Genetic Variability of Silver Birch (*Betula pendula* L.) Wood Hardness in Progeny Testing at Juvenile Age

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Abstract

17 out of 24 Lithuanian and 2 Swedish silver birch (*Betula pendula* L.) populations were studied for wood hardness by using 6 J Pilodyn measurements. Totally 100 Lithuanian and 14 Swedish half-sib families at age 7 are being tested in the plantation, but only 83 local families were included in this study. 3-7 families represented each population. Family effect appeared to be the largest for wood hardness and its variance component was 18.4±7.6% (P=0.1%). Family x diameter class interaction component was 16.0±7.7% (P=1%). Population effect, tested against the family effect as an error term, was not significant and its variance component did not exceed 1%. Additive genetic coefficients of variation for this trait ranged from 0 to 12.7% and only 4 of analysed 17 populations had estimates exceeding 10%. Genetic correlations with bud burst and growth cessation were negligible positive to weak negative. Though weak, but significant estimate indicates better wood trait quality in birch families with longer vegetation period. This type of estimates of wood hardness with height and diameter were 0.23±0.06 and 0.71±0.08, respectively.

Correlation of wood hardness with individual tree selection indexes was 0.15. It can be concluded that even performing birch breeding in the way when wood properties are not examined and used, wood quality in coming breeding cycles remains only slightly worse. Also including wood properties in selection process could lead to significant improvement of that trait.

Key words: *Betula pendula*, populations, open pollinated families, progeny testing, wood hardness, genetic diversity, breeding

Introduction

Birch is the third most spread forest tree species in Lithuania. Birch stands comprise 20 % of the area occupied by forest. Though State Forest Survey does not recognise separate birch species, there are 4 species naturally occurring in Lithuania: *B. pendula*, *B. pubescens*, *B. humilis*, *B. nana*. Silver birch (*B. pendula* L.) among them is the most common and perspective for tree breeding. Up to now there are only few silver birch field tests in Lithuania, where Lithuanian populations are represented only with open pollinated progeny from plus trees as it is the first breeding cycle for this species. The first evaluation of progeny growth, stem quality and survival was performed at age 6 in 2003. Wood properties were not taken into consideration at that time. But one of the main breeding objectives is to improve wood quality, so we made an attempt to evaluate progeny for wood hardness using Pilodyn 6J Forester.

A Pilodyn wood tester originally designed for assessing soft rot in wooden poles. Nowadays this non-destructive and cost-effective equipment is widely used by forest researchers for wood hardness measurement in standing trees, especially in field trials. Pilodyn 6J Forester allows rapid data generation in a large number of trees and obtained data are used as an indirect measure of wood density. Numerous studies of forest tree species (gymnosperms as well as angiosperms) have shown that the depth of pin penetration is negatively correlated with wood basic density (*e.g.* Cown 1981, Moura *et al.* 1987). Full or half sib families can be evaluated precisely enough by using Pilodyn, though the accuracy is less for individual trees. The disadvantages of using Pilodyn also are: poor representation of the stem; impossible or difficult application for small trees; measures only outer rings; inaccuracy of records due to the presence of the reaction of wood or tension wood; seasonal variability in wood characteristics (when the data of several years are analysed). On the other hand, the advantages are: the method is suitable for application on large numbers of samples; it is quick cost-efficient and non-destructive (especially at younger age, when bark does not require its removal); accuracy of readings can be regulated by taking multiple readings; good agreement with the more precise measurements of wood properties.

Optimum selection age for wood density can be very young according to the results presented by Gwaze *et al*. (2002). Proper age for measurement of the genetic variability of wood quality traits can vary from species to species. It was shown that for Norway spruce it would be reasonable to wait until trees will reach at least an age of between 5 and 7 years (Rozenberg and Cahalan 1997).

Accuracy of wood basic density prediction by Pilodyn data can be even better than using near-infrared spectroscopic method (Schimleck *et al.* 1999). It also can be more reliable than stress wave technique (Chui *et al.* 1997). Some other non-destructive equipment such as Resistograph can well be used for rapid assessment of relative wood density of trees in progeny trials (Isik and Li 2003). Large scale measurements using Pilodyn were done mainly on forest tree species of few genera during the last two decades. These genera are *Pinus*, *Picea* and *Eucalyptus*.

