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Vegetative Propagation of *Tilia* sp. Using Semi - Hardwood Cuttings

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Abstract

This study was conducted to investigate the effects of IBA, IAA and NAA on rooting power of semi - hardwood cuttings from *Tilia*. The cuttings were treated with 30, 40, 50 and 60 mg/l IBA, 100, 150, 200 and 250 mg/l IAA, and 20, 30, 40 and 50 mg/l NAA. The results were reported after three months. The following indicators were analyzed: percentage of rooted cuttings (%), number of roots of cutting and the average length of root (cm). The largest number of rooted cuttings was obtained using 40 mg/l IBA for most of the variants. The treatment with 200 mg/l IAA resulted in the roots with the greatest length. The average length of the roots is increased by treatment with 30 mg/l and 40 mg/l NAA. High doses of IBA, IAA, NAA will provide a weak, brittle root system, which reduces the chances of successful adaptation in replanting.

Key words: *Tilia*, vegetative propagation, rooting, indole-3-butyric acid, indolyl-acetic acid, naphthyl acetic acid

Introduction

The genus *Tilia* is the only representative of the family of *Tiliaceae*. *Tilia* is a common tree in the temperate latitudes of the northern hemisphere. The genus consists of approximately 40 large or medium-size deciduous species. Most of the American and European species have 82 chromosomes. The East Asiatic species have 164 chromosomes. Linden flowers contain a number of natural compounds, or phytochemicals, that are biologically active and responsible for some of its properties. Several of its components are antioxidant chemicals called flavonoids, including one called quercetin. Antioxidants help the body get rid of free radicals. In herbal medicine, linden tea is recommended to combat anxiety and promote relaxation. The ability of some linden compounds to cause sweating may have benefits in fighting against cold, cough or fever. Some components of linden flower tea might also help soothe irritated membranes in the mouth or throat that develop during an illness. Young shoots are characterized by a zigzag growth. Winter buds are prominent. The leaves alternate in two opposite rows on the branches, and are toothed and heart-shaped at the base. Flowers are small, bracteae are large. Fruits are nut-like. *Tilia* has a great recovery potential, as a result of which, trees may reach a very old age. *Tilia* prefers the sun and a rich, humid, loamy soil. The trees have a well-developed root system and are not sensitive to wind.

Originally, *Tilia* is a forest tree. Since its wood is soft and rots easily, *Tilia* is not suitable for wood production, only for musical instruments and woodcutting. *Tilia* species have an important ornamental value. They are commonly used in streets, avenues and parks. Because of its shape and its resistance to air pollution, severe pruning, and traffic, *Tilia* is a suitable tree in and around cities. Since little flowers produce large amounts of nectar, many species are important host plants for bees.

Most of the trees in culture are seedlings. A limited part is grafted, budded or propagated by layering. In The Netherlands, the total production of Tilia in 1988 was estimated to be 400 000 trees. Approximately 100.000 were specific cultivars which had been propagated vegetatively. The most important species are the common linden (*Tilia vulgaris*), the small – leaved European linden (*Tilia cordata*), the Crimean linden (*Tilia euchlora*), the large – leaved European linden (*Tilia platyphyllos*), and European with linden (*Tilia tomentosa*). The most important disease is early leaf dropping during summer. This disease is probably physiological and occurs mainly in street plantations near buildings where leaf temperatures rise by heat radiation. Leaf and stemspots disease like *Cercospoza microsora* may cause severe damage, especially in nurseries. *Tilia* is also affected by verticilium wilt.

Aphids and spider mites (*Eutetranychus tilarium*) sometimes cause damage. Aphid infections result in sooty mold infections on honeydew which drops from trees. Spider mites attack the trees in dry period during summer. Sawfly caterpillars (*Pristiphora geniculata*) sometimes skeletonize a greater part of the leaves (Dirr, 1983; Kunneman, 1991).

