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У журналі «Agricultural science and practice» публікуються результати фундаментальних і прикладних досліджень з питань грунтознавства, землеробства, рослинництва, ветеринарії, тваринництва, кормовиробництва, генетики, селекції та біотехнології, механізації, агроекології, радіології, меліорації, переробки та зберігання сільськогосподарської продукції, економіки, інноваційної діяльності.

Друкуються статті, які раніше не видавалися, огляди літератури, короткі повідомлення. На сторінках журналу провідні науковці висвітлюють актуальні проблеми сільського господарства.

Повідомлення публікуються англійською мовою; російською та українською – резюме. В електронній версії журналу (http://www.agrisp.com) розміщуються резюме (трьома мовами), список літератури англійською мовою.

Розрахований на науковців, викладачів, студентів.

Сфера розповсюдження – загальнодержавна і зарубіжна.

Періодичність журналу – 3 номери на рік.

Передплату приймають всі відділення зв’язку.

Індекс у Каталогу видань України – 86327

З питань передплати звертатися до поштових відділень.
SPATIAL HETEROGENEITY OF PHYSICAL PROPERTIES OF THE SOILS IN UKRAINE

V. V. Medvedev, I. V. Plysko

National Scientific Centre “Institute for Soil Science and Agrochemistry Research named after O. N. Sokolovsky”
4, Chaikovska Str., Kharkiv, Ukraine, 61024

E-mail: vvmedvedev@ukr.net

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Aim. To investigate structural composition (lumpiness), bulk density and penetration resistance in soils of Polissia, Forest-Steppe and Steppe.

Methods. The experimental plots were allocated on the fields in a regular network. The measurements performed before harvesting testified to an equilibrium condition of soil physical properties. The data were processed by the geostatistical method. Results. The key parameters of spatial heterogeneity (variation factors, histograms, autocorrelation function, variograms, 2-D and 3-D diagrams, etc.) were received. The heterogeneity of physical properties, revealed in all soils, is characterized by moderate and increased values. As a result the investigated fields were divided into three agrotechnological groups by qualitative parameters of their physical properties. Conclusions. The recommendations on pre-sowing or basic tillage of various intensity – without tillage (if parameters are close to the requirements of the sown culture), with moderate tillage of zone type (if parameters are close to modal values) and with tillage of the enhanced intensity (if parameters are unsatisfactory and more intensive pre-sowing tillage is required) were formulated for each group.

Keywords: soil spatial heterogeneity, physical properties, precise tillage.

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INTRODUCTION

Spatial heterogeneity of soil physical properties is not an intensively studied direction of science. There are singular publications on this topic. In particular, Shein et al. [1] discovered an especially wide range of bulk density fluctuations in the ploughed layer of gray podzolic soils – from 0.98 to 1.48 g/ccm and the density of a considerable part of the investigated allotment did not exceed the allowed limit for most crops, grown on loamy soils (1.30 g/ccm), therefore this part of the allotment did not require any pre-sowing tillage. The work of Tymbaev [2], performed on the same soil, demonstrated that the heterogeneity of bulk density was formed in the course of May–July; in addition, there was a considerable compaction (up to +0.35 g/ccm) in some parts of the allotment and loosening – in others. These processes were observed in the field of black fallow the least, and under the grain crop – the most. In the work of Bolenius et al. [3] soil penetration resistance varied at the depth of 10 cm (from 200 to 1,200 kPa) and 30 cm (from 700 to 2,700 kPa). The yield of barley on the experimental field fluctuated from 11.5 to 4.5 t/ha, respectively.

Regardless of evident shortage of materials about spatial heterogeneity of soil physical properties, it is obvious that the accumulation of such data is an urgent task, the solution to which will allow implementing considerable advantages of precise agriculture for the differentiation of soil tillage. Precise agriculture is still almost exclusively used only while administering fertilizers and chemical means of plant protection.

The aim of the work was to obtain the materials about spatial heterogeneity of soil physical properties, which could be used as a basis to formulate the suggestions on precise tillage of field soils.

MATERIALS AND METHODS

The studies were performed on six fields, three of which were located in Polissia (conventional names –
Romaniv, Kolky and Vediltsy), two – in the Forest-Steppe (Korotych and Kommunar) and one – in the Steppe (Donetsk).

