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A rare cultivar of horse chestnut (*Aesculus hippocastanum* L. ˈBaumaniiˈ) in Slovakia: morphological, molecular and antioxidant analysis

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Abstract: *Aesculus hippocastanum* L. ˈBaumaniiˈ (syn.: *A. hippocastanum* L. ˈFlore Plenoˈ; *A. hippocastanum* ˈPlenaˈ) is a rarely cultivated cultivar characterized by fully white and sterile flowers, and as a result it does not produce fruit. For identification and characterization of the morphological, molecular, and biochemical parameters of this cultivar, we performed the pollen viability test, morphological analysis of flowers, assay of miRNA-based markers and biochemical analyses. The results show that the germination of pollen grains in artificial media reached 0 % and the pistil in flowers was undeveloped. The antioxidant activity, total polyphenol and flavonoid content exhibited the highest values of all three tested parameters (1.5540 mg TEAC/g FM; 3.7278 mg GAE/g FM and 1.328 mg CAE/g FM) in the extracts from *A. hippocastanum* ˈBaumaniiˈ. The applied miRNA-based markers showed a sample-specific and tissue-specific genomic polymorphism. Various DNA fingerprinting profiles between the flowers and leaf samples of fruiting and barren trees were noted.
Keywords: horse chestnut, inflorescences, pollen, miRNA-based markers, antioxidant activity.

Introduction

Aesculus hippocastanum L. (horse chestnut) is native in the Balkan Peninsula where it occurs in a few small and isolated populations in southern Albania, the territory of the former Yugoslavia, Bulgaria and in Greece on both sides of the Pindos Mountains. This species grows in mixed forests at an altitude of 400 – 1300 m a. s. l. It was introduced into culture in 1557. It is a semi-shade-loving tree, it requires moisture and nutrient content in the soil, and it is resistant to frost. It is sensitive to air pollutants. The species is used in parks and urban greenery for its impressive appearance at the time of flowering (Ravazzi & Caudollo 2016).

In Slovakia, the cultivation of this species started at the end of the 17th century and it became the fourth most widespread non-native tree that is grown in all climatic areas. The total number of recorded specimens is more than 20 000, ranging from 10 to 130 years of age. There are altogether 1602 known locations in our territory in 33 counties. The main area of occurrence is the regions of Western Slovakia (630 locations). The species is suitable for the climatic region A/B – warm locations, C – protected locations up to 1000 m a. s. l. (Benčať 1982; Pagan & Randuška 1988).

The species blooms in May. This taxon has hermaphrodite, pentamerous zygomorphic flowers. The calyx is bell-shaped and hairy. The petals are white, with yellow and red spots and an inverted ovoid sized 12–15 × 8–10 mm. The flower has 7 stamens with filaments of the same length. The stamens are longer than the petals. The upper ovary is present. The flowers grow at the end of this year´s shoot in an upright panicle. The second flowering can be observed in September–October in some years (Thomas et al. 2019).

Bellini & Nin (2005) list 14 cultivated varieties of *Aesculus hippocastanum* L. The cultivar ˈBaumaniiˈ with double flowers is considered the best ornamental variety. *Aesculus hippocastanum* L. ˈBaumaniiˈ (syn. *Aesculus hippocastanum* L. ˈFlore Plenoˈ; ˈPlenaˈ) was introduced into cultivation in 1822 in France. This species is characterized by large dark-green leaves, full white flowers with a hint of pink and yellow marks that age to red. The flowers are sterile and do not have a developed pistil. The pollen grains do not germinate. There is no fruit development due to the sterility of the flowers. The inflorescences are shorter and more compact compared to the common species. The cultivar is used mainly in urban greenery where fertile cultivars are not suitable due to the fall of their fruits, which causes clutter on the roads and lawns (Van der Berk 2013). This rarely grown cultivar was recorded in Slovakia only in two localities: Banská Štiavnica – Botanical Garden of Secondary Forestry School and Malý Cetín (Nitra County) on the church yard. This tree was awarded as one of top trees in the competition “Slovak Tree of the Year“. It won the 3rd place in 2016 (Ekopolis 2022).

