

DIVERSITY, GENETIC RESOURCES AND CONSERVATION OF *TILIA* SPECIES IN BULGARIA

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Abstract

Species of genus *Tilia* (lime) still did not gain the attention they deserve by researchers and forest managers. Their importance as a significant part of forest biodiversity is expected to increase, especially in the light of changing environment, due to climate change and other factors. We present a mini-review about the genetic resources of *Tilia* species in Bulgaria. The survey starts with species diversity, including the standing taxonomic problems, and continues with genetic variation. A thorough evaluation of lime species' genetic resources and their conservation in Bulgaria is performed, facilitated by a specially developed database. Knowledge gaps are identified and some domains for prospective studies are outlined.

Key words: *ex situ*, genetic diversity, *in situ*, Lime species, taxonomy.

Introduction

Limes (genus *Tilia* L.), known also as Linden and Basswoods, are valuable noble hardwood species in Europe due to their economic importance and significance as an integral part of forest biodiversity. They are especially valued for the fragrant and nectar-producing flowers, valuable timber and excellent ornamental properties. The flowers are used in phytotherapy, production of herbal teas and tinctures, but are also exceptionally important for bee-keeping and honey production. The timber is soft, resistant to splitting and used for various purposes, ranging from wood carving to producing artists' charcoal and

fuel wood (Delkov 1992, Svejgaard Jensen 2003, Yurukov and Panayotov 2015). Even though currently the *Tilia* species do not enjoy great attention by researchers and forest managers, their significance as a part of the boreal and temperate broad-leaved forests is expected to increase, especially in the light of the changes in environmental conditions due to climate change (Radoglou et al. 2009, De Jaeger et al. 2016). Therefore, studies of their ecology, regeneration (natural and artificial), habitats and response to silvicultural treatments are essential for their successful management and conservation (Petkova et al. 2005, Sultanova et al. 2020). Central to these studies is the assess-

ment of their genetic resources (Maurer and Tabel 1995a, Alexandrov and Dobrev 2011, Black-Samuelsson 2012, Gil and Zajaczkowski 2017). The objective of the present study was to evaluate the state of the knowledge of *Tilia* species diversity and genetic resources in Bulgaria and to identify important knowledge gaps, in relation to their future sustainable use and conservation.

Species diversity was studied by an extensive literature survey and by our own field inventories. The main references were different floras (Browicz 1968, Jordanov and Peev 1979, Raab-Straube 2017+) and the latest comprehensive monograph of the genus (Pigott 2012). A special database was established containing detailed information about the stands with composition of *Tilia* species using information from the current forest management plans (FMPs). The database was organized to allow fast and complete retrieval of information about the available *Tilia* genetic resources.

Species diversity

Traditionally, genus *Tilia* L. has been classified to family Tiliaceae Juss. However, recent phylogenetic studies placed it within Malvaceae Juss. *sensu lato* (Angiosperm Phylogeny Group 2009), which was accepted also in Raab-Straube (2017+). Discussing the phylogenetic position of the genus is beyond the scope of the present review.

Despite the relatively clear-cut and stable morphological characteristics of *Tilia* species, their taxonomy was unnecessarily confusing, controversial and over-complicated. The comprehensive study of Pigott (2012) resulted in a revised taxonomic structure, consisting of 23 species

and 14 subspecies. A total of six species are recognized in Europe, but in spite of this modest diversity, some taxonomical problems still exist. This is at least partly due to the interspecific hybridization occurring in some parts of the species' natural range. The problem was first reviewed by Browicz (1968) in the volume 2 of Flora Europaea. He listed five 'good' species: *T. cordata* Mill., *T. dasystyla* Steven, *T. platyphyllos* Scop., *T. rubra* DC., and *T. tomentosa* Moench., and two species of hybrid origin: *T. × vulgaris* Hayne (*T. europaea* L. *pro parte*) and *T. × euchlora* C. Koch. Currently, the number of species in Europe was reduced to five and a new species, *T. begoniifolia* Steven was added, replacing *T. rubra* DC., whose taxonomic position and status was re-evaluated (Pigott 2012, Raab-Straube 2017+).

