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Full Length Research Paper

Characteristics and variability of half-sib progeny as the base for ginkgo breeding

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The analysis of several morphological traits and phenotypic characteristics of ginkgo seedlings in juvenile tests with half-sib lines in two successive years provide the bases for further planting stock breeding and production for the purposes of urban coenoses and for the establishment of target plantations of this species. The comparative analysis enables the identification of planting stock for further breeding programmes with the aim of getting new ginkgo varieties. Thus, the justification for the protection of the researched ginkgo genotypes and the need for their multiplication were confirmed.

Key words: *Ginkgo biloba* L., adaptation, age, selection.

INTRODUCTION

Nowadays, the practice of biotechnical disciplines in addition to species formed by natural processes in the field develops varieties with target characteristics obtained by the application of tree and shrub breeding methods. The basic unit of reconstruction in an individual is its genotype, and the reconstruction is realised by evolution factors such as inheritance, variability and selection (Isajev et al., 1998; Tucović, 1983). The decisive role in the synthesis of new varieties is assigned to a targeted selection for growing habit, branching method and crown form. The criterion for the selection of units from a great number of individuals depends not only on the future function, but also on the aesthetical-physiological characteristics. By selection and breeding, many ornamental cultivars with emphasized individual aesthetical features have been selected and described (Josifović, 1973; Ocokoljić, 2006; Ocokoljić et al., 2009; Šijačić-Nikolić et al., 2011).

By all parameters, ginkgo is classified in the order of the most resistant and most ornamental species, because of the fact that it is cultivated on green spaces worldwide. This species tolerates the living conditions in urban environments and shows high resistance to low temperature. The damaging of this species occurs rarely and most often it is the consequence of mechanical activities; there is no damage caused by pathogens (Vilotić, 2004; Ocokoljić et al., 2009). Ginkgo trees show efficiency in the storage of pollutant concentrations, and this justifies the utilisation of ginkgo trees on green spaces of urban coenoses. There is a great number of old ginkgo trees throughout the world and in our country (Ocokoljić, 2006). On the territory of Serbia, there are 55 protected trees above the age of 100 years. The origin of planting stock is not known, but it is supposed that it does not originate from Asia. The trend of plantation cultivation is increasingly greater for the production of foliage for pharmaceutical industry (Cohen et al., 1998; Lugasi et al., 1999; Ocokoljić et al., 2011). Since 1990, in China, 5,000 ha have been in use for ginkgo plantations for foliage production, and also for the production of seed (Schmid, 2001; Vilotić, 2004).

Taking into account the above facts, this paper analyzes the growth elements of ginkgo seedlings at the level of half-sib lines of the old and the younger trees. This enables the study of the genetic potential of the species *Ginkgo biloba* L. in Belgrade and the potential use of very old trees for the production of seeds and plant stock.