Systematic documentation and evaluation of the plant genome based on molecular markers is essential for the management of genetic resources of plants. Molecular markers are widely used to determine biodiversity, as they make it possible to obtain specific information on the variability of individual regions of the plant genome (Grover & Sharma 2016). A molecular marker essentially consists of a

sequence of nucleotides corresponding to a precise site in the genome. Its nucleotide sequences must be sufficiently polymorphic among the relevant plants so that hereditary characteristics can be easily traced (Schulman 2007). These markers complement the characteristics necessary to classify and identify plant genotypes within the species. Their importance and benefits are significant both for research and breeding, and in practice (Gálová et al. 2016).

The small molecules of microRNAs (miRNAs) have a significant regulatory potential in the genetic and epigenetic control of gene expression (Bartel et al. 2004). Their regulatory role is defined in the following processes: plant growth and development, leaf morphology and plant polarity, root formation, transition from the embryogenic to vegetative phase, flowering time, formation of flower organs, reproduction, defence mechanisms through the transfer of signalling molecules and in a stress response to (a)biotic factors (Jones-Rhoades et al. 2016; Xie et al. 2010). The genomic conservation of miRNA sequences – and especially the stem-loop structure of the precursor molecules of miRNA (pre-miRNA) – provided an opportunity to develop a novel type of molecular markers (Cuperus 2011). The advantages of this marker system include high polymorphism, reproducibility, and transferability across species (Fu et al. 2013; Yadav et al. 2014). The abundance of mature miRNAs varies greatly among miRNAs, tissue types and developmental stages, indicating the spatially and temporally regulated expression patterns of plant miRNAs (Htwe 2015).

Polyphenols are common secondary metabolites of plants. Plants produce polyphenols as a response to biotic and abiotic environmental stress conditions. The presence of polyphenols in the leaves, seeds, flowers, and bark of horse chestnut has been reported in many studies. According to the literature, the content of polyphenols is (7.81–24.48 mg/g FW) in the horse chestnuts leaves, (24.24–70.40 mg/g FW) in the seeds, (8.17 mg/g) in the flowers and (363.58 mg/g FW) in the bark. The flowers, fruits, seeds, and cortex are used for medicinal purposes. Extracts and concentrates are used in the manufacture of creams and ointments with anti-inflammatory properties to treat phlebitis and different kinds of cancer. However, culinary properties of horse chestnut flowers have been reported as well (Bielarska et al. 2022).

The aim of this study was to characterize the morphological, molecular and antioxidant properties of a rare cultivar of horse chestnut (*Aesculus hippocastanum* ˈBaumaniiˈ) in Slovakia.

Material and Methods

The specimen *Aesculus hippocastanum* ˈBaumaniiˈ growing in the village Malý Cetín (48°14'13"N 18°10'34"E) was used as a research subject. This specimen is specific for its abundant inflorescences, but it does not produce fruit – chestnuts (Fig. 1). The morphological, molecular and antioxidant parameters of flowers and leaf samples of this specimen have been compared to fruiting *Aesculus hippocastanum* (horse chestnut) tree growing in the Botanical Garden of Slovak University of Agriculture in Nitra).

The basic taxation data (trunk circumference, trunk diameter 1.3 m above the ground, diameter at breast height – DBH, tree height and crown width) have been determined based on the methodology introduced by Hrubík et al. (2011). The age of the tree has been estimated according to the circumference of the trunk (d 1.3 m).

The number and types of flowers in inflorescence were evaluated from five panicles of *A. hippocastanum* 'Baumanii' and the same number of inflorescences was sampled from five individuals of *A. hippocastanum*. The nomenclature of the taxa follows the publication by Marhold & Hindák (1998).

Sampling

The leaves and flowers for molecular and antioxidant analyses were collected randomly at the beginning of June from the lower branches on different sides of the tree crown. The samples were frozen at -20°C until further analyses. The samples for antioxidant and morphological analyses were stored at 4°C.

Genomic DNA isolation

The pooled samples of leaves and flowers were grinded in liquid nitrogen and aliquots of 100 mg were used for DNA isolation. The genomic DNA was isolated based on the Rogers-Bendich protocol (1984). The extracted DNA was quantified by the Implen NanoPhotometer™ and diluted to 70 ng×µl^{-1}.