Traditionally, three to four species have been recognized in Bulgaria (Stefanoff 1934, Černjavski et al. 1959, Jordanov and Peev 1979, Yurukov and Panayotov 2015). The systematics of three of them (*T. cordata*, *T. platyphyllos* and *T. tomentosa*) is not considered problematic, while the fourth species has undergone several nomenclatural changes, from *T. rubra* DC., (Černjavski et al. 1959, Jordanov and Peev 1979) through *T. corinthiaca* Bosc ex K.Koch, *T. caucasica* Rupr., and finally set as *T. begoniifolia* Stev. (syn. *T. platyphyllos* subsp. *caucasica* (V. Engl.) Loria) in Pigott (2012) and Raab-Straube (2017+).

Other *Tilia* species like *T. petiolaris* DC. have been reported from Bulgaria (Dončev 1964). Interestingly, it was described from a natural locality (Strandzha Mts) while Pigott (2012) considered this 'form' as only cultivated. Evidently, this needs further clarification. Raab-Straube (2017+) considers this taxon as synonym of *T. tomentosa* Moench.

There were some chromosome number reports of Bulgarian *Tilia* species (Petрова et al. 2007) confirming the previous findings that all European species are diploid ($2n=82$), with the exception of *T. dasystyla*, which is a tetraploid ($2n=164$) (see Pigott 2002, for review).

Interspecific hybridization between *Tilia* species was reported from different parts of the species' range; however, as pointed out by Pigott (2012), description of these hybrids in most cases was based on trees in cultivation. The topic is still scarcely studied in Bulgaria and adjacent regions. Our field studies revealed some populations (e.g., Golo Bardo, Vitosha (northern slope), Lozenska and Strandzha Mts., near the town of Trun, Pernik region, and in the valley of Yadenitza river, Pazardzhik region) with putative occurrence of *T. begoniifolia*, and with individuals expressing some intermediate characteristics between *T. platyphyllos* and *T. cordata*. These populations need further detailed investigations. Due to the existing taxonomic uncertainties, the inventory of lime species genetic resources was done only considering the three species – *T. cordata*, *T. platyphyllos* and *T. tomentosa*.

Genetic diversity

The studies on the genetic diversity of limes in Bulgaria to date are limited only to describing the variation among provenances and studies on the ecotype and phenotype variation (Peev 1970, Dobrinov et al. 1982; Kalmukov 1987, 1995). In fact, the studies on limes' genetic variation in Bulgaria by using genetic markers are still in their infancy. Some studies, employing genetic markers were performed in other parts of the natural distribution range of

limes in Europe. Isozymes were applied to document genetic variation in the species and to study the interspecific hybridization (Maurer and Tabel 1995b, Fromm 2001, Fromm and Hattemer 2003). Fineschi et al. (2003) found relatively low differentiation among 17 European populations of *T. cordata* (Balkan populations not included in the set) by applying markers based on restriction fragments length polymorphism amplified by polymerase chain reaction (PCR-RFLP), and the patterns of diversity were attributed to human activity in the past.

Random amplified polymorphic DNA (RAPDs) were used for documenting genetic diversity of *T. tomentosa* in urban trees (Filiz et al. 2015) and natural populations (Gabur et al. 2019), and for studying the interspecific hybridization (Liesebach and Sinkó 2008, Mylett 2015).

Barker (2017) studied fine-scale spatial genetic structure of *Tilia cordata* (microsatellite markers) and found that the species has a mixed reproductive system with approximately half of all individuals being of clonal origin. The studies on chloroplast genome of plants is considered very important both for studying the genetics of the species, and for systematic purposes. A complete sequence of the plastid genomes was performed of four Asian *Tilia* species (Cai et al. 2015). These approaches could be extended successfully to the European species.

Microsatellite markers have been successfully developed for *T. cordata* and *T. platyphyllos* (Phuekvilai and Wolff 2013, Phuekvilai 2014, Logan et al. 2015, Mylett 2015, Logan 2016, Vanden Broeck et al. 2018). These markers were applied to study genetic structure and differentiation of lime forests, hybridization and outcrossing. Lobo et al. (2018) found high genetic variation in phenology and fine-scale se-

lection in favor of local growth conditions in *T. cordata*. Erichsen et al. (2019) detected high degree of diversity but low degree of differentiation among the Danish populations. These results could be instructive for similar future studies of the Bulgarian and Balkan populations. Furthermore, Logan (2016) reported thousands of simple sequence repeats, which can be used to extend the marker set for the two species.