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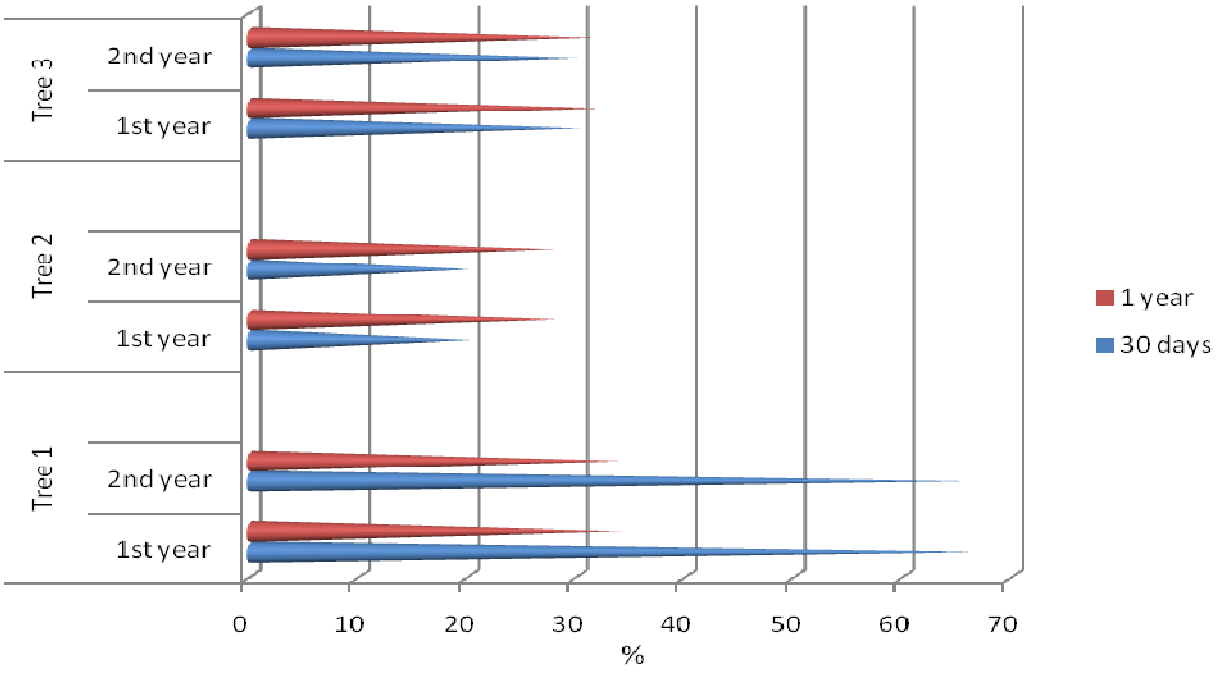

Figure 1. Seedling survival percentage 30 days after sowing and at the end of the vegetation periods.

MATERIALS AND METHODS

Ginkgo trees which were used as the main seed material for the establishment of the experiment and the production of half-sib progeny were grown in Belgrade. Aiming at the research of generative progeny from the selected trees (two old trees and one younger tree), the seeds in the phase of full (technical) maturity in two successive vegetation periods were collected, after which the seeds were dried for a short time and stored till spring. The seeds were controlled once a week. Based on the observed germination, seed storage was interrupted at the beginning of spring (Hartmann et al., 1990; Facciola, 1990). Seed sowing was performed in April, in seedbeds, in the nursery in Belgrade suburbs, after a previous soil preparation. The seedbeds were formed by randomized block design of half-sib families in each of the replications, and divided into four equal blocks. Intensive tending measures were applied during the plant's cultivation.

The seedbeds were established in the nursery in a Belgrade suburb. Its geographical position and coordinates are: 44° 48' east longitude and 20° 28' north-east latitude. The nursery is on a mild north-western slope, at an altitude of 112 m. The site of the sample plot, based on the sub-spontaneous flora, belongs to the climatogene community *Quercetum farnetto-cerris* Rud. The Belgrade fringe area along the southern edge of Pannonia, and the position of the nursery, belong to the sub-association *Quercetum-farnetto-cerris aculeatetosum* Jov.- community of Hungarian oak and Turkish oak with butcher's broom, variant with hornbeam, and represents a natural part of this forest community (Jovanović, 2000). Climate characteristics of the site on which ginkgo half-sib families were cultivated correspond with the characteristics of Belgrade climate. Based on the values of climate index, which is calculated after Thornthwaite, the climate of this site is moist subhumid type (Stojičić et al., 2010). In-depth soil analysis with physical and chemical characteristics was performed based on the open profiles. Soil type is chernozem, lessivé, or loess and loess-like sediments. The anthropogenic impacts are morphologically discernible, though they were first reflected in the solum thickness.

The degree of seedling survival in beds was determined by counting the seedlings 30 days after sowing and at the end of the vegetation period, and compared to the number of sown seeds. The analysis of growth elements included variability of seedling height and root collar diameter 30 days after sowing, in the middle and at the end of the vegetation period.