Fig. 1 Abundant inflorescences *A. hippocastanum* 'Baumanii' growing in the village Malý Cetín.

Tab. 1 Sequences of miRNA-based primers applied in the genomic analyses.

Primer identification	Sequences (5´- 3´)
gb-miR160_F	TTAGTCTGCCTGGCTCCCTGTATG
gb-miR160_R	AGTAAAGCTCTGCATGGCTCCCTC
gb-miR482_F	TGGGTTGTAGTCTTCAGGAGTGGG
gb-miR482_R	GAAGGCAATAGGAATGGGAGGATC
hyp-miR414_F	AGAGTACAGGGAAATGGAGGA
hyp-miR414_R	CACAGCGAAACCCAC
lus-miR168_F	CACGCATCGCTTGGTGCAGGT
lus-miR168_R	CCAGTGCAGGGTCCGAGGTA
lus-miR408_F	GGCTGGGAACAGACAGAGCATGGA
lus-miR408_R	GGGAAAAAGGCCAGGGAAGAGG
mdo-miR160_F	TGCCTGGCTCCCTGTATGCCA
mdo-miR160_R	TGGCATACAGGGAGCCAGGCA
mdo-miR398_F	TGTGTTCTCAGGTCAGGGGTT
mdo-miR398_R	AACCCCTGACCTGAGAACACA

Note: The two/three-letter abbreviations indicate the genomic origin of the sequences; gb – *Ginkgo biloba* L., hyp – *Hypericum perforatum* L., lus – *Linum usitatissimum* L., mdo – *Malus domestica* Borkh.

miRNA-based assay

The MiRNA-based analyses were performed based on Fu et al. (2013), Yadav et al. (2014) and Ražná (2020a). PCR was amplified in a 20 µL PCR mix containing 70 ng×µl^{-1} of genomic DNA, 10 pmol×dm^{-3} of each primer, 2U of DreamTaq DNA polymerase, 0.8 mmol×dm^{-3} dNTPs (Bioline) and 1× DreamTaq Buffer (KCl, $(NH_4)_2SO_4$, 20 mmol×dm^{-3} $MgCl_2$). The PCR amplification programme used the following 'touchdown' method: initial denaturation at 94°C for 5 min; 5 cycles of 30 s at 94°C, 45 s at 64°C (with a 1°C decrease in the annealing temperature per cycle), and 60 s at 72°C; 30 cycles of 30 s at 94°C, 45 s at 60°C, and 60 s at 72°C; and the final extension at 72°C for 10 min.

Seven different miRNA-based markers were selected based on our previous studies (Ražná et al. 2019, 2020a, b, 2021) (Tab. 1).

The amplified PCR products were separated on the 3 % and consequently 15 % TBE-Urea polyacrylamide gels (Novex™) running in 1 × TBE Running Buffer at a constant power of 90 V, 25 mA for 120 min. The 10 bp DNA ladder (Invitrogen™) was used for the size comparison. The polyacrylamide gels were stained with the GelRedTM Nucleic Acid Gel stain and visualised in the G-Box (Syngene™) electrophoresis documentation system. The gels were analysed by the GeneTools software – GeneSnap version 7.09.17 (Syngene™) to record the number of the loci and identify the unique fragments.

Analyses of Antioxidant Activity

Sample preparation

An amount of 1 g of sample was extracted with 20 ml of 80 % ethanol for 2 hours. After the centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, supernatant was used for the measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). The extraction was carried out in triplicate.

Free Radical Scavenging Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the free radical scavenging activity in accordance with the method described by Sánchez-Moreno et al. (1998). Briefly, the absorbance of samples (0.4 mL) and alcohol solution DPPH (3.6 mL) were measured at a wavelength of 515 nm using a spectrophotometer (6405 UV/Vis, Jenway, Stone, UK). The results of antioxidant activity were expressed as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent in mg/g. The analysis was carried out in triplicate.

Total Polyphenol Content

A standard method (Singleton et al. 1999) was used to measure the total polyphenol content in the analysed samples. The results of absorbance at 700 nm measured spectrophotometrically (6405 UV/Vis, Jenway, Stone, UK) were then calculated and expressed as the gallic acid equivalents expressed in mg/g. The analysis was carried out in triplicate.