Tillia is propagated by seed or vegetatively by layering, (chip) – budding or grafting. Production by seeds is relatively easy, but germination is irregular. Furthermore, unknown seed sources and hybridization between species give rise to variability among seedlings. In the past, the most important method of vegetative propagation was by layering; now *Tilia* is propagated by budding or grafting. In this way, new clones may be introduced in a shorter period of time, labor is reduced and variety in choice of cultivars is easier.

Material and Methods

Studies have been carried out during the period 2013-2015 in the experimental farm of the University Botanical Gardens - base Ecopark - Varna. Planting was made in polyethylene bed-tunnel loaded with double-layer substrate - 4 cm layer of pure perlite over 20 cm layer of standard mix. The substrate has a pH of 5.6 to 7.5. The tunnel 40 day after the bet of the cuttings was maintained by spraying mode Irrigation and air humidity in the 70-90% and a temperature of 25 ± 5 °C. The basic principle was the cuttings during the day from 7.30 to 21 hours to keep constantly wet. The following were carried periodically: nourishing environment with basic nitrogen, phosphorus and potassium fertilizers and disinfection with potassium permanganate solution with the addition of the "Fix B" in definite concentration.

Cuttings were taken from annual shoots taken from trees of different ages from big - leaf, small leaf and silver leaf lime. From big-leaf trees are used around 30-32 years of age, small-leaved - trees 25-28 years old and silver - trees 40-42 years old. Cuttings were taken a week between July 7 to 15 in the two years of study. The length of cuttings was 10-12 cm in the lower segment made of 2-4 mm below the lower bud and top - of 10-15 mm above the top bud. The top two leaves are preserved. Cuttings are selected from the middle part of the shoot with a thickness of 4.0 to 6.5 mm in diameter. After washing with running water, the cuttings were surface-stabilized with a 10% aqueous solution of Ca hypochlorite for 5 min and then washed in running water for 5 min three times. In each case they were set at 50. The lower segment of the cuttings was immersed for 1 min in 50% ethanol solution of 3-indolyl butyric acid at a concentration of 1, 2, 4, 6 and 8 g / l. Cuttings were placed for rooting in the substrate at a depth of 2 cm and watered.

The first recording was made three months after betting. At the end of the experiment the following indicators were reported: percentage of rooted cuttings (%); numbers for cutting roots (roots longer than 5 mm; pcs./plant and average root length (cm).

Statistical data processing was done with ANOVA test. The significant difference between the control and the variants was presented as: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$) and the non – significant as ^{ns}.

Results and Discussion

It is evident that the rooting of cuttings is a dynamic event and the root formation from cuttings for each *Tilia* species exhibited different outcomes (Table 1). The rooting rate and root quality of cuttings was changeable according to species. *Tilia* species gave different rooting percentage in different IBA, IAA and NAA doses and interaction between the species and IBA, IAA and NAA doses were found statistically important. As far as rooting percentage is concerned it was maximum with 40 mg/l IBA, 150 mg/l IAA and 30-40 mg/l NAA. Increasing doses of IBA, IAA and NAA reduced the rooting capacity of cuttings and 60 mg/l IBA had negative effects on rooting of species of Tilia. The rods showed breakable structure in high doses of IAA – 25- mg/l and in 50 mg/l NAA the roots were too small.

Tab. 1. Rooting of cuttings in *Tilia sp.*
Укорјењивање резница Tilia sp.