Inventory of *Tilia* genetic resources using database searches

As indicated above, the database inventories rely on information obtained from forest management plans (FMPs). By

regulation, the information in the FMPs is being updated every 10 years, and therefore, our database and the evaluations of the genetic resources based on it, reflect the current status of the genetic resources, a snapshot. Detecting finer temporal trends of their development requires additional studies and information.

The database contains information about all stands where species of genus *Tilia* occur including the location, administrative management unit (Forest service) and numerous stand characteristics. The database allows searches using different criteria. An illustrating example is provided in Figure 1, representing extraction of all stands with participation of *Tilia* species in one unit – Palamara Forestry Service and a map of the stands with occurrence of *Til-*

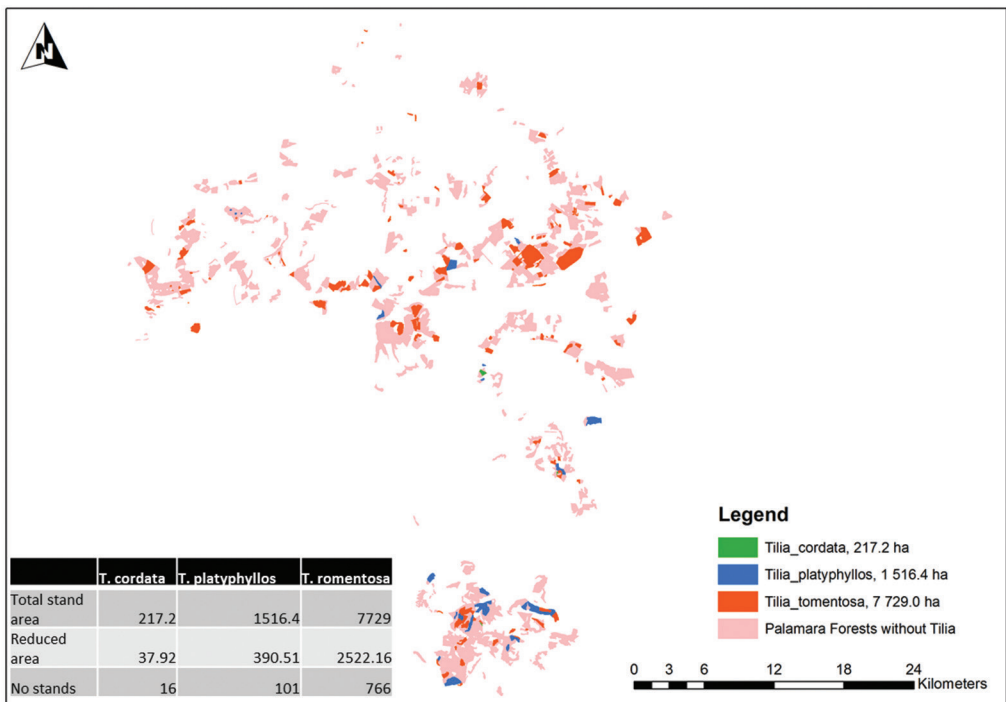


Fig. 1. Example of a database search of the total and reduced area covered by *Tilia* species in the Palamara Forestry Service. A spatial map of the distribution of the three species is also shown.

ia species (Palamara was chosen as an example because all three species occur in this district).

T. tomentosa is by far the most abundant species in Bulgaria (Table 1). The total area of the stands where it occurs, is 191,665.6 ha, 145,756.2 ha of which are natural stands, only 11.5 % of them being of seed origin and the remaining 88.5 % are coppice forests. The next species, in term of abundance by occupied

area, is *T. platyphyllos*, with total area of the stands where it occurs, 15,842.8 ha, 92.7 % (14,701 ha) of them being indigenous, either of coppice (88 %) or of seed origin (12 %) (calculation based on stand number). The least distributed species is *T. cordata*, with only 3421.5 ha total area of the stands, and 2698.6 ha of natural origin. About 84 % of the stands are of coppice, and 16 % – of seed origin.

