From *in vitro* clones to high-quality timber production: the Project "Wavy Grain Maple"

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Abstract

Wavy grain maple is a rare growth variation of sycamore maple (*Acer pseudoplatanus* L.) with wood fibres undulating in the tree rings. Due to its attractive timber, it belongs to the most expensive hardwoods in Central Europe. It has never been produced systematically for commercial use, although wavy grain maple has a great popularity and is in demand. In 2016, therefore, a national joint project started to explore possibilities for its commercial use, and to investigate the causes for the wavy grain structure in sycamore maple. Here, we report about first results achieved by one of the five project partners, the Thünen Institute. We give an overview on selected clones and their propagation by tissue culture, as well as the establishment of planned field trials. Furthermore, we present a method for the clone identification of sycamore maple using highly variable nuclear microsatellite markers.

Key words: wavy grain, figured wood, fiddleback maple, *Acer pseudoplatanus,* commercial use

Introduction

In wood, the normal orientation of longitudinal fibres is parallel to the longitudinal tree axis, which is called straight grain (BEALS and DAVIS 1977). Beside this, various deviations from this parallel growth pattern are known, one of which is wavy grain. Here, the wood fibres undulate in the tree rings creating a "washboard" effect in the split radial section of the log (RICHTER 2015). The result of such a wavy growth is timber with a longitudinal radial surface exhibiting a series of alternately bright and dark stripes shading into one another and giving an optical illusion of waves (BEALS and DAVIS 1977). The surface of standing trees with wavy grain shows no visible external symptoms, because the growth phenomenon is restricted primarily to the radial plane of wood (BEALS and DAVIS 1977).

Wavy grain is mentioned to occur in many hardwood species (e.g. CONRAD 1988). According to BEALS and DAVIS (1977), a well-developed wavy grain structure over the entire tree stem is rare in trees of most species, but can be found in the genera maple (*Acer*), ash (*Fraxinus*), birch (*Betula*), and walnut (*Juglans*). Within populations, the percentage of individuals with wavy grain may vary from location to location. For populations of sycamore maple, different frequencies of occurrence of wavy grain figure have been reported ranging between 1 – 7 % (e.g. ROHR and HANUS 1987, CONRAD 1988, KRAJNC et al. 2015).

Sycamore maple (*Acer pseudoplatanus* L.) exhibiting wavy grain is known under different names, for example wavy grain maple or wavy grain sycamore. In German, it is mainly referred as "Riegelahorn", in Russian as "явор с волнистой структурой". Another term is also fiddleback maple, because this quality of wood is extensively used for backs of string instruments since the sixteenth century (e.g. BEALS and DAVIS 1977, CONRAD 1988).

Still today, there is a strong demand for wavy grain maple in the musical instrument making. Moreover, the timber is used as veneer and has some importance for exclusive furniture industry (NAUJOKS et al. 2013). Therefore, maple logs with a regular, well-developed wavy grain are always sold for very high prices at auction sales, so-called submissions. In Germany, such logs regularly achieve prices over

10,000.00 Euro, e.g. 12,100.00 Euro at the submission Waging am See in 2017. According to KRAJNC et al. (2015), wavy grain maple achieves significantly higher prices than any other wood in Slovenia. Thus, wavy grain maple belongs to the most expensive hardwoods in Central Europe.

Although wavy grain maple has such a great popularity and is in demand, it has never been produced systematically for commercial use, because research concerning wavy grain maple has not been continuously promoted in many cases (KRABEL and WOLF 2013). In Germany, however, activities to preserve valuable sycamore material led to the micropropagation of a few wavy grain maple clones (EWALD and NAUJOKS 2015), as well as the establishment of seed orchards and progeny trails (KRABEL and WOLF 2013). Nevertheless, research into the reliable production of nursery trees of wavy grain maple on a larger scale with the aim to establish commercial plantations is lacking. Moreover, the factors causing wavy grain in sycamore maple are still unclear. Since there is accumulating evidence that figure in other tree species can be genetically inheritable, such as in curly birch (KÄRKKÄINEN et al. 2017) and curly poplar (FAN et al. 2013), the growth phenomenon may also have a genetic basis in wavy grain maple.

In order to explore possibilities for the commercial use of wavy grain maple, and also to investigate the causes for its wavy growth, a national joint project is funded by the Landwirtschaftliche Rentenbank (Federal Ministry of Food and Agriculture). In the frame of this project, methods for the identification, propagation and commercial use of wavy grain maple will be developed (https://www.thuenen.de/en/fg/projects/current-projects/maple-trees/). The project partners are: RLP AgroScience, Thünen Institute of Forest Genetics, Northwest German Forest Research Institute (NW-FVA), Institute for Plant Cultivation in Solkau, and the Reinhold Hummel GmbH & Co KG in Stuttgart.

