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Assessment of the Phytin Content in the Fruit of Five Species of Juglans when Introduced in Central Russian Upland

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ASSESSMENT OF THE PHYTIN CONTENT IN THE FRUIT OF FIVE SPECIES OF JUGLANS WHEN INTRODUCED IN CENTRAL RUSSIAN UPLAND

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Abstract. The phytin (myo-inositol-1,2,3,4,5,6-hexacystichydrophosphoric acid) content was studied in the endosperm of five species of *Juglans* grown in the Central Russian Upland conditions: 1) Persian walnut (*Juglans regia* L.); 2) Manchurian walnut (*Juglans mandshurica* Maxim); 3) heartnut (*Juglans cordiformis* Maxim); 4) grey walnut (*Juglans cinerea* L.); 5) black walnut (*Juglans nigra* L). The walnut is shown to have the highest phytin content (31.90 ± 0.67 mg/g wet weight) and a low coefficient of variation ($Cv=4.23\%$). Based on the research, we can conclude that walnut is well adapted to the conditions of the Central Russian Upland and is recommended for the creation of fruit plantations by seed.

1. Introduction

Juglans nuts have been successfully introduced and cultivated in the Central Russian Highland. They have high economic value and stable fertility in the new environment [1, 2]. Therefore they are subject to comprehensive research. Much attention is paid to the study of the quality of fruits, both from the point of view of their nutritional value, and from the point of view of the content of reserve nutrients necessary for seed germination. One of these chemical compounds is phytin (myo-inositol-1,2,3,4,5,6-hexakisdihydrophosphoric acid), which is the main intracellular source of phosphorus: up to 60-90% of total phosphorus is present in the cell as a mixture of calcium and magnesium salts of inositol phosphoric acids. Structural formula of phytin was shown in figure 1 [3].

The germination energy directly depends on the amount of phytin content in the seeds. Hydrolysis of phytin and dephosphorylation under the action of the enzyme 6-phytase (EC 3.1.3.26) contribute to the intensive growth of the plant and the accumulation of biomass at the initial stages of plant ontogenesis [4, 5].

Since seed reproduction significantly reduces the economic costs of creating plantations of introduced plants, the aim of our study was to assess the quantitative content of phytin in the fruits of five types of nuts that have been growing for a long time in the conditions of the Central Russian Upland.



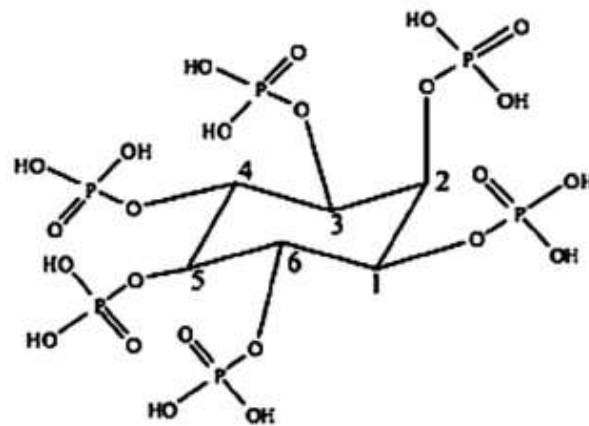


Figure 1. Structural formula of phytin

2. Materials and methods

The fruits of five species of nuts were growing in experimental plantings on the territory of the Central Black Earth region in the conditions of the Central Russian Upland (51° 42' north latitude; 39° 12' east longitude, height above sea level - 156 m): 1) Persian walnut (*Juglans regia* L.); 2) Manchurian walnut (*Juglans mandshurica* Maxim); 3) heartnut (*Juglans cordiformis* Maxim); 4) grey walnut (*Juglans cinerea* L.); 5) black walnut (*Juglans nigra* L.).

For the experiment, phenotypically normal, without damage by pests and fungal diseases, 30-35 years old nut trees were selected. The fruits of all the studied species of nuts are shown in figure 2 [1].

