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NEOTENY OF THE EUROPEAN PLANE TREE

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Abstract – The neoteny of 64-day-old European plane tree autovegetative progeny is described, starting from the fact that sexual maturity in the juvenile stage of tree development is a rare and significant process by which the period of synthesis of new lower taxonomic units is shortened. The observed formation of European plane tree reproductive organs has been classified as induced neoteny. Compared to the inflorescences of mature European plane trees, the number of precocious inflorescences was lower, flower structure was simpler, and the diameters of inflorescences were the same. As neoteny is a genetic and physiological marker for the tree genotype and the species, and as it is classified as an example of parallel-inherited variability explained by changed interactions of trophic, hormonal and genetic systems, this analysis of neoteny can be a contribution to its further scientific study and its practical utilization at a larger scale.

Key words: *Platanus x acerifolia* (Ait.) Willd., inflorescences, green cuttings, adaptation, taxon

INTRODUCTION

Neoteny is a form of tree and shrub diversity that has been insufficiently investigated and reported by very few authors in Serbia (Tucović, 1995; 2001; Tucović et al., 1996). Precocious sexual maturity (already in the juvenile stage) is especially significant because it enables early seed reproduction. From the morpho-physiological aspect, flowering induction is a change in the method of apical meristem differentiation, which in the vegetative stage of development produces the cells of leaves and lateral branches. By induction, the activity of the genes that produce the enzymes for leaves is stopped and the genes that produce the enzymes necessary for flowering are activated (Nešković, 1978). The explanation of the mechanism of interactions conditioning neoteny are some of the most complex parts of tree physiology, affected by the factors that regulate the development of higher plants: (1) chemical substances which take part in metabolic processes and are the products of enzymatic reactions, (2) plant hormones and (3) external factors, such as light and temperature regimes (Bernier et al., 1985). In the second half of the 20th century, precocious flowering and fructification of two-year-old trees was reported for arbor vitae, cypress, Douglas fir, and giant and maritime sequoia after treatment with gibberellin water solutions (Pharis, 1969; 1974), and for one-year-old shoots of buddleia and chaste tree after freezing their aboveground parts (Vukićević, 1996).

Precocious flowering and fructification shortens the breeding cycle and leads to faster synthesis of lower taxa and to the cultivation of self-fertilized lines and hybrid generations. Neoteny also enables the establishment of seed orchards for the production of genetically good-quality seeds and planting material from elite trees. Considering its general biological and practical significance, the precocious flowering of European plane tree autovegetative progeny was analyzed, taking into account the above facts and the fact that intraspecific variability is best observed in the juvenile stage.

MATERIALS AND METHODS

The comparative experiment with green cuttings was established in June. The cuttings were collected from 10 trees, five very old European plane trees (100 to 177 years) and five younger trees (40 to 60 years). *Platanus x acerifolia* (Ait.) Willd. belongs to the order *Hamamelidales* and family *Platanaceae* Lindl.; it was formed in 1640 as a subspontaneous hybrid of *Platanus occidentalis* L. and *Platanus orientalis* L., whereby in some references its scientific name is *Platanus x hybrida* Brot. The European plane tree, hybrid plane, or London plane tree is a monoecious broadleaf tree attaining the height of 35 meters and a diameter at breast height of 2.5 m (Vukićević, 1996; Ocokoljić et al., 2003). It is a fast-growing and long-living species (in Transcaucasia there are some 1000-year-old trees, and in the Mediterranean countries, there are trees older than 2000 years) with a wide ecological range. The highest viability is achieved on fresh alluvial soils of alkaline pH. Its adaptation capacity is good, so it can be grown on different soil types (even on saline soils). As a heliophyte, its shade tolerance is poor, but it tolerates high temperatures, dry air, dust and high concentrations of air pollution. In addition, it is resistant to low temperatures (Gerhold, 1993; Ocokoljić, 2006).