All biometric analyses were performed on sufficiently large samples. In the zone of collar, diameters were measured by using a micrometer to the nearest 0.1 mm, while height was measured by using a ruler from the root collar to the tip of the terminal bud, to the nearest 1 mm. The data were statistically processed using computer programme Excel. The analysis of variance was performed using the statistical programme Statistica 5.0.

RESULTS AND DISCUSSION

Comparative analysis of seedlings from half-sib lines during two successive vegetation periods included: Seedling survival, elements of growth, and the occurrence of special phenotypic characteristics. Based on the data analyses of the total number of plants sown after 30 days, the average survival percentage in the first year was 39.11, and in the second year, it was 26.54. The comparative analysis of survival percentage after 30 days in the two study years showed the decrease in survival percentage by 12.56. The greatest survival percentage per trees was measured for tree number I: 66.60% in the first year, and the lowest in tree II: 20.00%, also in the first year (Figure 1). By taking the mean value of survival percentage 30

Table 1. Analysis of variance for the survival percentage, seedling height and root collar thickness.

Factor variability	Sum of squares	F - Ratio	P - Value
Ist year (Age: 1 year)			
The survival percentage			
Tree	2993,278	1862,683	0,000000
Study year	1243,230	1743,640	0,000022
Replications	10,222	0,34543	0,754670
The seedling height			
Tree	143,7713	150,9510	0,000000
Study year	7,9550	8,3522	0,000307
Replications	0,7852	0,8245	0,439649
The root collar thickness			
Tree	89,53526	18,70247	0,000000
Study year	14,35834	2,99923	0,051608
Replications	13,53039	2,82628	0,611120
IInd year (Age: 1 year)			
The survival percentage			
Tree	3306,360	849,3911	0,000000
Study year	2978,345	790,000	0,000000
Replications	9,012	0,121876	0,907806
The seedling height			
Tree	116,7526	132,2203	0,000000
Study year	11,5064	13,0308	0,000004
Replications	4,0202	0,8653	0,583082
The root collar thickness			
Tree	102,3993	70,38136	0,000000
Study year	14,0969	9,68911	0,000088
Replications	0,5627	0,38676	0,679660

days after sowing in both study years and by comparing the obtained values with the reference seed germination percentage (Stilinović, 1987), it was concluded that the survival percentage was within the scope which is reported for this tree species. The differences between survival percentages expressed by the results of the analysis of variance confirm the highest level of significance of the differences between the trees and a high level between the study years, but between the replications there were no significant differences in both terms (Table 1). At the end of both vegetation periods, survival percentage was almost the same as the percentage determined 30 days after sowing, so these minimal differences can be neglected. The variability in the number of survived individuals inter and intra families (under the equal environmental conditions in the experiment) points to the differences in the genetic constitution of test trees.

The analysis of 30-day old seedlings in the first year shows the average height of 6.22 cm. The lowest average height at the individual level was attained by tree II (4.64 cm).The maximal average height was attained by tree III (7.8 cm). 60-day old seedlings in the first year reached the average height of 9.97 cm. The lowest average height at the individual level was reached by tree I (8.00 cm). The maximal average height was attained by tree III (12.45 cm). Average height increment from the first term to the second term amounted to 3.75 cm. Maximal height increment of 60-day old seedlings was attained by tree III (5.35 cm) and the lowest height increment was reached by tree I (1.76 cm). Average height of one-year old seedlings in the first year was 15.05 cm. Minimal average height was attained by tree II (14.16 cm) and maximal average height was attained by tree III (15.76 cm). In the second study year, the analysis of 30-