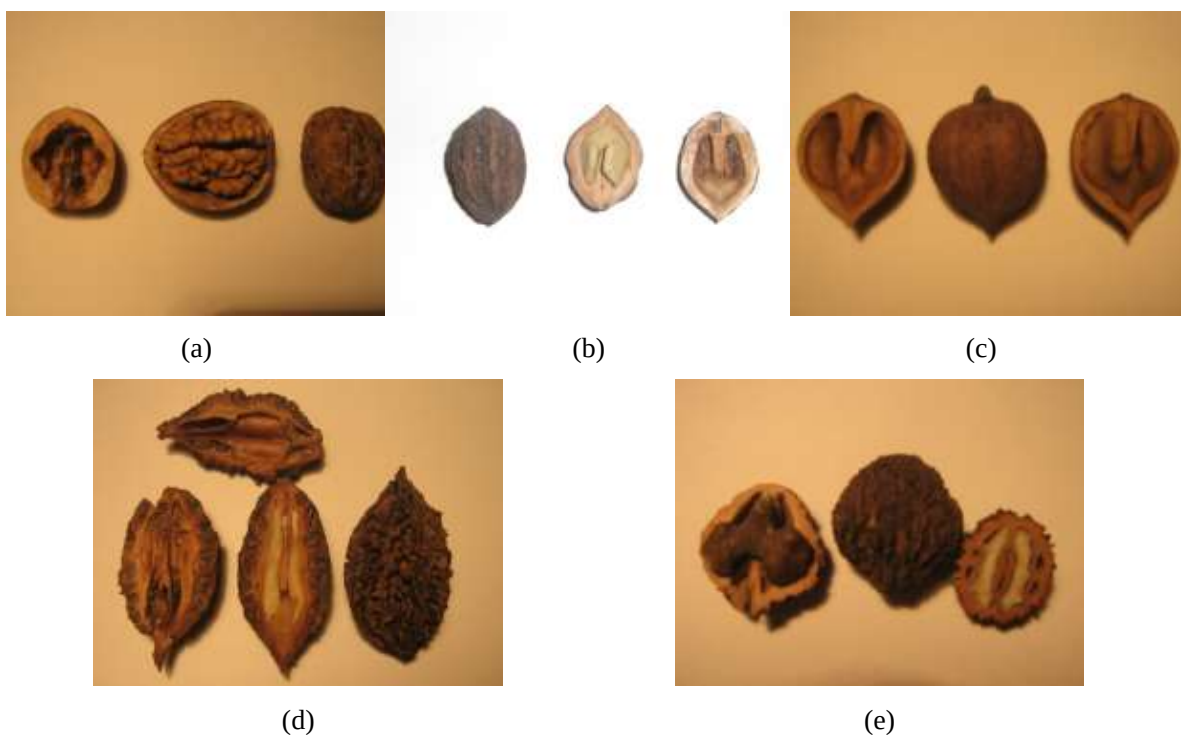


Figure 2. Fruits of *Juglans* species: (a) for *J. regia* L.; (b) for *J. mandshurica* L.; (c) for *J. cordiformis* L.; (d) for *J. cinerea* L.; (e) for *J. nigra* L.

The 2017 harvest nuts were studied. The harvested fruits were stored in the refrigerator at 0°C. Endosperm of the nuts were used to determine the phytin content. The method of removing protein and lipid fractions from plant tissue was similar to that used for DNA extraction. A quantity of the plant material (200-300 mg) was spread in 2 ml 0.5 mol/L of the hydrochloric acid solution HCl (solution 1) with broken glass. To the 1.5 ml of the resultant homogenate was added 0.5 ml of the solution of isoamidal alcohol with chloroform in a ratio of 1:24 in volumetric parts (solution 2) and then transferred to the eppendorfs. The mixture was shaken for 1 minute, then centrifuged for 3 min at 20,000 g on the microcentrifuge MM50 ELMI (Latvia). The samples from the pre-treatment were completely transparent, which improved the quality of the results [6].