Romaniv, Volyn region. Gray podzolic (Albeluvisols Umbric), sod-podzol (Albeluvisols Gleyic) and gleyed black soils (Umbrisols Gleyic) prevail in the soil cover. The terrain is flattened. The soil texture is light clay loam. The size of the field is 63 ha, the number of experimental plots is 35. Cereal and forage crops are cultivated. The agrotechnical methods of crop cultivation are not differentiated, regardless of evident mottling of the field.

Kolky, Volyn region. The soil cover is a complex of sod-podzol gleyed (Albeluvisols Gleyic), sod-gley (Gleysols Listic) and meadow-bog (Leptosols Umbric) soils. The terrain is flattened. The soil texture is argil sand. The size of the field is 11 ha, the number of experimental plots is 27. Forage crops are cultivated (on the non-boggy part). The field has been dewatered by an open network of partially non-functioning channels. The studies have been conducted only on the non-boggy part. The agrotechnical methods are not differentiated in the field.

Vediltsy, Chernihiv region. Sod-mesopodzol (Albeluvisols Gleyic) loamy soils. The terrain is flattened. The size of the field is 105 ha, some part of the field is grassy, the number of experimental plots on the non-grassy part is 117. Cereal and forage crops are cultivated.

Korotych, Kharkiv region. Dark gray heavy loamy podzolic soil prevails. The terrain is slightly sloping. The size of the field is 31 ha, the number of experimental plots is 35. Cereal and forage crops are cultivated by the method, traditional for the Forest-Steppe.

Kommunar, Kharkiv region. Typical low humus leached (Chernozems Chernic) heavy loamy chernozem. The terrain is flattened. The size of the field is 30 ha, the number of experimental plots is 26. Cereal and forage crops are cultivated.

Donetsk, Donetsk region. Ordinarily (Chernozems Chernic) heavy loamy chernozem. The terrain is flattened. The size of the field is 105 ha, the network of experimental plots (51) is established on the part of the field of 50 ha. Cereal and forage crops are cultivated.

Field experiments. The main research method, used in the work, was a geostatistical approach. A regular network of experimental plots, 5 × 5 m each, was established in the field. The plots were geopositioned for the agreed sampling and registration of the yield. The bulk density was measured in the ploughed and sub-surface layers (the method of rings, the ring volume is 100 ccm, 5 repeats), the penetration resistance – up to the depth of 35 cm (the Reviakin’s method, a plunger of a flat type, 10 repeats), soil humidity – at the depth, similar to that for the bulk density (the method of drying at 105 °C, 5 repeats), soil samples were selected to determine the structural composition. The time of field experiments is 2–2.5 months after the last treatment, i.e. the measurements characterized the balanced state of soil physical properties [4, 5]. The yield per 1 sq.m. was determined in field conditions in 5 repeats.

LABORATORY EXPERIMENTS AND DATA PROCESSING

The laboratory conditions were used to determine the structural composition, including the definition of the content of aggregates (Savvinov’s method, 4 repeats), and to perform statistical and geostatistical data processing. In particular, the variation coefficient and the range of fluctuations were found, the histogram was drawn (to determine the type of distribution and the degree of its deviation from the Gaussian – normal distribution), the variogramme was made (to estimate specific geostatistical parameters – the threshold of dispersion, correlation radius and nugget-effect), 2-D and 3-D-diagrams were built (to establish the contours with different parameters of physical properties and consequently to define their areas), the autocorrelation function and its spectral dispersion density was estimated (to assess the credibility of heterogeneity availability and its fluctuations). The standard Surfer program and the interpolational Kriging method were used in the estimation.

The ultimate aim of the mathematical processing was to discover the working sites and to formulate directions for precise pre-sowing and basic tillage of soil.

RESULTS AND DISCUSSION

Structural composition. The structural composition and especially the availability of separate aggregates therein is the most important indicator of the physical state of the sowing layer prior to the tillage and sowing. According to agricultural requirements [6], not more than 20% of aggregates are allowed after the tillage, and there should be none prior to sowing. It should be noted that in the experiment, described in [7], even 10% of aggregates in the sowing layer diminished the advantages of the structural composition of chernozem soil completely.
Table 1 demonstrates statistical and geostatistical characteristics of lumpiness in the investigated objects. In Polissia, only Vediltsy was used due to its better expressed structure. Noteworthy are extremely high variation coefficients. The variation coefficient for Kommunar object reached 0.70 at a very considerable range of fluctuations between the maximal and minimal values. The agronomic consequences of high variability of the lumpy fraction of the structure in the upper soil layer are extremely unfavorable, as even its insignificant presence in the soil leads to the deterioration of the sowing quality, the increased evaporation of available moisture and the decrease in the rate of emergence and fullness of seedlings. All these affect the development of plants until the harvest. In all the objects (Fig. 1–4) the content of aggregates above 20 % was observed on the area of 33 % (Vediltsy), 29 % (Korotych), 26 % (Kommunar) and 65 % (Donetsk). Thus, the removal of aggregates from the surface (sowing) layer is a very important task of tillage. Taking into consideration the fact that this work requires considerable energy consumption, it is cost-efficient to have precise differentiated tillage only on the needed part of the allotment.