Total phenolic acid content

The total phenolic acids content was determined using the method specified by Jain et al. (2017). A 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 mol×dm^{-3} hydrochloric acid, 0.5 ml Arnova reagent (10% $NaNO_2$ +10 % Na_2MoO_4), 0.5 ml of 1 mol×dm^{-3} sodium hydroxide (w/v) and 0.5 ml of water. The absorbance at 490 nm was measured using a Jenway (6405 UV/Vis, England) spectrophotometer. Caffeic acid was used as a standard and the results were expressed in mg/g caffeic acid equivalents. The analysis was carried out in triplicate.

Statistical analysis

All antioxidant experiments were carried out in triplicate and the mean of replications with standard deviations was reported. The experimental data were subjected to the analysis of variance (Duncan's test) at the significance level of 0.05 in the SAS software (2009).

Pollen viability

The pollen grains were collected from the flowers shortly before opening. The anthers were dissected from the flowers and dried at a room temperature for 24 hours. The estimation of viability was performed on two variants:

I. Determination of aborted and non-aborted pollen grains

The pollen before staining was fixed in the Carnoy´s fixative solution (6 ethanol:3 chloroform:1 acetic acid). The fixed pollen grains were placed on microscopic slides, thoroughly dried, and stained with a solution of Malachite green, Acid fuchsin and Orange G (Peterson et al. 2010). The developed pollen grains of normal size and shape whose protoplasm was dyed magenta-red were considered non-aborted, and other pollen grains of blue-green colour were counted as aborted. The percentage of non-aborted pollen grains was evaluated from approximately 1000 pollen grains per sample.

II. Germination of pollen grains

The pollen grains were cultivated in Petri dishes on a 1 % agar medium with the addition of 20 % sucrose. The culture medium was optimized by adding 0.01 % boric acid (H_3BO_3) and 0.15 % calcium nitrate ($Ca(NO_3)_2$). The germination was carried out at a temperature of 25°C and a humidity of 80 %. The percentage of germinated pollen grains was evaluated after 24 and 48 hours. The sample of 100 pollen grains from 3 random fields in each Petri dish was used to calculate.

Results and Discussion

The analyses of polymorphism in flowers and leaf samples of fruiting and barren horse chestnut tree (*Aesculus hippocastanum* L.) were done by seven miRNA-based functional markers (Tab. 1). The markers have been selected based on the previous studies (Ražná et al. 2019, 2020a, b, 2021) where the species transferability of these markers have been verified. In total, 80 miRNA loci were amplified by the touchdown PCR approach, which represent approximately 13 DNA fragments per markers. The amplification effectivity varied from 0 amplified products (mdo-miR160) up to 24 amplified products (hyp-miR414).

In terms of genomic characterization, one of our goals was to identify a marker that could distinguish between the samples of fruiting and barren horse chestnut tree with the help of sample-specific DNA fingerprinting. Two markers, hyp-miR414 and gb-miR160, have shown this capacity and mediated the amplification not only in a sample-specific but also in a tissue-specific manner (Fig. 2). At the same time, the highest number of miRNAs loci (with 56% of the total number of loci) was amplified by these two markers.

Various DNA fingerprinting profiles between the flower samples of fruiting and barren trees and/or between the leaf samples of fruiting and barren trees are obvious (Fig. 3).

The lus-miR168, gb-miR482 and mdo-miR398 markers provided tissue-specific polymorphism (Fig. 4).

In the sample of flowers from a fruit-bearing horse chestnut a distinctive fragment of the size around 150 bp has been amplified by the lus-miR168 marker, which is stress-sensitive marker (Li et al. 2012; Neutelings et al. 2012; Barvkar et al. 2013; Bej & Basak 2014). The role of miR168 was identified in several protective mechanisms of plants (Melnikova et al. 2015, 2016). Another tissue- specific DNA fragment, amplified by marker gb-miR482 (Wang et al. 2015), has been observed in the leaf

sample of a barren horse chestnut tree, and tissue-specific DNA fingerprinting was also observed in the leaf samples in contrast to flowers in both types (fruit-bearing and barren) of the horse chestnut tree (Figure not shown).