Table 1. Area and origin of *Tilia* species stands in Bulgaria.

Species	Total stand area, ha	Natural stands only, ha	Reduced area natural stands, ha	Origin: coppice stands; seed stands, %
<i>Tilia cordata</i>	3421.5	2698.6	528.59	84; 16
<i>Tilia platyphyllos</i>	15842.8	14701.6	5095.9	88; 12
<i>Tilia tomentosa</i>	191665.6	145756.3	46893.9	85.5; 11.5

Note: percentage calculations based on the stand number, not on area.

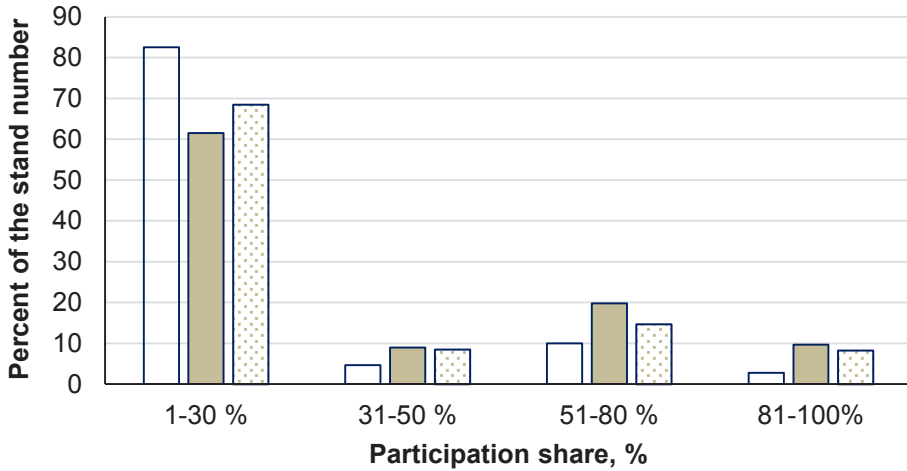
The species of genus *Tilia* are seldom dominant in the forest communities, which is due to their ecological and life-history characteristics. The only species tending to form pure stands is *T. tomentosa*; however, most of them are of secondary origin, formed after cutting the main oak stands. A more precise estimate of the amount of their genetic resources provides the reduced area, obtained by multiplying the respective share of the species by the stand area (Table 1).

The distribution of limes' proportion in mixed stands was calculated based on the number of stands and area. Results based on number of stands revealed that in 60 to 80 % of the stands lime species comprise up to 30 % of the total stand composition (Fig. 2A). This is especially true for *T. cordata* (83 % of the stands). The cases where the limes participate more substantially in the stands are few – in less than 10 % of the stands the lime species play dominant role, with more than

80 % participation ratio. This is valid even for *T. tomentosa*, which is more prone to form pure stands. However, these distributions changed when the calculations were based on occupied area (Fig. 2B). Then the distributions became more even, with greater *Tilia* share of the total stand areas, both where the species have dominant and sub-dominant position.

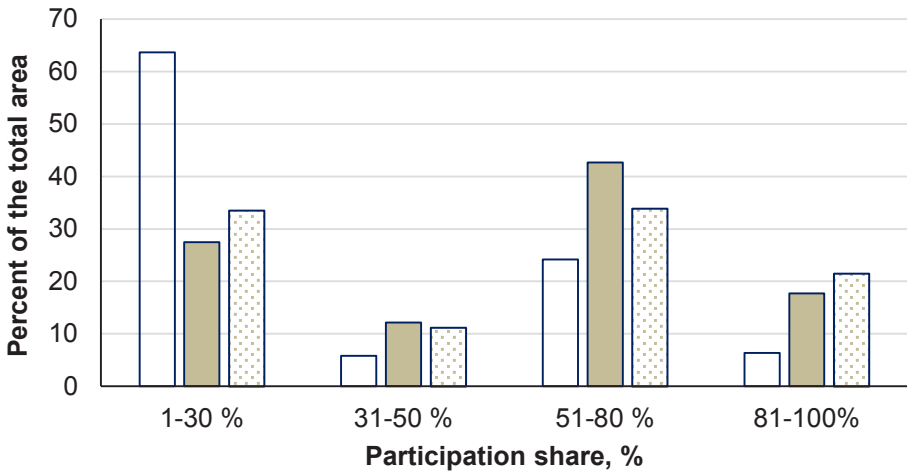
Distribution regarding the altitude and age classes was calculated based on the reduced area (Table 1). Generally, the species occur below 1000 m a.s.l., with some insignificant exceptions (Fig. 3). *T. cordata* is distributed mostly within the altitudinal range of 500–1000 m, *T. platyphyllos* within the range of 200–500 m and of *T. tomentosa* below 200 m. These patterns correspond well with the ecological characteristics of the species, even though some stands with lime species could reach up to 1300–1400 m a.s.l. (Fig. 3).