The trees selected for cuttings were cultivated at altitudes between 88 and 101 m on deposol (deposited soils) on alluvium. This soil type is characterized by its still insufficiently developed soil formation processes (Živković et al., 1979). The composition and other properties of the study alluvial soils on which European plane trees are grown are as follows: sandy soil (near river island Veliko Ratno Ostrvo), clayey soil (along the river Topčiderska Reka), and loamy soil (along the Sava). There is little difference in the chemical characteristics of clayey and loamy alluviums, whereas sandy alluviums differ significantly (Živković et al., 1979). As the properties of alluvial soils are changed in all soil horizons due to building and other utility activities, a high number of pollution factors, the effect of rainfall water and street-washing water, and the effect of industrial salt from winter de-icing (Sun, 1992), the type of soil in the study area is described as deposol on alluvium. The potential hydrologically conditioned vegetation of the study localities in Belgrade belongs to the alliance *Alno-Ulmion* Br. Bl. et Tuxen (Tomić, 2004), and includes the willow and poplar communities in the widest sense (*Saliceto-Populetum sensu lato*), and the common oak community (*Genisto-Quercetum roboris* Horv.). The analysis of meteorological data includes the measurements of climate elements at the Belgrade weather station of the Republic Hydrometeorological Service of Serbia from the very beginning of measurements in Belgrade until 2010 (when the experiment was established). Based on the mean monthly air temperatures per year (Table 1), the average mean annual air temperature over the last thirty-year period in Belgrade was 12.5°C. The average mean annual air temperature in the last three decades was 0.4°C higher compared to the previous thirty-year period; i.e. 0.7°C compared to the period 1887 - 2010. The coldest month over the studied thirty-year period was January and the warmest month July. The average mean air temperatures per seasons indicate favourable conditions for vegetation development in spring and summer, while autumn and winter temperatures can cause damage to vegetation. The average mean air temperature over the growth season in the last three decades was 19.3°C, which is 0.4°C higher than in the previous thirty-year period; i.e. 0.7°C higher than in the period 1887 - 2010 (Table 2).

After collection, the cuttings were shortened to a length of about 10 cm, the leaf plates were reduced, treated with 1% concentration of Rhizopon AA (powdery Indolebutyric acid) and then inserted in frames with a 4:1 mixture of peat and sand. The frames were covered 90 cm above the cuttings with plastic foil, which was removed after the onset of rooting. The experiment consisted of 4 x 100 cuttings from each mother tree, arranged in randomized block design with four replications. Altogether 4000 cuttings were

Table 1. Mean monthly air temperatures (°C) in Belgrade for the period 1887 - 2010 (annual reports, RHMS Serbia).

Month Year Parameters	I	II	III	IV	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**Year** $(\bar{x})$
$(\bar{x})$ 1981/2010	1.4	3.1	7.6	12.9	18.1	21.0	23.0	22.7	18.0	12.9	7.1	2.7	**12.5**
$(\bar{x})$ 1971-2000	1.3	3.1	7.5	12.3	17.5	20.4	22.1	21.9	17.6	12.3	6.5	2.7	**12.1**
$(\bar{x})$ 1961/1990	0.4	2.8	7.2	12.4	17.2	20.1	21.8	21.4	17.7	12.5	7.0	2.3	**11.9**
max	7.6	9.1	11.8	16.2	21.5	25.0	26.0	26.8	22.6	17.7	13.0	6.6	14.2
yr.max	2007	1966	2001	2000	2003	2003	1928	1992	1942	1907	1926	1960	2000
min	-9.4	-9.2	0.0	8.2	11.0	17.5	18.2	18.1	12.0	7.6	1.2	-4.1	9.8
yr.min	1893	1929	1932	1912	1919	1933	1913	1940	1912	1905	1888	1933	1940
$(\bar{x})$-series	0.2	2.0	6.9	12.1	17.1	20.3	22.3	21.8	17.8	12.5	6.8	2.2	11.8

monitored for neoteny, flowering time, number of inflorescences, inflorescence sex and morphological characters. Quantitative data were biometrically processed using the software Statistica 9.0.