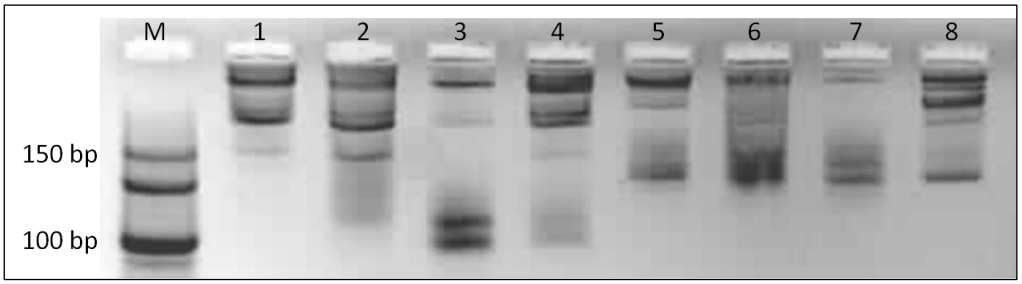

Fig. 2 DNA polymorphism detected by the hyp-miR414 (samples 1–4) and gb-miR160 (samples 5–8) marker. 1 and 5 - flowers from a fruiting tree, 2 and 6 - flowers from a barren tree, 3 and 7 - leaves from a fruiting tree, 4 and 8 - leaves from a barren tree. M - O´Range Ruler 10 bp DNA (Thermo Scientific™).