Distribution of genetic resources by



A

□ *Tilia cordata* ■ *Tilia platyphyllos* ▨ *Tilia tomentosa*



B

□ *Tilia cordata* ■ *Tilia platyphyllos* ▨ *Tilia tomentosa*

Fig. 2. Proportion of *Tilia* species in mixed stands: **A** – calculations based on the stand number; **B** – calculations based on the reduced area (see the text).

age classes revealed similar patterns in the three species. Predominant part of the area for all species is within the age classes 40–60 and 60–80 years (Fig. 4). It corresponds with the legacy of forest management in Bulgaria – there was a

strong harvesting pressure during the 1950s and 1960s together with the large-scale afforestation occurring during the same period. Forests regenerated post this period now represent the two predominant age classes. Regardless, the age

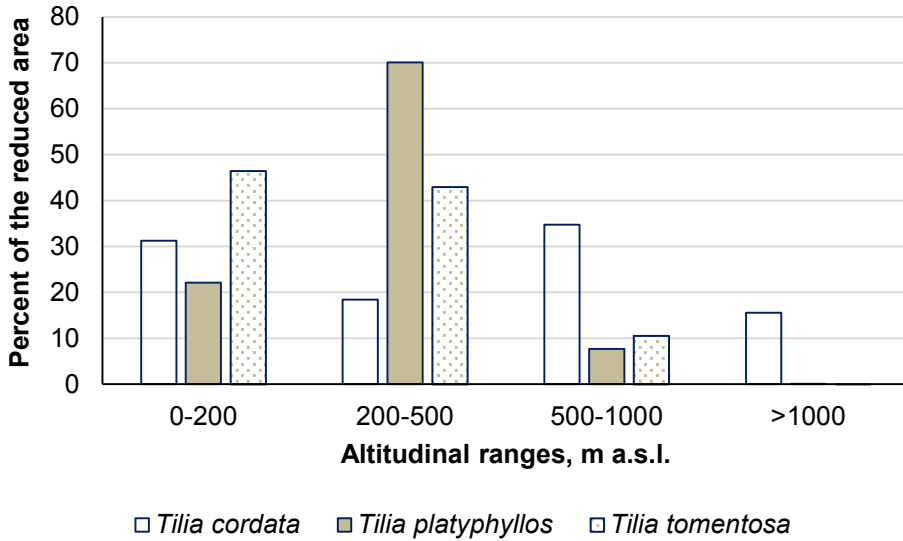


Fig. 3. Distribution of *Tilia* species by altitude.