Other specificities of spatial heterogeneity of the structural composition are the emergence of nugget-effect in some cases, the termination of variation at the dispersion threshold value of 30–117 and the correlation radius of 150–300 m (the parameter, defining the distance on the spherical variogramme, after which there is no dispersion increase). It means that structure heterogeneity is traced in different distances, and the sizes of allotment sites, similar in structure properties, should be sought in the same range. The sites with increased lumpiness are well observed on 2- and 3-D diagrams. The sizes of working sites for differentiated tillage are usually changed depending on the content of the lumpy fraction on different sites of the field. For instance, if the main autumn tillage is intended for sowing a spring crop in the following year, the differentiation may be related only to the sites with over 20 % of aggregates. If the pre-sowing tillage is intended, tillage differentiation is obligatory. It is clear on 2-D diagrams, which sites require enhanced and more qualitative division of the sowing layer.

Structure variability of agronomically valuable size is much lower. Therefore, its mottling should be considered less in terms of precise tillage. Moreover, the increase of the number of aggregates of this size in the structural composition is achieved not so much due to mechanical tillage, as due to long-term and systematic application of the elements of precise agricultural technology.

Finally, the share of dust is negligible and, according to our data, is not relevant for precise tillage planning. At the same time, the parameters presented may be used as monitoring criteria – tillage should be performed in a way that would not increase soil dispersion.

Below are the specificities of geostatistical characteristics of the structure in the investigated fields. The histograms of the distribution of indices for all the fractions are remarkable for weak left or right asymmetry, but the deviations from the classic Gaussian curve are still small. Autocorrelation function and its reliability are noted on practically

Table 1. Statistical and geostatistical indices of the lumpy fraction of the structural composition (%) on the investigated objects*

<table>
<thead>
<tr>
<th>Index</th>
<th>Vediltsy</th>
<th>Korotych</th>
<th>Kommunar</th>
<th>Donetsk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of fluctuations</td>
<td>37.5</td>
<td>46.4</td>
<td>47.1</td>
<td>34.7</td>
</tr>
<tr>
<td>Average value</td>
<td>16.6</td>
<td>18.3</td>
<td>16.9</td>
<td>22.6</td>
</tr>
<tr>
<td>Median</td>
<td>15.3</td>
<td>16.3</td>
<td>13.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Variation coefficient</td>
<td>0.64</td>
<td>0.57</td>
<td>0.70</td>
<td>0.43</td>
</tr>
<tr>
<td>Dispersion</td>
<td>114.3</td>
<td>108.6</td>
<td>142.3</td>
<td>95.4</td>
</tr>
<tr>
<td>Asymmetry coefficient</td>
<td>0.39</td>
<td>1.42</td>
<td>1.19</td>
<td>0.04</td>
</tr>
<tr>
<td>Nugget-effect</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>22.2</td>
</tr>
<tr>
<td>Dispersion threshold</td>
<td>117</td>
<td>30</td>
<td>–</td>
<td>35</td>
</tr>
<tr>
<td>Correlation radius, m</td>
<td>300</td>
<td>150</td>
<td>–</td>
<td>250</td>
</tr>
</tbody>
</table>

*The geostatistical indices (nugget, threshold and radius) were obtained on condition of variogramme approximation using a spherical model.
all the lags (steps). The variogram is more frequently approximated using the linear or spherical model, and the spectral density of dispersion usually has several peaks of fluctuations with a wide range of frequencies. In general, the spatial heterogeneity of the investigated land allotments is absolutely evident. There are also remarkable differences between the sites, which is evident while comparing 2-D and 3-D diagrams. Due to more complex topography, the allotment with dark gray soil (Korotych) has a wider network of isolines of structural fraction parameters. It requires more precise differentiation of agrotechnologies on this allotment compared to another allotment (Kommunar) with dominating typical chernozem.

