaved and large-leaved lindens (Schmit, 1995). Milev and Iliev (2010) also reported that there is an opportunity to obtain lime seedlings fitted for planting within one year, but this is more likely to happen when they grow for two years when their development is better.

In order to better sprouting of the seeds and developing of seedlings, Zahariev (1977) pointed out that it is appropriate to cover with sands, tree bran or straw after sowing. It is also necessary to keep the lime seedlings wet until the cold starts. With the onset of spring, periodic observation is made of the bursting of the fruit (walnut) and in the presence of the first sprouts, the mulch partially removed, and subsequently completely.

One of the main activities in growing seedlings of decorative species is their passage through separate schools. For the training of linden seedlings, Wilson et al. (2006) recommend the use of biennial plants from silver linden, large-leaved lime and small-leaved lime with a height of 25 cm and a root collar thickness of 6-8 mm. Large-dimensional lime saplings can be produced by applying prick out of seeding at a distance of 80 cm between the rows and 30-40 cm inside the row (Stnwe and Sons, 2014).

The young lime seedlings are strongly sensitive to early spring and late autumn frosts (Milev, 2007). Apart from the nutrient regime and irrigation according to Suszka et al. 1996), one of the main agricultural practices in the cultivation of lime seedlings is mulching, which protects against the harmful effects of late spring and early autumn frosts. The authors also recommend shading, regular weeding and loosening in the first eight weeks.

4. Behaviours of morphological development of seedlings of main linden species.

The morphological responses between the separate species of limes occur at the beginning of seedlings development, especially with regard to their seed, and the information is not unidirectional. The lime sprouts are characterized by feather-chopped seeds of 5–7 parts. If an asymmetric partition is present, they can also be categorized as 6-sided. Vassilchenko (1990) believes that the first true leaves are usually opposite, slightly longer than the first typical leaves. They develop consistently, non-simultaneously, that leading to the incorrect conclusion that the first true leaves are consistently located. Due to cross-pollination, hybrid forms are often found in which there are deviations from the mentioned traits (Iliev and Alexandrov, 2004).

In *Tilia cordata*, according to Иветић (2013), the cotyledons are seven-dimensional, with the individual parts are with a more rounded shape and short peaks, the base is a straight line and the bottom is weakly hairy.

In the large-leaved linden, Maier (1994) and Grossinckle (2005) specify that the cotyledons have elongated ellipsoidal shape, most often five-pointed, with relatively narrow and sharply to the top parts, and the periphery slightly indented. The base is wedge-shaped, acuminate or rounded. In this linden, the primary leaves are also hairy by the underside, and the nervation is directed to the parts, but according to the first researcher the hairy exists on both sides. A different opinion is expressed by Glovanneti et al. (2010), he observed the common morphological features in the cotyledons among the large-leaved and small-leaf lime.

line base, with broad and rounded tops. In difference from the previous two species, they are nude and shiny on both sides (Guidin et al., 1982). Despite the identity between the morphology of the first true leaves between the three linden species, more significant differences between them, according to Hartman (1990), are expressed in the hairy of silver linden.

In the in small-leaved linden for the production of 2600-2800 annual seedlings, approximately 1 kg of seeds are needed (Regulation №80, 2006). In the case of broadleaf *Tilia platyphyllos*, according to Landis et al. (2010), however, this quantity of seeds of 1 kg is sufficient to produce significantly more annual seedlings - 3000-3200 units, whereas for silver linden the number of seedlings is the smallest, only 2250 (Popkov, 2009).

CONCLUSIONS

1. The review of the existing information in the literature on the propagation and mass production of planting material of the different

species of limes used in decorative gardening in our country indicates that it is scanty and in most cases contradictory. A number of issues concerning the maturation of seeds and dormancy in the major and widely applied limes in our country such as *Tilia platyphyllos* Scop., *Tilia cordata* Mill., *Tilia tomentosa* Moench is not clear.

2. The analyzed literature suggests that seed propagation through the seeds is a difficult and complex process that requires specific knowledge about seed dormancy, the ways of it overcoming and pre-seed treatment.

3. Considering that linden is one of the most preferred and widely used deciduous species in European and world decorative horticulture, the clarification of these issues is of particular topicality.

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