Within the project, the Thünen Institute and the NW-FVA work closely together. Their tasks include the search for valuable material of wavy grain maple and its genetic conservation with methods of *in vitro* culturing and grafting. In this context, the clone collection of both Institutes will be enlarged, the *in vitro* cultivation of wavy grain maple will be improved, and clone material is exchanged among all project partners.

Beside this, a fingerprint method for the genetic identification of sycamore maple clones will be established by the Thünen Institute. Based on this method, the identification of *in vitro* propagated clone material can be ensured. Furthermore, the establishment of field trails testing tissue culture propagated material is included in the joint project, because the commercial use of clonal propagated material of sycamore as a forest tree species needs an official approval procedure. This implies the evaluation of field testing results according to the regulations on forest reproductive material (FRM) in Germany.

In the following, we report about first results of the work package of the Thünen Institute.

Material and methods

Search for material of wavy grain maple

The Thünen Institute and the NW-FVA are responsible to find new material of wavy grain maple in different regions of the German area. In the winter of 2015/2016 and 2016/2017, the institutes contacted the organisers of submissions, and asked for sycamore maple logs with wavy pattern. In case, logs exhibiting clear evidence of wavy grain were offered for sale, efforts were made to find the original tree stump and the remains of the tree crown outside in the forest. For a few logs, the corresponding stumps and also remains of the crowns could be clearly identified with the help of district foresters or private forest owners. Scions from the tree crown were harvested only if their belonging to the felled wavy grain maple tree was undoubtedly determined. If possible, the tree stumps were protected to facilitate the development of stump sprouts.

In vitro cultivation of wavy grain maple

Vegetative winter buds from scions of the tree crown were used as starting material for the establishment of tissue cultures of wavy grain maple in many cases. If existing, we also used vegetative buds from stump sprouts or grafted plants. The buds were surface-disinfected for 20 minutes with 0.4 % "FINK - Antisept P" followed by another disinfection step with 0.05 % silver nitrate for five minutes. After three rinses with autoclaved water, buds were prepared under a binocular microscope as described by EWALD and NAUJOKS (2015). For vegetative propagation of the wavy grain clones, we also followed the method published by EWALD and NAUJOKS (2015).

Planned field trails

To investigate the long-term growth behaviour and the manifestation of the wavy grain structure in the clone material selected and propagated by tissue culture, two field trials are planned by the Thünen Institute. One of those trails will be located in Saxony, district Großröhrsdorf near Dresden. We will test as many as possible clones originating from material of wavy grain maple trees and two approved seed standards of *A. pseudoplatanus* as controls.

Genetic characterization of the wavy grain clones

Genetic markers are a very important tool for the identification of clones in tree breeding, as morphological traits are not sufficient for that purpose. To genotype material of sycamore maple, we selected 12 nuclear microsatellite markers, which were known from the literature. The markers were used in three different sets of multiplex PCR (Table 1) and analysed using DNA fragment length analyses of PCR-amplified repetitive DNA sequences (Beckman Coulter CEQ-8000 Genetic Analysis System).

Table 1: Overview of the 12 nuclear microsatellite markers selected for genetic characterization of *A. pseudoplatanus* and their use in three different sets of multiplex PCR

Set	Marker	Fluorescent dye	Size range published	Motif	Reference
Set 1	MAP12	Cyanine 5	142-178	$(GT)_7$	PANDEY et al. (2004)
	MAP33	BMN-6	146-182	$(GT)_{18}$	PANDEY et al. (2004)
	Aop122	DY-751	185-199	$(CT)_{11}$	SEGARRA-MORAGUES et al. (2008)
	MAP40	Cyanine 5	238-246	$(GT)_6$	PANDEY et al. (2004)
Set2	MAP9	BMN-6	96-110	$(GA)_8$	PANDEY et al. (2004)
	Am118	Cyanine 5	140-190	$(CT)_{16}$	KIKUCHI and SHIBATA (2008)
	SM21A	DY-751	179-243	$(GAT)_{14}$	GRAIGNIC et al. (2013)
	SM60	BMN-6	231-237	$(AAC)_6$	GRAIGNIC et al. (2013)
	SM29	Cyanine 5	281-301	$(CTT)_{10}$	GRAIGNIC et al. (2013)
Set3	Aop116	Cyanine 5	109-139	$(GA)_{16}$	SEGARRA-MORAGUES et al. (2008)
	Aop943	DY-751	142-164	$(GA)_8$	SEGARRA-MORAGUES et al. (2008)
	MAP2	BMN-6	144-198	$(GT)_{23}$	PANDEY et al. (2004)

Results and discussion

Selected clones and their propagation by tissue culture

During the first half of the project, material from eight wavy grain maple trees was established using methods of tissue culture (Table 2). Together with *in vitro* clone material, which had already been cultivated by the Thünen Institute before the project started and clones received from the NW-FVA, the Thünen collection was extended to a number of 27 *in vitro* clones in total. From these *in vitro* clones, 17 might be regarded as stable clones, while the others are still in the establishment phase, which is characterized by a very small shoot elongation.