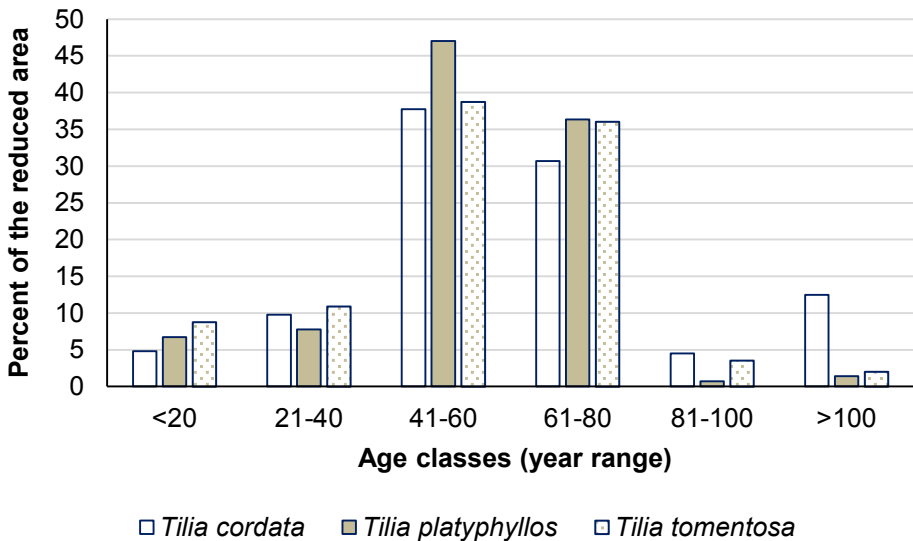


Fig. 4. Age distribution of *Tilia* species.

structure is far from optimal (Figure 4). However, having in mind the current undertaken measures aimed at sustainable and nature-oriented forest management, we could expect improving the age structure in the next decades.

Coppice management system is applied more frequently to the stands with participation of *T. tomentosa*, while *T. platyphyllos*, and particularly *T. cordata*, tend to be of seed origin more frequently (they have lower sprouting capacity).

Conservation of *Tilia* genetic resources

The measures for conservation of genetic resources of *Tilia* species have been usually part of larger-scale measures for conservation of forest ecosystems where *Tilia* species are part of and were the focus of the Noble Hardwoods network of the European conservation programme (Bozzano et al. 2006), with Bulgaria participating in the programme until 2014.

In situ conservation measures at national level include different kinds of protection and special management regimes. Typical example is the protection category known as a seed production stand. Such stands are selected based on phenotype superiority and are used for harvesting seeds for the afforestation purposes. They are managed in a way promoting flowering and seed production and not for timber. There are 121 permanent seed production stands of *T. tomentosa* with an area of 1433.8 ha. The respective figures for the other two species are: 22 permanent seed production stands of *T. platyphyllos* (239.7 ha) and only 2 of *T. cordata* (12.5 ha).

Some natural habitats where *Tilia* species play substantial role, are important from general nature conservation perspective. Two of them are included in the Red Data Book of Bulgaria, vol. 3 Natural Habitats: 23G1 Forest of Silver Lime (*Tilia tomentosa*) (Tzonev 2015) and 28G1 Ravine and slope woodlands (Tashev 2015). The same habitats are considered important for Europe and are included in the priority habitats of Natura 2000: 91Z0 Moesian Silver lime woods (Tzonev 2003, 2009) and 9180 *Tilio-Acerion* forests of slopes, screes and ravines (Dimitrov 2009). Numerous stands with participation of *Tilia* species fall into other protect-

ed territories (natural reserves, national parks) and/or Natura 2000 zones, but their amount and effectiveness of conservation measures still remain to be studied.

Ex situ measures are practically lacking and are undertaken for only *T. tomentosa*. There are three provenance trials, two clonal seed orchards (4.9 ha), and one seedling seed orchard (1.7 ha).

Knowledge gaps and needs of studies

Based on this review, we could identify and summarize some important knowledge gaps concerning Bulgarian *Tilia* species. The distribution of genetic diversity within and among populations is still largely unknown. This is true both concerning the application of genetic markers, and adaptive quantitative traits. There are still some unresolved taxonomic problems, whose investigation could benefit greatly from new DNA-based methods (Hörandl and Appelhans 2015, Soltis et al. 2018). The effectiveness of conservation measures is unknown, and the conservation units are not properly identified and described.

The identified knowledge gaps suggest some important and necessary activities and studies, briefly:

- documenting the existing genetic diversity by using a combination of neutral and adaptive genetic markers and characteristics;
- extending the ex situ conservation activities by establishing provenance/progeny trials and thus combining the conservation measures with studies on the adaptive quantitative traits;
- identification and detailed documentation of the existing gene conservation units;
- popularizing the economic, environ-

mental and conservation importance of the *Tilia* species among the experts and public.

Conclusion

Tilia species in Bulgaria are traditionally highly valued but still neglected by forestry professionals. The present review outlined the state of the art and existing gap knowledges and drew focus on areas that should be addressed in the near future, including detailed study on the genetic diversity of natural populations. These could be beneficial from both economic and conservation viewpoints.

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