**Bulk density.** The coefficients of bulk density variation in all the investigated fields may be considered not high and moderate, as their value is in the range of 5–11 % (Table 2). It should be noted that in the work [8] the soil was called heterogeneous,
if the variation coefficient for any property exceeded 25%. The moderation of variability is also confirmed with insignificant dispersion and relatively balanced 2-D and 3-D diagrams (Fig. 5, 6). At the same time, for instance, the allotment site in Kolky had the density of over 1.5 g/ccm, which is unfavorable for most crops, grown here, especially prior to sowing.

But the values up to and exceeding 1.6 g/ccm are even more unfavorable. It is impossible to work the soil using common tools with good quality, to sow
and to receive any emerging crops in such conditions. This is one of the main reasons of mottling, remarkable for Polissia. If such sites are permanent in time, they should be either excluded from tillage at all or worked on using more active (for instance, rotor) tools.

At the same time 10–25 % of the allotment area in Kolky and Vediltsy and over 50 % of other investigated fields have parameters of bulk density, similar to optimal ones (below 1.3 – in the Forest-Steppe and below 1.4 g/ccm – in Polissia). Therefore, tillage on these sites of allotments is not required prior to sowing more demanding crops.

As the median and the average value of indices almost coincide, and histograms are close to Gaussian...
ones, one may state the normality of this index distribution. At the same time there are some, at least insignificant, asymmetry coefficients. Their sign and value (especially evident in the upper part of the pre-sowing layer) are obvious evidence of their anthropogenic origin. The matter is the overthickening in the tillage pan, which turned out not to have any complete manifestation and is locally limited only to specific (rather more humid and low) spots (hatched site of allotment in Fig. 6).

The autocorrelation function tends to the reliable deviation from zero. It confirms the regular character of spatial variability of physical properties.
Based mainly on 2-D diagram of spatial information about bulk density, it is possible to recommend the tillage after the division of allotments into three parts – for usual tillage, minimal tillage and no tillage.

Penetration Resistance. The variation coefficient fluctuates in the range of 0.10–0.27 (Table 3) for the investigated objects which is considered to be mode-rate and increased estimate and almost always guarantees the presence of spatial heterogeneity. Actually the autocorrelation function is present, which is irrefutable evidence of the presence of a specific spatial structure of penetration resistance on all the fields and the substantiation of future plotting (division) of the field. In other words, working sites for differentiated administration of different tillage methods may be found on each investigated field. However, it pertains to theory, whereas in practice the working sites should have a specific size and configuration to ensure work cost-efficiency according to the principles of precise agriculture.

Other specificities of spatial heterogeneity of penetration resistance are also remarkable. The similarity between median and average values confirms the

### Table 2. Statistical and geostatistical indices of bulk density in the sowing layer (g/ccm) on the investigated objects

<table>
<thead>
<tr>
<th>Index</th>
<th>Romaniv</th>
<th>Kolky</th>
<th>Vediltsy</th>
<th>Korotych</th>
<th>Kommunar</th>
<th>Donetsk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of fluctuations</td>
<td>0.60</td>
<td>0.45</td>
<td>0.25</td>
<td>0.47</td>
<td>0.31</td>
<td>0.61</td>
</tr>
<tr>
<td>Average value</td>
<td>1.19</td>
<td>1.47</td>
<td>1.40</td>
<td>1.31</td>
<td>1.30</td>
<td>1.16</td>
</tr>
<tr>
<td>Median</td>
<td>1.17</td>
<td>1.47</td>
<td>1.39</td>
<td>1.31</td>
<td>1.28</td>
<td>1.17</td>
</tr>
<tr>
<td>Variation coefficient</td>
<td>0.11</td>
<td>0.06</td>
<td>0.05</td>
<td>0.08</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Dispersion</td>
<td>0.02</td>
<td>0.007</td>
<td>0.004</td>
<td>0.01</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>Asymmetry coefficient</td>
<td>−0.10</td>
<td>−0.046</td>
<td>0.22</td>
<td>0.43</td>
<td>0.61</td>
<td>−0.12</td>
</tr>
<tr>
<td>Nugget-effect</td>
<td>0</td>
<td>0</td>
<td>0.004</td>
<td>0</td>
<td>0.0004</td>
<td>0</td>
</tr>
<tr>
<td>Dispersion threshold</td>
<td>0.009</td>
<td>−</td>
<td>0.045</td>
<td>0.004</td>
<td>0.017</td>
<td>0.08</td>
</tr>
<tr>
<td>Correlation radius, m</td>
<td>250</td>
<td>−</td>
<td>450</td>
<td>300</td>
<td>120</td>
<td>350</td>
</tr>
</tbody>
</table>