The difficulties in shoot multiplication of wavy grain maple are often related to the maturity of the material used (Ewald and Naujoks 2015). The rejuvenation process, therefore, may be considered as the most important aspect for the success of *in vitro* cultures of sycamore. In this context, rejuvenation may be induced by regular cutting of grafted plants (EWALD and NAUJOKS 2015), or continuous subculturing and regeneration of shoots in tissue cultures of wavy grain maple. Another possibility may result from using a juvenile starting material, such as buds from stump sprouts. For the clone "Poldi", for example, our attempts to establish a tissue culture arising from buds of scions of the tree crown failed, but we successfully established material from stump sprouts using the same method (i.e. preparation of buds). Therefore, the physiological status of material from stump sprouts may be more suitable for *in vitro* cultivation of *A. pseudoplatanus* compared to material harvested from the tree crown. The time period, furthermore, in which scions of the crown of felled wavy grain maple trees are influenced by changing weather conditions and fungal attack outside in the field, may also be of significant importance for the success of *in vitro* cultures originating from those material.

Table 2: Overview of wavy grain maple clones, which were selected or re-established during the first half of the project by the Thünen Institute.

Clone	Submission	Price per cubic metres in Euro	Material used for *in vitro* culture
Bonn	2001, Bonn	4,000.00	buds from grafted plant
Haini	2017, Erfurt-Egstedt	3,740.00	buds from tree crown scions
Isen	2017, Waging am See	7,160.00	buds from tree crown scions
Pfull	2003, Bad Waldsee	5,768.00	buds from grafted plant
Poldi	2016, Waging am See	2,290.00	buds from stump sprouts
Rhön	2016, Sailershausen	2,741.00	buds from tree crown scions
Schussi	2012, Bad Schussenried	2,730.00	buds from stump sprouts
Staig	2001, Bad Waldsee	6,952.00	buds from grafted plant

Planned field trails

Within the first year of the project, *in vitro* material of eight clones, which originated from buds of wavy grain maple trees, was successfully propagated by tissue culture in larger quantities. In 2017, therefore, we already produced *in vitro* plants from these well-performing clones (Figure 1). In addition, control plants of two approved seed standards were also grown. For 2018, we expect to produce *in vitro* plants of several more clones cultivated from material of wavy grain maple (ca. seven clones), and to test them on the field trails planned.

Figure 1: Produced *in vitro* plants of sycamore clones, which originated from buds of wavy grain maple, in the tree nursery of the Thünen Institute in Waldsieversdorf.

Genetic characterization of wavy grain clones and planned parentage analysis

A method for genotyping and clone identification of wavy grain maple was established using highly variable nuclear microsatellite markers. Because *A. pseudoplatanus* is a tetraploid tree species, a maximum rate of four different alleles was detected within a single gene locus. As expected, we found extended size ranges of alleles for some loci in comparison to the size ranges reported in the literature, e.g. loci of MAP12, MAP40, Aop122, Aop943, and SM60.

The developed microsatellite analysis will further be used to conduct a parentage analysis, since microsatellite markers are optimal for clone identification, as well as for parentage and sibship analysis. As parent generation of sycamore maple, we use 21 genotypes including seven clones with wavy grain from the seed orchard in Reinhardshagen, which was planted from 1959 to 1964 by the NW-FVA. Regarding the offspring generation, we will analyse individuals of two progeny testings, which were established with open-pollinated seeds from Reinhardshagen in 1986.

Conclusions

Within the project, valuable material of wavy grain maple was selected during submissions or re-established from grafted plants using methods of tissue culture. As starting material for the establishment of *in vitro* cultures of wavy grain maple, dormant buds from stump sprouts seem to be more suitable than vegetative buds from scions of tree crowns and grafts, respectively. In future, material from stump sprouts will be preferred. Therefore, the protection of tree stumps of felled wavy grain maple is highly recommended to avoid browsing of stump sprouts by animals. A method of tissue culture propagation of sycamore maple was successfully applied, and nursery plants of wavy grain maple clones were produced in larger quantities to test their growth performance and the manifestation of wavy grain on field trails. A reliable genotyping method with nuclear microsatellite markers was established and can be used for routine genotyping.

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