Variants/Species *Варијанте/Врсте*	*Tilia cordata*	*Tilia platyphyllos*	*Tilia tomentosa*
Control	53.75	64.58	86.67
30 mg/l IBA	58.43*	69.43*	90.45*
40 mg/l IBA	68.43**	79.81***	93.14**
50 mg/l IBA	69.01***	77.54**	94.05***
60 mg/l IBA	56.81*	64.11^{ns}	73.18^{ns}
100 mg/l IAA	60.14*	68.48*	88.15*
150 mg/l IAA	63.18***	71.83**	89.86*
200 mg/l IAA	59.34**	72.43**	89.01*
250 mg/l IAA	57.18*	70.43*	75.43^{ns}
20 mg/l NAA	54.81^{ns}	65.45*	88.83*
30 mg/l NAA	58.43*	67.13**	92.13**
40 mg/l NAA	60.04*	68.07**	94.18***
50 mg/l NAA	59.34**	64.93^{ns}	87.15^{ns}

*($P \leq 0.05$), ** ($P \leq 0.01$), ***($P \leq 0.001$) and not – significant ns

The best rooting in the experiment was observed with *Tilia cordata* and the best rooting percentage occurred in 40 mg/l IBA generally.

Significant interaction occurred between *Tilia* species and IBA, IAA and NAA doses for root number produced per cutting. IBA, IAA and NAA doses increased the root number according to control except 250 mg/l IAA and 50 mg/l NAA. *Tilia platyphyllos* and *Tilia tomentosa* produced higher numbers of roots in 50 mg/l IBA and 150 mg/l IAA and *Tilia cordata* – in 50 mg/l IBA (Table 2). Results regarding number of roots showed that *Tilia tomentosa* produced maximum average numbers of root per cuttings, *Tilia platyphyllos* and *Tilia cordata* as second and third respectively. *Tilia cordata* produced the minimum number of roots – 7.45 per cutting in 50 mg/l NAA dose. All IBA, IAA and NAA doses increased the rooting rates compared with control, but just 40 mg/l IBA, 150 mg/l IAA and 30 mg/l NAA increased the root length statistically (Table 3).

Significant importance occurred between *Tilia* species in terms of length of roots produced per cutting and the longest main root was recorded in *Tilia platyphyllos* cuttings – 9.41 cm and the shortest in *Tilia cordata* – 2.51 cm. As far as doses were concerned, length of roots was increased in 40 mg/l IBA; 150 mg/l IAA and 30 mg/l NAA and increasing doses reduced the root length.

Tilia is a common tree in gardening practice in Bulgaria and in Southeast Europe. An efficient propagation method for commercial propagation and production of plant material needs to be determined. It is generally accepted that auxins play an important role in the process of root formation (Davis et al. 1989; Klerk et al. 1999). For successful root induction plants need to contain a certain quantity of IBA, IAA or NAA. It is common to use IBA for rooting of tree type plants because it has a greater ability to promote adventitious root formation in comparison to IAA. (Spethman and Hamzah, 1988; Riov, 1993; de Klerk et al., 1999; Ludwing – Müller, 2000). It is more stable and less sensitive to the auxin degrading enzymes (Nordstrom et al., 1991; Riov, 1993).

The results confirmed the convenience of cuttings for economical propagation and the importance of exogenous IBA application too. All IBA, IAA and NAA doses improved the off-cuttings. Research with species of Tilia also showed that the addition of IBA, IAA and NAA was deemed effective for promoting rooting of cutting (Deghan et al., 1990; Tchoundjeu et al., 2002; Tofanelli et al., 2002). The most important factor in successful propagation was the use of the rigat doses of plant growth regulator which increased the root development. The results reported herein indicate that the IBA, IAA and NAA treatment improved the rooting percentage, root number and main root length.

The most adequate doses to propagate *Tilia* cuttings 40-50 mg/l IBA or 150 mg/l IAA or 30 mg/l NAA. IBA at 60 mg/l or IAA at 200 and 250 mg/l or NAA at 40 and 50 mg/l showed lower rooting percentage and root quality.

Tab. 2. Number of roots of *Tilia* sp. seedlings
Број корјенова за укорјењене резнице Tilia sp.