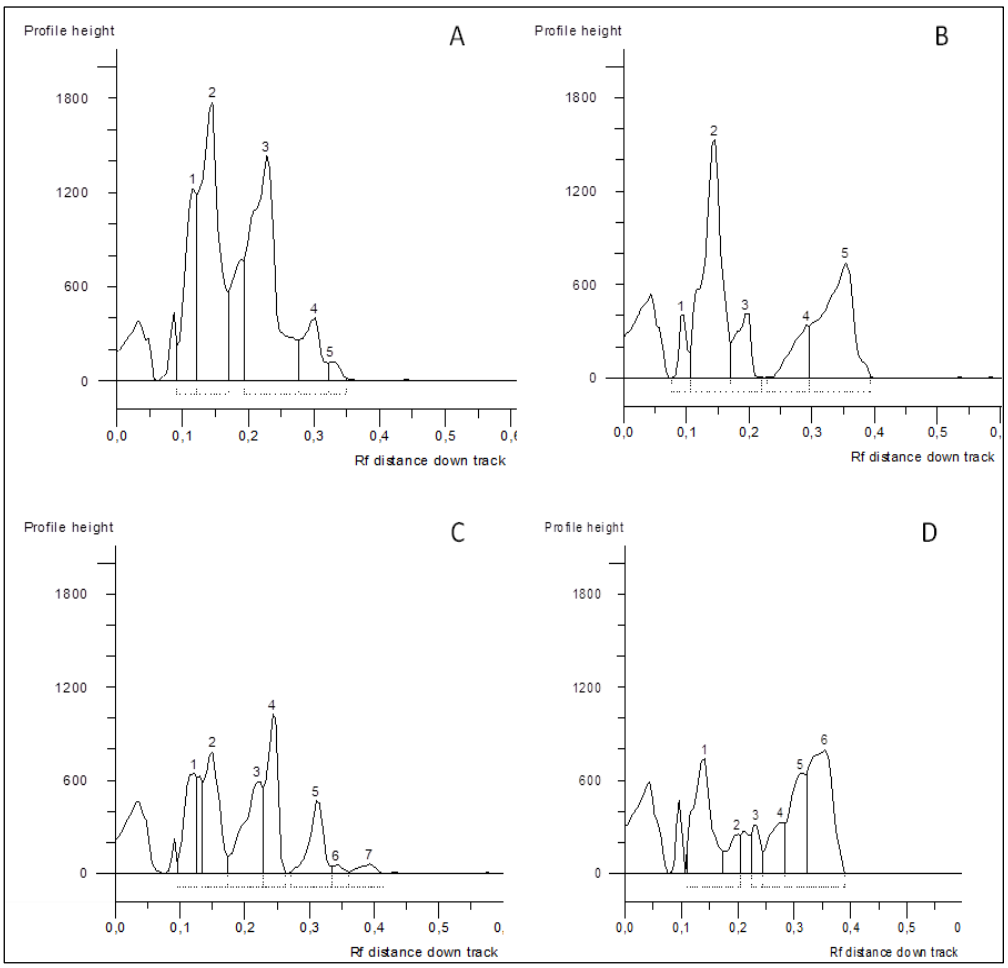

Fig. 3 Profiles of miR414 (A) and miR160 (B) loci of flowers from a fruiting tree and flowers from a barren tree (C, D).

Generally, it can be stated that a higher representation of the miRNA loci in comparison with the samples from a fruit-bearing tree was observed in the flower and leaf samples from a barren chestnut tree.

The study and comparison of individual organisms/genotypes at a molecular level involves the search for DNA polymorphisms (Malik et al. 2017). Polymorphism is represented by the differences in DNA sequences and can be identified by molecular marker techniques (Clark 2005). A molecular marker is a specific identified fragment of the DNA, inherited to the next generation, which is located in or close to a gene or in the noncoding regions. The presence of polymorphisms between the individuals will lead to a different pattern, "DNA fingerprinting", of markers after electrophoresis. These patterns reveal the DNA polymorphisms and the genetic diversity among the studied individuals (Batley 2015).

The markers based on the sequences of microRNA meet several attributes that make them a suitable diversity detection tool (Fu et al. 2013; Yadav et al. 2014): (a) good stability due to a direct PCR-based marker system, (b) improved reproducibility and sequence specificity due to high annealing temperature followed by the "touchdown" PCR approach, (c) relatively high polymorphism, (d) putative functionality due to their polymorphic nature and the ability to predict the phenotypes controlled by miRNAs, (e) the cross-genera transferability potential due to the conservation level of miRNAs between the species and the way of deriving the markers from the consensus sequences of miRNAs.

Antioxidant activity, total polyphenol and phenolic acids content

The horse chestnut flowers represent an excellent source of bioactive compounds, including flavanols, phenolic acids and anthocyanins (Celep et al. 2012; Kędzierski et al. 2016; Owczarek et al. 2021; Bielarska et al. 2022). The analysis of flowers of both horse chestnut taxa have showed higher values of all tested parameters in the extract from the barren tree compared to the fruiting tree (Tab 2).

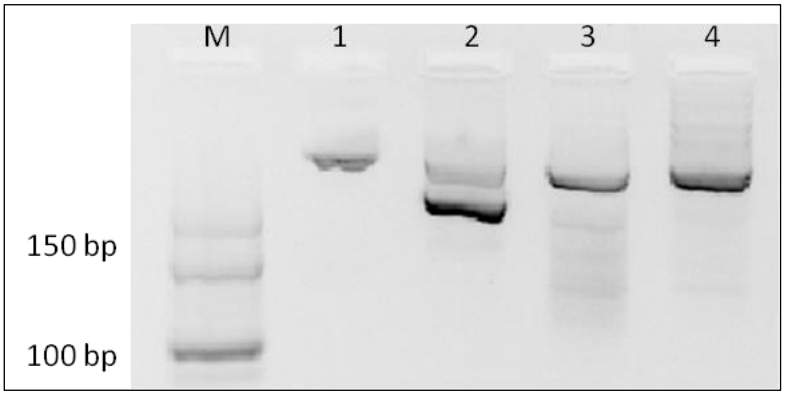

Fig. 4. The tissue-specific locus of miR168 detected in flowers from a fruit-bearing tree (2). 1 - sample of leaves form a fruiting tree, 3 - sample of leaves from a barren tree, 4 - sample of flowers from a fruiting tree.

Tab. 2 Antioxidant activity, total polyphenol and phenolic acid content of flower samples of barren and fruiting *Aesculus hippocastanum* trees.