Medium to strong heritability is characteristic of many wood properties in forest trees (*e.g.* Einspahr *et al.* 1967, Nepveu *et al.* 1981, Malan 1988, Gea *et al.* 1998). It is known that wood hardness and density are strongly related traits. Wood density is usually considered as the most important wood property. Fibber and vessels characteristics in birch are closely related to wood density, which is in close relation with pin penetration depth. Such relation is well described by Cown (1978).

Wood density is influenced by provenance and also by site, as it was shown in *Eucalyptus globulus* study (Miranda *et al.* 2001). Similar results should be considered in tree breeding implementation programs. Neighbouring trees of different clones growing in the natural stand of *Populus tremuloides* may be significantly different by wood density (Yanchuk *et al.* 1984). Clonal effect on wood density was larger than growth traits effects in hybrid poplar (*Populus* spp.) trial at age 3 (Zhang *et al.* 2003).

The objectives of this study were to estimate genotypic variation in wood hardness, growth and growth rhythm traits of *Betula pendula* half-sib progenies, and to evaluate the possibilities of inclusion of new economically important trait into the breeding program.

Materials and methods

A total of 100 open-pollinated silver birch families from 24 Lithuanian populations (Figure 1) and 14 sib families from 2 Swedish populations were studied in a field trial at age 7 (only total height was measured at age 6). Each Lithuanian population was represented by 3-7 families originated from plus trees (the least distance between selected trees was not less 30 m) and each Swedish population by 14 families from controlled crosses of tested plus trees. The field trial

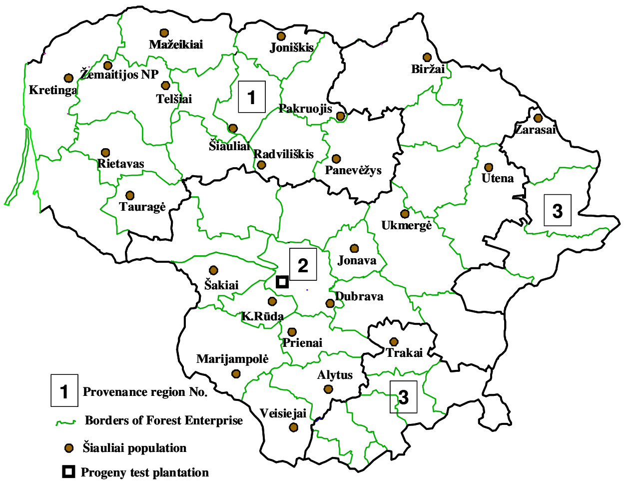

Figure 1. The origin of Lithuanian populations studied

is situated on the site surrounded by forest in Ezerelis forest district. Previously there was wet forest grassland, and the experiment was established a few years after melioration measures were applied. Soil conditions are not optimal, but suitable for birch: permanently overmoistured decomposed peat. The geographic position of the trial is 54°54´27´´ N, 23°36´57´´ E, elevation 76 m. The progeny trial has randomised complete-block design with 5 replicates (data were collected only in 3 blocks due to stem size) and 10-12 tree lines plots per family at spacing of 1 x 1.5 m.

Traits assessed were: bud flushing (classes 1-5), leaf autumn colouring (1-5), total height (cm), stem diameter (mm), stem straightness (classes 1-5), branch thickness (classes 1-5), branch angle (classes 1-5), branch number (classes 1-5), wood hardness or penetration (mm). The higher the figure used in assessment of bud flushing (or growth initiation) and leaf autumn colouring (or growth cessation) the more advanced stage of the trait progress. The higher class in evaluation of the traits that are assessed in classes means better quality. Bud flushing and leaf autumn colouring in combination also will be referred as growth rhythm.

The data were filtered by excluding all single tree records if total height of tree value was less than the value of standard deviation from family mean multiplied by 1.75. Families having less than 3 individuals and also populations represented by less than three families were excluded from the analysis, so 17 out of 24 populations and 83 out of 100 families have been analysed. Data transformation was not applied as deviations from distribution normality were negligible and typical transformations used could not improve it much.

Wood hardness was measured using 'Pilodyn 6J Forest' by taking measure of a striker pin penetration depth. The two measurements on each tree were taken from southeast direction at height 0.6 m. Right angle was kept between the points of penetrations on birch stem. If one of two measurements differed from each other more than 3 mm, the third measurement was taken. The average of two or three measurements was used in the statistical analysis. Pin penetrations were applied only on trees with undamaged stems and on trees, the diameter of which exceeded or was equal to 4 centimetres at 0.6 m height above the ground. Growth rhythm traits were recorded in due time, which means the stage when overall trial value did not deviate much from the midpoint of the scale used for the trait.