Variants/Species *Варијанте/Врсте*	*Tilia cordata*	*Tilia platyphyllos*	*Tilia tomentosa*
Control	10.33	15.96	22.23
30 mg/l IBA	14.72**	17.45*	25.46**
40 mg/l IBA	15.46**	18.71**	27.51***
50 mg/l IBA	18.98***	21.45***	29.58***
60 mg/l IBA	16.31**	17.18*	25.14***
100 mg/l IAA	11.54*	16.42*	23.48**
150 mg/l IAA	12.81*	17.81*	25.14***
200 mg/l IAA	14.53**	14.51 ns	23.13**
250 mg/l IAA	9.47 ns	13.21 ns	20.41*
20 mg/l NAA	11.58*	16.81*	24.53**
30 mg/l NAA	12.34*	18.34**	26.81***
40 mg/l NAA	12.58*	19.51**	23.77**
50 mg/l NAA	7.45 ns	12.81 ns	19.34 ns

*($P \leq 0.05$), **($P \leq 0.01$), ***($P \leq 0.001$) and not – significant ns

Tab. 3. Root length of *Tilia sp.* seedlings
Дужина коријена за укоријењене резнице Tilia sp.

Variants/Species *Варијанте/Врсте*	*Tilia cordata*	*Tilia platyphyllos*	*Tilia tomentosa*
Control	2.51	7.16	5.27
30 mg/l IBA	2.81*	8.13*	4.34ns
40 mg/l IBA	4.32***	9.41**	6.75**
50 mg/l IBA	3.31**	8.01*	4.31 ns
60 mg/l IBA	3.27**	7.54*	4.71 ns
100 mg/l IAA	3.45**	8.12*	6.43*
150 mg/l IAA	4.81***	8.87**	6.88*
200 mg/l IAA	3.31**	7.51ns	5.47 ns
250 mg/l IAA	3.38**	6.34 ns	5.34 ns
20 mg/l NAA	3.34*	7.54 ns	6.11*
30 mg/l NAA	3.89**	8.93*	7.17**
40 mg/l NAA	3.21ns	7.45*	7.00**
50 mg/l NAA	3.01ns	7.79*	6.89*

*($P \leq 0.05$), **($P \leq 0.01$), ***($P \leq 0.001$) and not – significant ns

The increase IBA or IAA or NAA concentration was accompanied by the decreased rooting percentage, suggesting that high concentration of three examined growth regulators were not suitable for the root formation process (Singh et al., 2003; Moreira et al. 2009).

Weaver (1973) reported that the increasing doses of IBA could encourage the rooting ability of cuttings but could also change the rooting structure.

Conclusion

Based on the results of the present study the following was concluded: according to the results it appeared that 40 or 50 mg/l IBA or 150 mg/l NAA or 30 mg/l NAA are good for rooting of *Tilia* species cutting. We suggest putting the cuttings in rooting media for 90 days.

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Вегетативно размножавање врста рода *Tilia* полу-зрелим резницама

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Сажетак

Ово испитивање је спроведено у циљу испитивања утицаја различитих доза регулатора раста IBA, IAA и NAA на способности укорјењавања полу-зрелих резница врста рода *Tilia*. Резнице су третиране са 30, 40, 50 и 60 mg/l индол-3-бутерне киселине (IBA), 100, 150, 200 и 250 mg/l индолил-сирћетне киселине (IAA) и 20, 30, 40, и 50 mg/l нафтил сирћетне киселине (NAA). Резултати су очитани након три мјесеца. Анализирани су сљедећи показатељи: проценат укорјењених резница (%), број корјенова по резници и просјечна дужина коријена (cm). Највећи број укорјењених резница је добијен коришћењем 40 mg/l IBA у већини варијанти. Третман са 200 mg/l IAA дао је корјенове највеће дужине. Просјечна дужина корјена се повећава третманом са 30 mg/l и 40 mg/l NAA. Високе дозе IBA, IAA i NAA резултују формирањем слабог и крхког корјеновог система, што смањује шансе за успјешну адаптацију на поновну садњу.

Кључне ријечи: Tilia, вегетативно размножавање, укорјењавање, индол 3-бутерна киселина, индолил-сирћетна киселина, нафтил-сирћетна киселина

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