Parameter	Barren tree	Fruiting tree
Antioxidant activity DPPH (mg TEAC/g FM)	1.55 ±0.99a	0.83 ±0.76b
Total polyphenol content (mg GAE/g FM)	3.73 ±0.54a	2.61 ±0.32b
Total phenolic acids content (mg CAE/g FM)	1.33 ±0.21a	0.38 ±0.43b

Notes: FM – fresh matter; TEAC – Trolox equivalent antioxidant capacity; GAE – gallic acid equivalent, CAE - caffeic acid equivalent; mean ±standard deviation; different letters in a column denote mean values that statistically differ from each other

The study of Bielarska et al. (2022) compares the amounts of bioactive compounds present in *Aesculus hippocastanum* and *Aesculus* × *carnea* flowers and leaves from six independent locations. The content of polyphenol was measured in dry matter by HPLC. Their observation was that the horse chestnut flowers were characterized by a higher total concentration of polyphenol (9.45 mg/g FW) compared to the red horse chestnut flowers. However, the red horse chestnut flowers contained significantly more total carotenoids (40.6 µg/g FW) and higher levels of anthocyanins (5.41 µg/g FW). Another study also speaks in favour of the interesting biochemical composition of flowers, in which among the 43 constituents detected in the horse chestnut flower by UHPLC-PDA-ESI-TQ-MS/MS (flavonoids, phenolic acids, flavanols, and coumarins) altogether 31 were reported in the flower for the first time (Owczarek et al. 2021).

Celep et al. (2012) determined the antioxidant capacity of the horse chestnut bark, seeds, leaves, and flowers for its 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging capacity, microsomal lipid peroxidation inhibition capacity and total phenolic content. The bark extract of *A. hippocastanum* revealed the highest antioxidant capacity (IC50 value of 0.025 mg/mL). Kędzierski et al. (2016) measured the antioxidant activity and total phenolic content values in DPPH and Folin–Ciocalteu tests in the horse chestnut fruits, and they found that the antioxidant capacity was dependant on the harvest time.

Morphological characteristics of *Aesculus hippocastanum* ˈBaumaniiˈ in Malý Cetín

The individual in Malý Cetín is a tree with a dominant main trunk and a multi-branched crown ranging from ovoid to spherical shape (Fig. 5). The tree has a compact habitus. The measured dendrometric parameters (23 May 2021) of the Aesculus hippocastanum ˈBaumaniiˈ individual were as follows: DBH – 315 cm; trunk diameter - 100.7 cm; tree height – 20 m; tree crown width – 20 x 18 m. The tree was approximately 150 years old.

In the inflorescence, 150 - 200 flowers were developed (Fig. 8). The flowers had usually more than 20 petals, most often around 50. The flowers were yellow white in colour (Fig. 10). The flowers are characterized by a delicate fragrance. The cultivar

'Baumanii' is considered sterile. Despite this fact, we found that the observed individual produced 48.32 % of non-aborted pollen grains (Fig. 6). However, the germination of pollen grains of this individual in artificial media reached 0 %. In comparison, the pollen germination of fertile individuals *A. hippocastanum* was 44.23 % after 24 hours and 46.53 % after 48 hours (Fig. 7). Even the pollen viability reached up to 98,33 % after the staining process. Lower pollen viability ranging from 56 to 68 % after the fluorescein staining was recorded by Ćalić & Radojević (2017). However, they noted that the germination rate of the horse chestnut pollen on the base medium was 50–66 % and up to 76–91 % on the medium with the highest PEG concentration. A high percentage of pollen germination was also recorded in other species of the *Aesculus* genus. The viability of the *A. turbinata* Blume pollen reached more than 90 % (Suo et al. 1995). Pablo & Scheerens (2006) tested the germination of fresh and stored pollen of 11 genotypes from five *Aesculus* species and the hybrid *Aesculus* ×*carnea*. Fresh pollen of all tested genotypes exceeded 80 %. Even after 3 months of pollen storage at -20°C and -80°C, the germination remained above 60 %.

Fig. 5 Tree *Aesculus hippocastanum* L. 'Baumanii' in Malý Cetín.