RESULTS AND DISCUSSION

Trees in the subdivision *Magnoliophyta* in natural conditions reach sexual maturity and start the flowering phenophase depending on genetic and environmental variables (Table 3). In most broadleaf tree species in the temperate belt (*Acer* L., *Fraxinus* L., *Quercus* L., etc.*)*, the onset of flower or inflorescence buds occurs in the preceding year and continues through the following year. Solitary trees or trees in small plantations are characterized by shorter periods prior to sexual maturity (Misnik, 1976), while in urban coenoses, the flowering phenophase of woody plants occurs after 4 to 8 years (Vukićević, 1996). Cultivated trees (due to drought, pruning, or transplanting) are characterized by a lower intensity of the traits that control the first flowering (Wzreing et al., 1984; Tucović et al., 1996). Exceptionally, flowering can occur in the first year, for example in 33-day-old rooted cuttings of *Ailanthus altissima* Swingle half-sib lines, and 2-3-month-old shoots from chaste tree roots – *Vitex agnus castus* L. (Tucović, 1995).

Neoteny was detected in 64-day old rooted cuttings in 10 lines of European plane tree autovegetative progeny. There were 72 rooted cuttings with neoteny from five lines originating from the oldest trees. Precocious flowering was initiated on 10 rooted cuttings of the test tree No. 6; 8 rooted cuttings of test tree No. 7; 12 rooted cuttings of test tree No. 8; 6 rooted cuttings of test tree No. 9 and 36 rooted cuttings of test tree No. 10. Based on the presented results, it can be concluded that under the same conditions, neoteny was expressed in a small number of European plane tree rooted cuttings, i.e. in 2.5% of test tree No. 6; 2% of test tree No. 7; 3% of test tree No. 8; 1.5% of test tree No. 9 and 9% of test tree No. 10. Flower bud differentiation started already at the moment of the first internode formation. There were one female and one male inflorescence in each of 72 rooted cuttings. Only one globose male inflorescence, averaging 9 mm in diameter, yellow-green in color, developed laterally

Table 2. Mean monthly air temperatures (°C) in Belgrade for the vegetation periods 1887 - 2010 (annual reports, RHMS Serbia).

Month Year Parameters	IV	V	VI	VII	VIII	IX	IV -IX ($\bar{x}$)
($\bar{x}$) 1981/2010	12.9	18.1	21.0	23.0	22.7	18.0	19.3
($\bar{x}$) 1971-2000	12.3	17.5	20.4	22.1	21.9	17.6	18.6
($\bar{x}$) 1961/1990	12.4	17.2	20.1	21.8	21.4	17.7	18.4
max	16.2	21.5	25.0	26.0	26.8	22.6	21.5
yr.max	2000	2003	2003	1928	1992	1942	1946
min	8.2	11.0	17.5	18.2	18.1	12.0	16.1
yr.min	1912	1919	1933	1913	1940	1912	1912
($\bar{x}$)-series	12.1	17.1	20.3	22.3	21.8	17.8	18.6

Table 3. Years of attaining the sexual maturity - subdivision *Magnoliophyta* (Misnik, 1976).

Species	External conditions	
	Stands	Small plantations or individual trees
Alnus glutinosa (L.) Gaertn.	20-25	12-20
Betula verrucosa Ehrh.	20-25	10-15
Fagus silvatica L.	60-80	30-50
Fraxinus excelsior L.	20-25	15-20
Qercus robur L.	40-50	30-50

Table 4. Biometrical characters of A. male and B. female inflorescences of 64-day old European plane trees.

Test tree No.	Locality	Character	
		Diameter of male inflorescence (mm)	Diameter of female inflorescence (mm)
6	Belgrade (Ada Ciganlija)	8 ± 1.02	12 ± 1.80
7	Belgrade (Topčiderski Park)	10 ± 0.99	12 ± 0.98
8	Belgrade (Topčiderski Park)	9 ± 1.01	13 ± 1.21
9	Belgrade (Boulevard Vojvode Mišića)	9 ± 0.25	11 ± 1.00
10	New Belgrade	10 ± 0.76	13 ± 1.94

on the 8.6 cm long peduncle. In addition, only one globose female inflorescence, average diameter 12 mm, reddish in color, developed terminally on peduncles averaging 10.4 cm in length (Table 4). The staminate flower had 4 stamens twice as long as the petals and a double perianth with 4 pointed petals. The pistillate flower had 4 free pistils and a double perianth with 4 pointed petals.