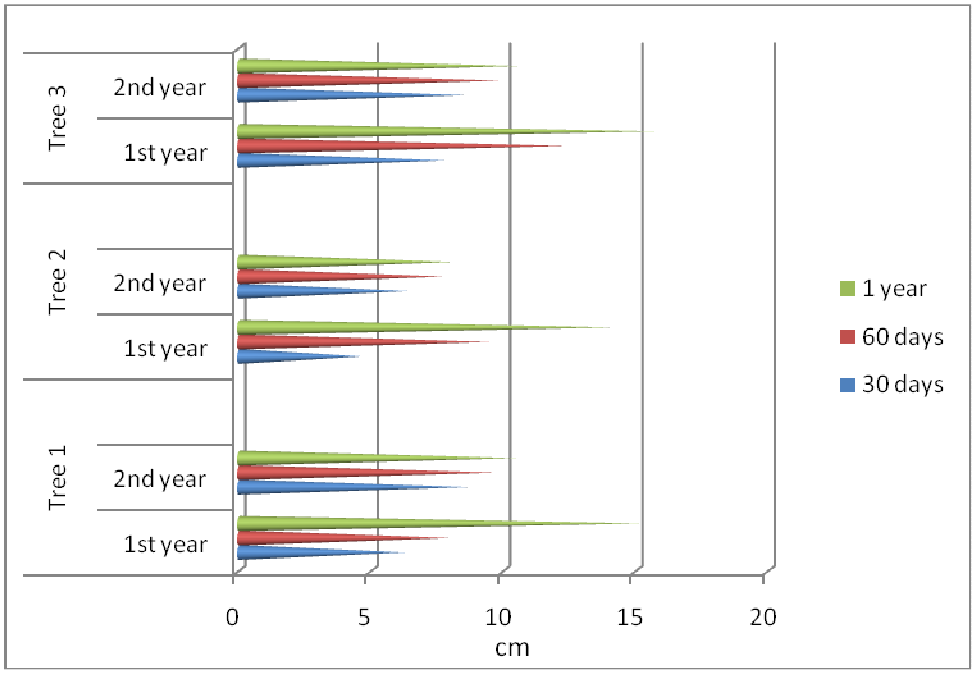

Figure 2. Height increment of 30-day, 60-day and one-year old ginkgo seedlings.

day old seedling height showed the average value of 7.88 cm. The lowest average height at the individual level was attained by tree II (6.38 cm) and maximal average height was reached by tree I (8.68 cm). The analysis of 60-day old seedling height in the second year showed the average height of 9.11 cm. The lowest average height at the individual level was attained by tree II (7.72 cm), and the maximal average seedling height was reached by tree III (9.89 cm). The average height of one-year old seedlings in the second year was 9.72 cm. Minimal average height was measured for tree I (8.11 cm), while the maximal average height was measured for tree III (10.57 cm). The comparative analysis of seedling height in the first and the second years showed that in the second year, the average height was lower than 5.77 cm. At the individual level, the maximal average height in the first study year was attained by the old tree III. The maximal height increment in both study years was reached by the old tree III (Figure 2). Statistical differences in the seedling heights between the old and the younger trees confirm the hypothesis that the differences in seedling height are of a genetic nature, that is, that the old age of parent trees did not affect the seedling height, which confirmed the good constitution of the old trees. This hypothesis is also confirmed by the results of the analysis of variance for 30-day old, 60 days old and one-year old seedling heights in both study years (Table 1).

The experience in practice shows that root collar diameter is an important indicator for root development, which by all means also affects the development of the entire plant. Taking into account the above fact, morphometric analysis in the juvenile experiment also included the analysis of root collar diameter in the progeny of the analyzed families at the ages of 30 days, 60 days and one year, for two successive growing seasons. The results of these analyses were processed at the population level and at the individual level, and they are presented in Figure 3.