Swedish families and also families represented by less than three records of one trait were discarded from the analysis of variance. All statistical analysis, except genetic correlation, was done using SAS Software Release 8e (SAS 1999). No transformations were applied in order to get improved distribution normality of residuals. For analysis of variance PROC MIXED (mixed model equations) and the REML (restricted maximum likelihood) option was used.

Variance components were calculated for family and interaction of family with diameter class:

$$y_{ijlm} = \mu + p_i + b_j + f_{l(i)} + e_{ijlm} \qquad (1)$$

where y_{ijlm} is the value of a single observation, μ is the grand mean, p_i refers to the random effect of population i, b_j is the fixed effect of block j, $f_{l(i)}$ is the random effect of family l within population i, e_{ijlm} is the random error term.

Variance components for pin penetration were also calculated for family and interaction of family with diameter class:

$$y_{ijlmn} = \mu + p_i + b_j + f_{l(i)} + fd_{ln} + e_{ijlmn} \qquad (2)$$

where fd_{ln} refers to the random effect of interaction between family l and diameter class.

Population effect was tested against the family effect as an error term. Diameter records were classified to 7 classes. The first class included diameters up to 40 mm, the second 40-50 mm, and so on.

The model used for calculation of variance components in separate population:

$$y_{kl} = \mu_o + b_k + f_l + \varepsilon_{kl} \qquad (3)$$

where μ_o – the grand mean, b_k – effect of block as fixed, k=1,..., 5, f_l – random effect of family, l=1,...,7 (the numbers of open pollinated families/population varied), ε_{kl} – the error term.

Formula for calculation of genetic correlations:

$$r_A = \frac{\sigma_{A_1A_2}}{\sqrt{\sigma^2_{A_1} \times \sigma^2_{A_2}}} \qquad (4)$$

$\sigma_{A_1A_2}$ is the covariance between trait 1 and 2, $\sigma^2_{A_1}$ and $\sigma^2_{A_2}$ are the additive genetic variance of traits 1 and 2, respectively.

The formula used for the narrow-sense heritability was:

$$h_a^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_e^2} \qquad (5)$$

Additive genetic coefficients of variation (in percents) for population were calculated as follows:

$$CV_A = \frac{\sqrt{4 \cdot \sigma_f^2}}{\overline{X}} \cdot 100 \qquad (6)$$

where σ_f^2 – family variance component of population, σ_e^2 – the error variance component, $\overline{X}$ – trait mean value.

Programs “Dfprep”, “Dxmux” and method AI-REML of DFREML Software 3.0 a (MEYER 1997) were used for calculations of genetic correlation coefficients.

Breeding values for individual trees were calculated by summation of standardised (mean=0, standard deviation=1) tree breeding values for height, diameter, stem straightness and branch quality (the average of three traits: branch thickness, angle and number). The only height was given an economic weight of 1.5.

Results

Model (2) presented in M & M was used for the calculation of pin penetration and diameter interaction. Family variance component was 20.0±8.0 % (p=0.006) and only slightly smaller compared to the estimate obtained using model (1) (see Table 1). The interaction variance component was 13.2±7.4 % (p=0.038). Branch quality trait was made from the three other traits: branch thickness, branch angle and branch number. That artificially made trait gave family variance component estimate 12.4±6.2 %, and this value is close to the average of all three estimates of composing traits. So, the use of one trait instead of three for calculation of breeding value is quite rational.

Block effect was significant for all traits studied. There is some gradient in water surplus in the field trial with the patches where only a few trees have survived. This and also selective recordings of the trees resulted in block significance. Less dependent on soil conditions traits, as bud flushing and stem straightness had smaller block effect.

