

# Amount of Total Protein and fluctuating asymmetry of *Betula pendula* in Various Ecological Conditions

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Abstract—The study was made about parameters of fluctuating asymmetry of the birch leaf blade and amount of total soluble protein of the seed progeny of *Betula pendula* in areas of Central Black Earth Region in different environmental conditions. The environmental impact on trees of weeping birch and its seed progeny in areas with different ecological pressure was shown. The high level of fluctuating asymmetry was revealed in maternal samples growing in conditions of anthropogenic pressure. The seed progeny of *Betula pendula* has the increased amount of total protein in polluted areas as compared to the control group (maternal trees and its seed progeny collected in the ecologically clean territory).

Keywords—fluctuating asymmetry; total protein; seed progeny; maternal plant

# I. INTRODUCTION

In connection with the deterioration of the environmental situation, the effects of anthropogenic impact on living organisms, including at the morphophysiological and biochemical levels, are comprehensively studied [1-2]. The concept of fluctuating asymmetry, the quantitative expression of which is the coefficient of fluctuating asymmetry, is more often associated with developmental stability, which is studied under various conditions, especially unfavorable for the body, in plants [3-7].

Fluctuating asymmetry is understood to mean insignificant and random deviations from the strict bilateral symmetry of biological objects [8]. According to A. T. Chubinishvili [9], the absence of absolutely symmetrical organisms is a consequence of the imperfection of the mechanisms that control ontogenesis, manifested in their inability to withstand the negative effects of environmental factors. In this regard, the fluctuating asymmetry of organisms according to bilateral signs is a macroscopic event, which consists in the independent manifestation of signs of varying degrees of manifestation on either the left or right or on both sides of the body [10]. The indicated property allows the use of fluctuating asymmetry at the macroscopic level as a measure in assessing the stability of the development of an organism [11]. The level of morphogenetic deviations from the norm is minimal only under certain (optimal) environmental conditions and increases non-specifically with any stressful effects. In this regard, the assessment of the level of fluctuating asymmetry

makes it possible to diagnose deviations from the conditional norm at earlier stages of the pathological state of the tree, when, according to other criteria, it is still "healthy" [10–12]. Thus, developmental stability, assessed by the level of fluctuating asymmetry, is a sensitive indicator of the state of organisms [9–14].

Using the assessment of the level of fluctuating asymmetry is effective, since it is fairly simple and fast. This method determines developmental stability by phenotypic features of parent organisms. But it is possible to assess environmental tension in the collection area of the plant material according to the level of fluctuating asymmetry. This is the main area of use of the method. Morphometric indicators allow to suggest genotypic changes in parental organisms and make a prediction about disturbances in the offspring, but they cannot give an accurate quantitative and qualitative determination of characteristics. A more adequate assessment of disturbances in the offspring and a prognosis of further changes in the genotype (by phenotypic characteristics) are possible using the physiological-biochemical method. One of its sides is the assessment of the amount of total soluble protein.

The study of the changing amount and composition of soluble protein in seeds is important as it is related to their quality and can be used to find the best storage conditions, and to study the agronomic features of woody plants. The data about the changing content of total protein in the seeds of woody plants is scarce: *Acer negundo* L., *A. pseudoplatanus* L., *Fraxinus lanceolata* Borkh. [2]. Earlier research did not show a correlation between the parameters of fluctuating asymmetry of the birch leaf in maternal plants and the amount of total soluble protein in seed progeny.

The timeframes of a single study often do not allow for the mass selection of selection-valuable trees based on the anatomical and morphological features of several generations of the progeny. Experiments using herbaceous plants are faster. They allow you to see the results of research in several generations of offspring, to identify modified specimens, especially when using the cytogenetic method [15]. The cytogenetic method makes possible to adequately determine stability of the genetic material of the parent plant and its seed offspring, and, at the same time, to assess environmental tension in the collection area [16–17]. This property of the method is valuable because the environment affects the genotype not only of plants, but also of humans [18–19] and especially the phenotype [20–21].

For the breeding process, it is necessary to identify and select mother plants with certain characteristics, and then to carry out the check by offspring [22]. But in order to accelerate the selection of woody plants, which sometimes takes decades, it is possible to conduct research in shorter time periods.