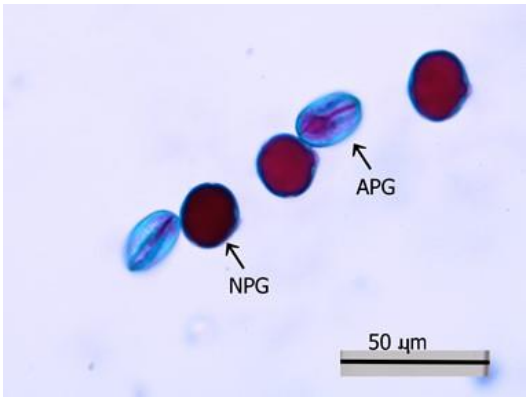

Fig. 6 Non-aborted (NPG) and aborted (APG) pollen grains of *Aesculus hippocastanum* L. 'Baumanii'

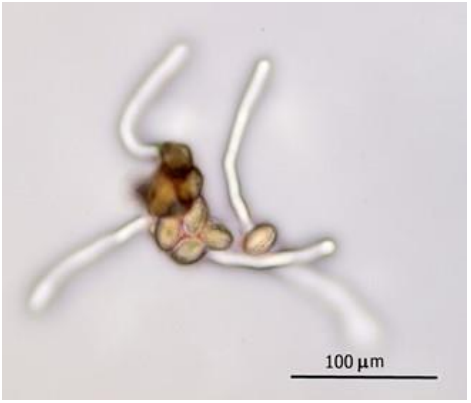

Fig. 7 Pollen germination of *Aesculus hippocastanum* L.

Fig. 8 Inflorescence of *Aesculus hippocastanum* L. 'Baumanii'

Fig. 9 Inflorescence of *Aesculus hippocastanum* L.

Fig. 10 Flower cross-section of *Aesculus hippocastanum* L. 'Baumanii' with undeveloped pistil.

Fig. 11 Flower cross-section of *Aesculus hippocastanum* L. with developed pistil

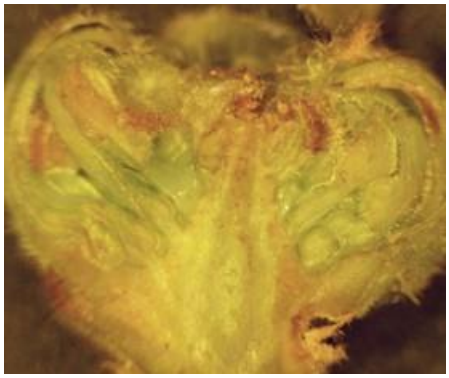

Fig. 12 Bud cross-section of *Aesculus hippocastanum* L. 'Baumanii'

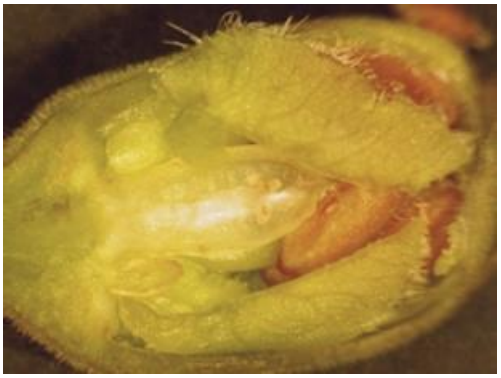

Fig. 13 Bud cross-section of *Aesculus hippocastanum* L.

The pistil in flowers of the studied cultivar ˈBaumaniiˈ was undeveloped (Fig. 12). Due to the above-mentioned facts, fruit production was not recorded in this tree. In contrast, 23 % of flowers in the inflorescence of *A. hippocastanum* were hermaphrodite (Fig. 13) on the base and 77 % were male in the upper half panicle (Fig. 9, 11). Similar results were obtained by Weryszko-Chmielewska & Chwill (2017) in the Polish horse chestnut samples whose inflorescences were divided into 27 % hermaphrodite and 73 % male flowers. The number of developed fruits in the observed individuals was 1–7 per panicle.

The specimen in Malý Cetín is exceptionally beautiful - an aesthetic tree with a multiple branched crown with a dominant main trunk. Full-flowered yellow white to white flowers, blooming on almost every branch, sometimes branched at the end of the branches and pleasantly scented. As it does not produce fruit or seeds, it can be used wherever it is necessary to prevent pollution of roads or lawns by fruit drop in autumn, e.g., in urban plantings.

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