Additive genetic coefficients of variation were the largest for bud flushing and height (Table 1), the extreme value in some populations reaching 59.6 % and 44.0 % respectively. Quite high value of CV_A was obtained for stem straightness. Additive genetic coef-

Table 1. Variance components for random effect of family as percent of the total random variation, significance of fixed effects, and mean value of CV_A. Level of significance is denoted by *: 0.05>P>0.01, **: 0.01> P>0.001, ***: P<0.001

Trait	Variance component of random effect, %	Significance of fixed effect		CV_A, %
	σ_f^2	*population*	*block*	
Pin penetration[1]	21.3±6.3**	.	**	4.6
Bud flushing	15.1±6.2**	.	*	30.7
Autumn leaf colouring	20.0±7.5**	**	**	13.0
Height	24.4±7.8***	.	*	20.3
Diameter	19.9±7.5**	.	***	9.3
Stem straightness	8.5±5.0*	.	*	15.1
Branch number	8.1±5.3	.	***	9.8
Branch angle	11.5±5.7*	.	**	11.6
Branch thickness	11.5±5.5*	.	***	10.4

[1] – tree diameter was used as a covariate in this case

ficients of variation for pin penetration ranged from 0 to 12.7% and only 4 of analysed 17 populations had estimates exceeding 10 %. It is notable that population CV_A values for the traits studied were mostly not correlated in between, except pin penetration and height (r=-0.51, p=0.035, see Figure 2), height and diameter (r=0.48, p=0.053), branch angle and bud flushing (r=-0.53, p=0.030). Medium positive correlation was obtained for pin penetration and bud flushing, though not significant.

The mean wood hardness of the Swedish population is higher than the average of Lithuanian populations studied. Some Lithuanian populations are equally hard, but the majority are less (Figure 2). Standard deviation in the Swedish population is less than in most Lithuanian populations, though these values are not directly comparable, because the Swedish population consists of full sib families. It is evident from Figure 2 that families with higher wood properties are not necessarily inferior in height growth.

Genetic correlations of pin penetration with bud burst and growth cessation were 0.07±0.05 and -0.15±0.06, respectively. This type of estimates of pin penetration with height and diameter were 0.23±0.06 and 0.71±0.08. Spearman correlation at an individual level was medium positive and highly significant only with height and diameter, and weak negative, but significant, with branch thickness and autumn leaf colouring.

Spearman rank correlation of pin penetration with individual tree selection indexes was 0.15. The correlation between family means for pin penetration and family selection indexes was 0.25 (p=0.013).

Discussion and conclusions

Significance of population effect for the majority of analysed traits would be expected as *Betula pendula* is a pioneer species as regards successional status of a species. Variance component estimates of pin penetration for population could be significant in case all trees at the trial were recorded. Usually experimental results are much dependent on the genetic entries studied as was pointed out and discussed in the paper by Persson and Persson (1996) and demonstrated in Scots pine half-sib progeny study by Haapanen *et al.* (1997).

Inclusion of the diameter as a covariate for calculation of block effect significance for wood hardness

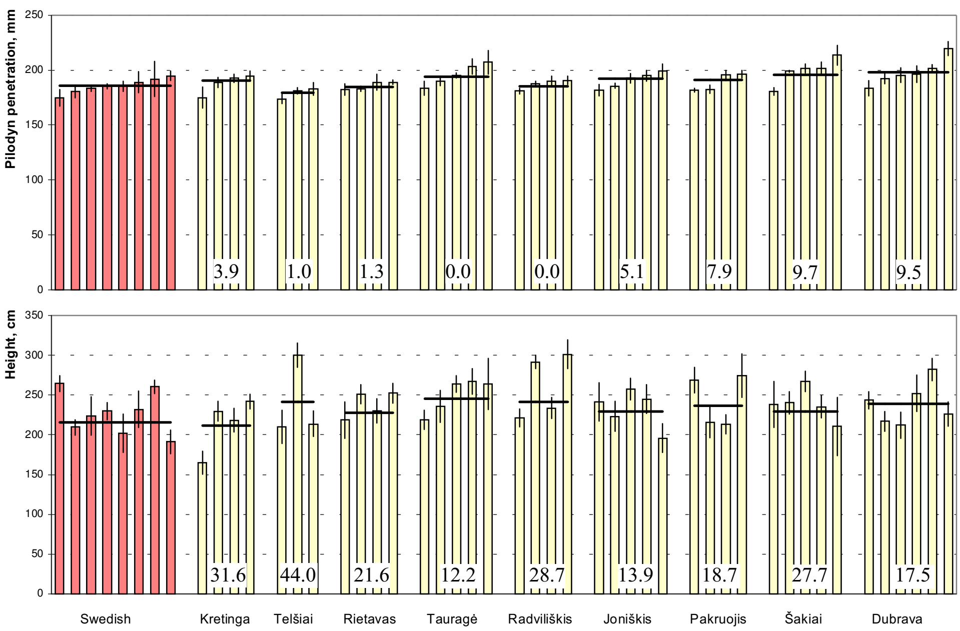

Figure 2. Variation in wood hardness at age 7 and height at age 8 among open pollinated families of nine Lithuanian and one Swedish *Betula pendula* L. populations (names given at the bottom) at the progeny field trial. Families are ordered by increasing value of family mean (vertical lines show mean standard errors) in penetration depth by populations (horizontal solid line indicates population mean). The order of families in the upper part of Figure corresponds to the order in the lower part. CV_A estimates are presented in the background of each population.