Some studies relate morphometric and physiological parameters [23-26], including the lipid peroxidation rate and the fluctuating asymmetry of the leaf in Betula pendula [3, 27] or specific leaf area, asymmetry of the leaf surface, stomata resistance, and chlorophyll content in Quercus robur [28]. Few works on the study of the stability of show genetic material the possibility of using morphophysiological traits in breeding, although this direction is quite promising. To accelerate the breeding process of woody physiological parameters were used. Using the example of scots pine, a fundamentally new method has been developed for the early diagnosis of adaptive properties of plus trees by seed progeny, called the "Method of Functional Tests" [29-30]. However, morphometric and biochemical parameters in the complex were not investigated.

Purpose of the study is to determine the parameters of fluctuating asymmetry of the birch leaf blade and the amount of total protein of the seed progeny of *Betula pendula* in areas of Central Black Earth Region in different environmental conditions.

### II. EXPERIMENTAL

Weeping birch (Betula pendula Roth) is a prevalent woody plant known for its medicinal properties and classified among main forest-forming species in European Russia. This species is a valuable material for the timber, wood chemical, pulp and paper, and other industries. It also has a high decorative value. Due to the biologically active substances, weeping birch has a higher or lower phytoncidal activity in different periods of the growing season and is widely used in sustainable building [3-4, 27]. The phytoncidal properties are especially important for planting in urban areas affected by technogenic pollution. Therefore, to fulfil this function, it is highly important to select plants resistant to anthropogenic pressure. Weeping birch is being actively studied in the area of anatomy [27], morphology [3–5, 10–14], biochemistry [27] and cytogenetics [16–17]. As Betula pendula is a perennial plant, it may experience chronic impact from environmental mutagens, accumulate doses of mutagens, and be used in cytogenetic studies, being one of the most sensitive species for bioindication [3-6, 10-14].

Object of the studies were approximately 30–40-year old phenotypically normal plants (without visual damage from parasites) from six areas of Central Black Earth Region. Three of them were ecologically clean (control) and another had environmental pollution. The research was conducted in two districts of Voronezh, Belgorod and Zadonsk (Lipetsk region). The following pollutants affect living organisms in Voronezh: SO<sub>2</sub>, NO<sub>2</sub> and heavy metals of various concentrations depending on how polluted the area is. These areas were ranked (by the level of pollution) in earlier research [31]. But environmental pollution is within permissible values on the ecologically clean area [31]. Ecologically clean (control) and contaminated districts presented in noted territories.

Plant material collected from 30 (15 in the ecologically clean and 15 in polluted areas) trees of weeping birch (Betula pendula Roth). Seeds and leaves were used as plant material for the research. To analyze the fluctuating asymmetry, 50 leaves (from each tree) of approximately the same size were collected from the maximum number of available shortened shoots of the lower part of the crown, relatively evenly around the tree. We measured five parameters of each leaf (the width of the left and right leaf halves; the length of the veins of the second order, the second from the base of the leaf; the distance between the bases of the first and second veins of the second order, the distance between the ends of the same veins, the coner between the main vein and the second from the base leaf vein of the second order) and the calculation of fluctuating asymmetry according to the method of V.M. Zakharov described earlier [5, 10].

In order to obtain protein preparations, a weighed portion of one seed (10 seeds from every tree were studied) was ground with glass sand in 0.1 M tris-NS1 buffer at pH 7.5, and centrifuged for 10 min at 20,000 g, 4°C, in a CM50 ELMI centrifuge (Latvia). The total soluble protein content was measured in the supernatant following a standard method [32]. Bovine serum albumin (Sigma) was used as a standard.

The results were processed statistically using the Stadia 7.0 software package. The procedure of data grouping and processing were described by A.P. Kulaichev [33]. A comparison of samples based on the level of fluctuating asymmetry was conducted using Student's t-test. A comparison of the samples was performed using the Van der Varden rank X-test, based on the amount of total soluble protein, as the distribution of these indicators is nonparametric. To calculate the correlation dependence, Spearman's rank correlation coefficient ( $r_s$ ) was used.

## III. RESULTS AND DISCUSSION

Quantitative data on the level of fluctuating asymmetry and the amount of total soluble protein are presented as average values in the table 1.