30-day old seedlings in the first year reached the average root collar diameter of 2.44 mm. At the individual level, the maximal average values were reached by tree III (2.57 mm), and the minimal values, by tree I (2.34 mm). 60-day old seedlings reached mean values of root collar diameter of 3.84 mm. The lowest average value at the individual level was reached by tree I (3.45 mm). The maximal average value of root collar diameter was attained by tree III (4.40 mm). Diameter increment of 60-day old seedlings was 1.40 mm. At the individual level, the maximal diameter increment was attained by tree III (1.83 mm), and the lowest diameter increment was that of the tree I (1.11 mm). One year-old seedlings in the first study year had the mean value of root collar diameter of 7.59. The lowest average root collar diameter, at the individual level, was attained by tree II (6.51 mm). The maximal average value of root collar diameter was that of the tree II (6.72 mm). Diameter increment of one-year old seedlings between the last two measurements was 3.75. At the individual level, the maximal diameter increment was that of the trees I (4.95 mm); the lowest diameter increment was attained by tree II. In the second study

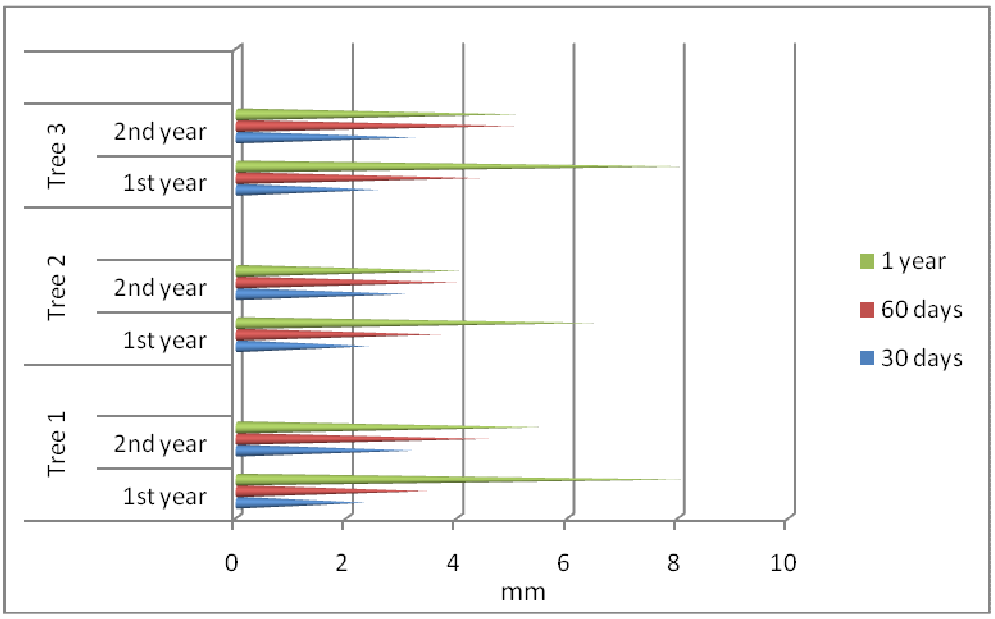

Figure 3. Thickness in root collar of 30-day, 60-day and one-year old ginkgo seedlings.

year, average root collar diameter of 30-day old seedlings was 3.18 mm. At the individual level, the maximal average value was attained by tree III (3.25 mm). The minimal average value of root collar diameter was reached by tree II (3.09 mm). 60-day old seedlings attained the mean root collar diameter values of 4.52 mm. The lowest average root collar diameter, at the individual level, was that of the tree II (3.98 mm). The maximal average value of root collar diameter was that of tree III (5.02 mm). Diameter increment in the second measurement term in the second year was 1.34 mm. At the individual level, the maximal diameter increment was reached by tree III (1.77 mm). The lowest diameter increment was reached by tree II (0.89 mm). One-year old seedlings in the second year had the mean value of root collar diameter of 4.79 mm. The lowest average root collar diameter, at the individual level, was reached by tree II (4.07 mm). The maximal root collar diameter was reached by tree I (5.50 mm). Diameter increment of one-year old seedlings at the end of the growing season in the second study year from the age of 60 days to the last measurement amounted to 0.29 mm. At the individual level, the maximal diameter increment was reached by tree I (0.94 mm). The lowest diameter increment was measured for tree III (1.63 mm). The analysis of root collar diameter of one-year old seedlings in the two study years shows that attained root collar diameters were larger in the first year. The maximal root collar diameter in the first year was reached by the old tree II. In the second year, the maximal root collar diameter was reached by the seedlings of the younger tree I. The analysis of the growth of one-year old seedlings in the nursery, in both study years shows high variability in diameter increment. Taking into account that height and diameter growth is genetically determined, it can be concluded that the variability in the genetic predispositions of the analyzed trees was pronounced. This is also confirmed by the results of the analysis of variance of root collar diameters of 30-day, 60-day and one-year old seedlings in both years (Table 1). Just as in the case of the analysis of variance of seedling height, these analyses confirm the high levels of significance of the differences between the trees, whereas between replications, there are no significant differences. The statistical differences in the root collar diameters of one-year old seedlings, in both study years, between the old and the younger trees indicate that the old age of parent trees did not affect their root collar diameters. The results of the analysis of variance for root collar diameters of one-year old seedlings, confirm the hypothesis that the differences in root collar diameter are, first of all, genetically conditioned.