helped to increase the precision of the estimates, because the diameter is correlated with wood density and quite sensitive to spacing at that age. Though, the effect of tree competition on wood genotypic parameters was weaker than for growth parameters in the study of Scots pine full-sib families (Hannrup *et al.* 1998). Age-age correlation coefficients are usually reported to be strong and highly significant (*e.g.* Greaves *et al.* 1997, Hannrup and Ekberg 1998), although actual correlations are species-specific. A combination of environmental factors influence wood density in trees more than its radial growth rate (Hillis and Brown 1984). As it was shown in *Populus deltoides* clone study, moisture-stress regime influences wood properties to a great extent, but genotypic differences within the treatment are not so pronounced (Farmer 1970). The decrease in wood density in response to water stress release was reported for *Eucalyptus nitens* (Wimmer *et al.* 2002).

CV_As of adaptive growth rhythm traits are not correlated and genetic parameters of both traits should be used for selection of valuable populations as candidates to gene resources conservation *in situ* and *ex situ*. Growth traits, in contrast to wood properties, usually show lower heritability, but larger CV_A (reviewed by Cornelius 1994). The estimate for wood hardness in our study (see Table 1) is comparable to that reported by Cornelius (1994). The ranking of the estimates of variance components (adequate to heritability) for traits to a great extent were similar to those reported for the other species (e.g. Haapanen *et al.* 1997), except that branch thickness in our study showed almost similar heritability as branch angle. Low specific combining ability for birch wood traits (Nepveu and Velling 1983) justifies the use of half-sib families, instead of full-sibs, as genetic entries for long-term breeding and possibly not only in the first cycle. Although, progenies from some combinations of birch plus tree crossings can be superior to open-pollinated progeny in growth and stem form (Koski and Rousi 2005).

Though weak, but significant genetic correlation of wood hardness with growth cessation indicates higher wood trait quality in birch families with a longer vegetation period. Silver birch can be attributed to the cold hardy species, so selection of genotypes with a comparatively longer vegetation period for breeding purposes would be possible. The confirmation that growth of *Betula pendula* and *B. pubescens* of northern origin is inferior in southern districts to that of local populations is repeated in several reports (see Eriksson and Jonsson 1986). Birch provenance experiment in Latvia has revealed that local populations are superior to Finnish and Polish ones (Pirags 1992). Our local half-sib families exhibit superiority to Swedish full-sib families in our experiments at age 8.

Zhang (1995) reported that, in general, hardwood (broadleaved species, including *Betula* sp., with diffuse-porous wood) properties were less affected by the growth rate than softwood (coniferous species) properties. Wood hardness is an important wood property and an object of tree breeding. Height and wood hardness traits were correlated negatively (it means that pin penetration depth and tree height are correlated positively), but there is some fluctuation in traits relationship by families (Figure 2), which means that families with good growth are not necessarily characterised by less wood hardness. Similar findings were made in black spruce half-sib families study (Zhang *et al*. 1996) and *Eucalyptus globulus* provenance study (Miranda et al. 2001). The conclusion that the relationship between wood density and growth rhythm is not generally present for families was drawn in the study of Norway spruce families (Skrøppa *et al*. 1999). Rozenberg *et al.* (2001) inferred that most of the observed variability in the wood density and growth relationship of Douglas-fir populations has a genetic origin. The results from several silver birch studies have indicated almost no relation of wood density with growth traits (Helinska-Raczkowska and Fabisiak 1995, Eriksson and Jonsson 1986). Stener and Hedenberg (2003) have found significant negative genetic correlation of wood density with stem diameter, but the use of diameter as a selection criterion did not affect wood density much. In our study we have found that multi-trait selection indexes (see M&M) for individual tree were only slightly negatively correlated with wood hardness. Negative relationship was more pronounced on family level, but still, if wood hardness were neglected in calculation of selection indexes, it would have slight impact on a decrease in wood density.