TP, MG/ML	CFA	TP, MG/ML	CFA
LIPETSK REGION (CONTROL)		LIPETSK REGION (EXPERIENCE)	
0.17±0.02	$0.040 \pm 0.004$	0.37±0.02в	$0.059\pm0.005^{\circ}$
Belgorod (control)		<b>Belgorod</b> (experience)	
0.18±0.02	0.028±0.003	0.29±0.03 <sup>^</sup>	$0.061 \pm 0.004$ <sup>c</sup>
VORONEZH (CONTROL)		VORONEZH (EXPERIENCE)	
0.31±0.03	0.020±0.005	0.73±0.04 <sup>c</sup>	$0.060\pm0.005^{\circ}$

 
 TABLE I.
 The Amount of Total Protein in the Seeds and the Level Of Fluctuating Asymmetry in Betula Pendula in Various Environmental Conditions

<sup>a.</sup> Note: TP, mg/ml – Total protein in seeds, mg/ml; CFA – coefficient of fluctuating asymmetry; <sup>a</sup> – differences with the control are reliable (P<0.05); <sup>b</sup> – differences with the control are reliable (P<0.01); <sup>c</sup> – differences with the control are reliable (P<0.001)</p>

In the control districts of Voronezh and Belgorod, a low level of fluctuating asymmetry is noted, corresponding to a conditionally normal state or I point of an assessment of the quality of the environment according to the scale of V.M. Zakharov [5, 10]. A higher level of fluctuating asymmetry was seen in the control of the Lipetsk region. Initial (insignificant) deviations from the norm or II point of environmental quality assessment on the scale of V.M. Zakharov [5, 10] are revealed. It is take place due to the emission of harmful substances from the Novolipetsk Metallurgical Combinat, which makes the main contribution to the environmental pollution of the city of Lipetsk, the region and the Central Black Sea Region, in general [34]. An increase in the level of fluctuating asymmetry indicates a deterioration of the environmental situation in the experimental territories, which, according to the scale of V.M. Zakharov [5, 10] corresponds to a critical state or V point of environmental quality assessment.

There was an increase in the coefficient of fluctuating asymmetry and, correspondingly, a decrease in the stability of development in plants [3–6, 10–14] and animals [8–9, 12] in unfavorable conditions for the body. It was established that fluctuations in weather conditions had a significant effect on the value of fluctuating asymmetry in *Betula pendula* trees of the control plot, as well as at a moderate level of motor pollution. At a high level of technogenic pressure, the anthropogenic factor makes the main contribution to the formation of fluctuating asymmetries [4, 27].

However, the amount of total soluble protein in the seeds of *Betula pendula* in the control region of Voronezh was the highest compared to others (control) (differences are reliable, P<0.05), which may indicate strong offspring and good quality of seeds.

The results of our study show an increase in the level of fluctuating asymmetry of the leaf blade and the amount of total soluble protein in *Betula pendula* seeds in environmentally polluted areas compared to clean ones. The largest amount of protein was found among the seed progeny from Voronezh (Table I). Birch trees grow in the conditions of low anthropogenic pressure [31]. Particularly increased the amount of total protein in seed offspring from Voronezh and the Lipetsk region (more than 2 times), which indicates the influence of environmental pollutants, causing a stimulating effect.

Analysis of the amount of total protein in the seeds showed a connection with the morphometric parameter "level of fluctuating asymmetry" in maternal birch plants. We found high positive correlations between the parameters "level of fluctuating asymmetry" and "amount of total soluble protein" for the studied regions  $r_s = 0.9$  (P <0.05). An increase in the amount of total soluble protein, indicating an increase in metabolic activity, is possibly an adaptive mechanism under conditions of anthropogenic pressure. The results of our research are consistent with the literature. Studying physiological and biochemical parameters, in particular, the bioelectric rest potential (BEP) of scots pine, it was shown that mild stress causes a stimulating effect [30].

### IV. CONCLUSION

Thus, according to the level of fluctuating asymmetry, it is possible not only a rapid assessment of the ecological situation at the tree habitats and the quality of plantings, but also selection of maternal samples. Subsequent verification of the offspring can be carried out according to cytogenetic and physiological-biochemical characteristics. The combined study of morphometric and biochemical parameters of plants can be used for the selection of source material.

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