1.5 ml of indicative acid solution of ammonium rodanide NHSCN (0.03%) was added to the 1 ml of the supernatant liquid in 6% HCl (solution 3), then 50 µL of the acidic competitive solution of iron chloride FeCl₃ (III) (solution 4). The solution of iron chloride was prepared from FeCl₃ salt, dried in the desiccator with concentrated sulphuric acid. The solution contained 0.86%±0.002% FeCl₃ and 0.2% HCl. As a control, we used a mixture of solutions No. 3 and No. 4 in the same proportions as for the experimental samples, replacing the volume of the experimental sample with an equal volume of water. Knowing the ratio of interacting reaction components (4 Fe:1 phytate), we can determine the concentration of phytic acid from the concentration of residual iron in solution [7]. Samples of phytin extracts were colorimed at 337 nm on the UNICO 2800 (USA) spectrophotometer in quartz ditches with an optical path length of 10 mm. The phytin content of the test samples was calculated according to a calibration schedule using as a standard the aqueous solution of the sodium salt of phytinic acid (Sigma-Aldrich, USA).

The measurements were carried out in four biological replications. The statistical processing of the data was carried out with the help of a package of «Stadia» applications. The data grouping and processing procedure is described in the scientific work [8]. The influence of the factor «species belonging» on the phytin content of the nuts endosperm was determined using single-factor dispersion analysis. The effect of the factor was calculated by Snedecor (%). The coefficient of variation (C_v) was calculated according to Lakin's recommendations [9]. The C_v was considered low when the value was no more than 10%, the average value was 10-25%, the high one was over 25%. Comparisons of the average phytin content of the nut endosperm were made according to the Student t-criterion. Variance comparisons were made using the Fisher F-criterion.

3. Results and discussion

The results of the phytin content study in the endosperm of the fruit of the nut species studied are presented in table 1.

Table 1. Phytin content in the endosperm fruit of five species of Juglans

Species of nuts	Phytin content (mg/g of wet weight)	Coefficient of variation (C _v , %)
<i>J. regia</i> L.	31.90±0.67 ^a	4.23 ^b
<i>J. nigra</i> L.	21.92±1.52	13.91
<i>J. mandshurica</i> Maxim.	20.49±1.43	13.98
<i>J. cordiformis</i> Maxim.	18.18±3.41	37.57
<i>J. cinerea</i> L.	12.20±2.55 ^a	41.35 ^c

^a differences with other species are significant (P<0.05)

^b differences in variances with *J. mandshurica* Maxim., *J. cordiformis* Maxim., *J. cinerea* L. are significant (P<0.05)

^c differences in variances with *J. regia* L., *J. nigra* L., *J. mandshurica* Maxim. are significant (P<0.05)

Using factor analysis techniques, the influence of species on the phytin content of the fruit of the studied nut species was shown to be statistically reliable (P<0.05) (Snedecor influence was 19.9%). The highest phytin content is found in the Persian walnut endosperm and the lowest in the grey nut. The differences with other nut types are significant (P<0.05). It can be assumed that the high phytin

content of the walnut endosperm will give the *J. regia* L. an advantage in the early stages of ontogenesis (seed germination) over other nut species.

Studies have shown that at low phytin content in the endosperm of the fruit, gray walnut and heartnut nuts exhibit significant variability on this basis (variation coefficients (C_v) exceed 35%). For Manchurian and black nuts, the phytin content and variability of this characteristic is at the average level. The walnut has the lowest value of the coefficient of variation, which makes it possible to speak of the high stability of the test feature in the offspring of the above-mentioned plant species and its good adaptation to the conditions of introduction. Thus, of all the nuts studied, Persian walnut can be recommended to establish fruit plantations by seed under conditions of introduction in the Central Russian Uplands.

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