Compared to the flowers of mature European plane trees, in which inconspicuous, spherical inflorescences, grouped 1 to 8 to a stalk, emerged in April (Xuan, 2003), the inflorescences of autovegetative progeny were separately stalked and emerged in mid-August. The morphological characters of the flowers partly coincide with the reference data, according to which male flowers are borne in hairy clusters less than 10 mm in diameter, yellow-green in color and formed laterally on the shoots, while female flowers are borne in hairy spherical clusters up to 13 mm in diameter, reddish in color, and formed terminally on branchlets. Precocious flowering is characterized by a simpler flower structure (four parts), while the perianth of adult trees is doubled, with 3 to 8 pointed petals. The stamens are half as long as the petals, with anthers that are almost sessile. There are between 3 and 8 stamens in the male flowers and most often 4 pistils in the female flowers (Xuan, 2003). The incidence of individual inflorescences on two-month-old rooted cuttings indicates that European plane tree flowering intensity changed depending on different factors, and that its character is both qualitative and quantitative. The observed precocious shedding of flower heads indicates that neoteny is based on complex morphophysiological processes.

Cluster analysis performed to determine the genetic base of the similarity, i.e. the distance of the analyzed mother trees based on the mean diameters of male and female inflorescences, shows the variability of European plane test trees and some regularity in their clustering. The hypothesis that in cluster analysis all mother trees will be grouped in one group, as neoteny was observed in the plants propagated from green cuttings of very old trees, was not confirmed. The hypothesis that in cluster analysis all mother trees will be grouped in four groups based on the four study sites was partly confirmed. Mother trees No. 7 and 8 were clustered as a homologous group, and the other three trees clustered at small distances among each other and at a great distance from the homologous pair.

Assessment of the quality and quantity of the male and female inflorescences of European two-month-old plane trees formed without an interval between the juvenile and the adult stages, confirms that very old European plane mother trees can be used as the basic material for the comparative study of the characteristics of mother tree genotypes, aiming at the assessment of the genetic and physiological control of neoteny. In addition, the very old European plane tree genotypes justified their utilization in vegetative propagation.

CONCLUSIONS

Induced neoteny of European plane tree autovegetative progeny occurred at the age of 64 days, in mid-August. On 72 plants, i.e. on 3.6% of the plants in the experiment with green cuttings taken from five mother trees 100 to 177 years old, there were one female and one male inflorescences, which is a significantly smaller number compared to the number of inflorescences in mature trees. Precocious flowering was also characterized by a simpler flower structure consisting of four members, i.e. 4 petals, 4 stamens or 4 pistils. The stamens were twice as long as the petals with anthers that were almost sessile. Flower bud differentiation started at the moment of the first internode formation. Precocious shedding of inflorescences was also observed.

Cluster analysis confirmed the variability of European plane mother trees. The trees did not cluster in one group based on age, but in four groups based on the study sites. The trees from Topčiderski Park clustered at a small distance as a homologous group, and the other three trees clustered at small distances among each other and at great distance from the homologous pair. This analysis proves that neoteny is a genetic marker of the species as a whole and that it is a process of tree adaptation that enables early seed reproduction. The studies performed to date still cannot confirm the structural or functional relationship between neoteny and genotype; they are still based solely on experimental evidence.

The European plane tree is a species suitable for experimental study, thanks to its fast growth, abundant yield, wide ecological range and the competition of trees on the alluvial sites of urban environments in Serbia, including streets and parking spaces. For this reason, neoteny, in addition to its biological significance, has also a high practical significance, as it shortens the breeding cycle and accelerates the synthesis of new taxa and enables the earlier establishment of seed orchards. The results are also very significant for the selection and production of a European plane tree nursery stock of good genetic quality.

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