Based on the above observations, it can be concluded that there is an expression of genotypic variability. Figures 2 and 3 show clearly the variability in the growth dynamics during the two study years of the progeny studied.

The analysis of the phenotypic characteristics of individuals within the same half-sib line as well as between the families shows the variability of leaf colour and dimensions, branching form and type. Seedlings with differently pigmented leaves were present in small numbers. The highest number of seedlings belongs to the type *atroviridis,* that is, its leaves are dark green. Also, the type *viridis* with light green leaves occurs in rather great numbers, whereas the seedling types *albovariegata*

and *aureovariegata* with variegated leaves are present in four individuals each. Based on the type of branching, there are two phenogroups: With monopodial growth, and with forked growth. In the progeny of the selected test trees in the first year, there are clearly differentiated individuals with early beginning of branching, which points to the fact that this trait is under strong genetic control. In the breeding programme for dwarf phenotype, the initial material is plants with poor growth. Dwarf plants can have horizontal - creeping stems based on which the forms *prostrata, repens, horizontalis* and *procumbens* are selected. The habit type *prostrata* is recorded in three seedlings of different half-sib lines. Taking into account that the individuals with special phenotypic traits occur rarely, the expressed variability in leaf colour and form, growth and branching habit is exceptionally significant. Genotypes with special phenotypic traits should be fixed by selection, and the selected genotypes should be vegetatively propagated.

Conclusion

Comparative analysis of seedlings from half-sib families enables the identification of the planting stock which is significant for further propagation. The seedlings were grown in the areas which are more or less ecologically homogeneous, so the factor of environmental impact can be eliminated and the resulting variability can be considered as the result of the differences in genotypes. The analyses included the quantitative characters controlled by polymer genes, that is, numerous multiple genes whose effects are added. As father trees are unknown because the reproduction material originated from free pollination, the good genetic constitution of test trees for mothers can be accepted because the results of the analyzed characters were repeated in their half-sib progeny during two successive vegetations.

The comparative analysis of seedling growth elements in the first and the second study years shows that lower values were attained in the second year. The maximal average values of growth elements in both study years were assigned to the old trees III and II. Based on the presented observations, it can be concluded that individual variability was expressed, which based on the confirmed statistical differences between the old trees and the younger trees confirms the hypothesis that the differences are of a genetic nature, that is, the old age of mother trees did not affect the growth elements.

The incidence of different phenotypic characters is very valuable not only for landscape architecture and horticulture, but also for other biotechnical disciplines. Bearing in mind that the individuals of special phenotypic characters are rare, the expressed variability in colour and leaf form, as well as in growth habit and branching method, is extremely significant. The results of the study of ginkgo half-sib progeny can serve as the base for the selection of mother trees which could be the sources of reproductive material for the production of seedlings with target characteristics.

The results obtained from the experiments confirm that it is necessary, prior to mass production, to previously identify parent trees with the superior combination capacity, that is, the trees with the best quality and the most productive progeny.

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