Selection with restrictions in one trait could be applied in silver birch breeding strategy without or with little influence on improvement success in one or another trait in long-term perspective. The situation when family rankings in wood hardness are not followed by the rankings in height (Figure 2) can be population or provenance specific. This can hardly be proved in our study, but in the study of provenances of Scots pine Dorn (1969) has found that the effect of the growth rate on wood properties in some provenances was different than in others.

The combination of population CV_A and heritability or variance component of the trait can provide the information on efficiency for using tandem selection or to select populations suitable for breeding in case of multiple population breeding strategy being used (Baliuckas *et al*. 2004). CV_A as a property of population is a good indicator of long-term potential of population in any trait of interest (Houle 1992). Larger estimates indicate higher potential for breeding. Heritability is an appropriate indicator for short-term breeding efficiency. In our experiment heritability for Pilodyn penetration was among the highest estimates (Table 1) and CV_A – the least. This means high genetic gain in tree breeding for this trait, while it is possible also to combine in an efficient way with tree breeding for other traits that exhibit potential in longer perspective (see results on genetic correlations).

Multiple population breeding strategy (Namkoong 1976) provides some advantage in case of uncertainty in estimating optimum selection index weights, because of the possibility to combine selection and intercrossing among subpopulations. Recurrent long-term breeding of the main forest tree species (based on Multiple Population Breeding System) combined with gene conservation is under implementation in Lithuania. Dynamic gene conservation can be the best way under rapid climate changes and uncertainty in the future use of forest resources (Eriksson *et al*. 1993).

In case of this study, multitrait index selection with restrictions on wood hardness (not lower than mean overall value) would be appropriate strategy for promotion of silver birch breeding in Lithuania. Koski and Rousi (2005) have analysed the advantages and disadvantages of all silver birch breeding work done in Finland. They came to the conclusion that conventional selection, crossing and testing remained the only large-scale method, despite that various modern techniques are available nowadays.

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ГЕНЕТИЧЕСКАЯ ИЗМЕНЧИВОСТЬ ВЕЛИЧИНЫ ТВЁРДОСТИ ДРЕВЕСИНЫ БЕРЁЗЫ ПОВИСЛОЙ (*BETULA PENDULA* L.) НА ОПЫТНЫХ НАСАЖДЕНИЯХ В РАННЕМ ВОЗРАСТЕ

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Резюме

Твёрдость древесины берёзы повислой (*Betula pendula* L.) была изучена в экспериментальных насаждениях полусибсового потомства 24-х Литовских и 2-х Шведский популяций. Измерения при помощи прибора 'Pilodyn 6J' были проведены в возрасте 7 лет у 100 семей с Литовских и 14 с Шведских популяций. Каждую популяцию представляло 3-7 семей. Эффект семьи в дисперсионном анализе оказался самым высоко достоверным и его компонент дисперсии получен 18.4±7.6 % (достоверность 0.1 %). Компонент дисперсии взаимодействия семьи и класса диаметра составил 16.0±7.7 % (достоверность 1%). Эффект популяции, вычисленный в соотношении к эффекту семьи, был недостоверным, а популяционный компонент дисперсии не превышал 1 %. Значения генетического коэффициента аддитивной дисперсии для твёрдости древесины у популяций достигали 12.7 %, но только у четырёх из семьнадцати анализированных популяций значения превышали 10 %. Коэффициенты генетической корреляции этого признака с распусканием и осенним окрасом листьев получены незначительные и отрицательные. Даже незначительная, но достоверная, корреляция с окрасом листьев или же с продолжительностью вегетационного периода указывает на более высокие качества древесины у дольше несбрасывающих листьев семей. Коэффициенты генетической корреляции с высотой и диаметром (0.23±0.06 и 0.71±0.08) указывают на связь между быстротой роста и показателями свойств древесины, особенно с ростом в диаметр. Тем не менее коэффициент ранговой корреляции Спирмена с индивидуальным индексом селекции был получен только 0.15. Это указывает на незначительное ухудшение свойств древесины при проведении селекционного отбора, не включая признак твёрдости древесины в подсчёт индекса селекции. Включение же этого признака помогло бы существенно улучшить качественные свойства древесины берёзы повислой.

Ключевые слова: *Betula pendula*, популяции, полусибсовые семьи, исследование потомства, твёрдость древесины, генетическая изменчивость, селекция.