### Table 3. Statistical and geostatistical indices of penetration resistance (kgf/sq.cm) of the ploughed layer and tillage pan in the soils of the investigated objects

<table>
<thead>
<tr>
<th>Object, layers</th>
<th>Range of fluctuations</th>
<th>Median</th>
<th>Average value</th>
<th>Standard deviation</th>
<th>Dispersion</th>
<th>Variation coefficient</th>
<th>Presence of reliable autocorrelation function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kolky:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ploughed</td>
<td>14</td>
<td>21</td>
<td>21</td>
<td>3.7</td>
<td>13.4</td>
<td>0.18</td>
<td>+</td>
</tr>
<tr>
<td>tillage pan</td>
<td>20</td>
<td>34</td>
<td>32</td>
<td>6.0</td>
<td>35.9</td>
<td>0.19</td>
<td>+</td>
</tr>
<tr>
<td>Romaniv:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ploughed</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>4.8</td>
<td>22.8</td>
<td>0.27</td>
<td>+</td>
</tr>
<tr>
<td>tillage pan</td>
<td>25</td>
<td>28</td>
<td>29</td>
<td>7.4</td>
<td>2.0</td>
<td>0.25</td>
<td>+</td>
</tr>
<tr>
<td>Vediltsy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ploughed</td>
<td>27</td>
<td>23</td>
<td>23</td>
<td>0.2</td>
<td>38.9</td>
<td>0.27</td>
<td>+</td>
</tr>
<tr>
<td>tillage pan</td>
<td>25</td>
<td>40</td>
<td>39</td>
<td>4.0</td>
<td>15.7</td>
<td>0.10</td>
<td>+</td>
</tr>
<tr>
<td>Korotych:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ploughed</td>
<td>7</td>
<td>10</td>
<td>11</td>
<td>2.1</td>
<td>4.5</td>
<td>0.19</td>
<td>+</td>
</tr>
<tr>
<td>tillage pan</td>
<td>23</td>
<td>24</td>
<td>24</td>
<td>4.5</td>
<td>20.2</td>
<td>0.18</td>
<td>+</td>
</tr>
<tr>
<td>Kommunar:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ploughed</td>
<td>13</td>
<td>22</td>
<td>21</td>
<td>3.3</td>
<td>10.9</td>
<td>0.16</td>
<td>+</td>
</tr>
<tr>
<td>tillage pan</td>
<td>20</td>
<td>39</td>
<td>37</td>
<td>3.1</td>
<td>26.4</td>
<td>0.14</td>
<td>+</td>
</tr>
</tbody>
</table>
mality of the distribution curve and generally insignificant impact of asymmetry and excess. It also testifies in favor of precise agriculture technology as one should not expect the domination of allotments with maverick solidness values on the fields.

Penetration resistance has considerable differences in the sowing layer and in the tillage pan. This regularity is found in loamy and heavy loamy soils, in soils with high and low fertility levels. It should also be noted that the penetration resistance of the investigated fields is mostly remarkable for considerable values. Certainly, it should be also considered that this is an equilibrium state and a plunger was a flat disk. But even taking it into account, one should admit that such solidness values do not testify in favor of complete tillage minimization for these fields.
The visualization of penetration resistance on the investigated fields brings clear demonstration of the specificities of spatial mottling of this index. Almost all 1-D diagrams of soil penetration resistance of elementary sites are one-type (the data are not presented), only the tillage pan is revealed in a different way — according to the depth and value of the drop compared to adjacent layers. It is remarkable that the tillage pan

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**Fig. 6.** Heterogeneity of bulk density of the plow pan pan, g/ccm (Kolky)
does not have any omission, therefore, only due to this reason it does not require any complete tillage.

The range of the indices obtained fluctuates from the penetration resistance values, comparatively easily overcome by the roots of practically all the cultivated crops (not exceeding 20 kgf/sq.cm), to the values, evidently hindering their growth and functioning (in the range of 30–40 kgf/sq.cm). Only some roots can overcome this solidity level on condition of sufficient humidity [5]. However, even in this case, as the mentioned high indices do not have any complete configuration, deep tillage also requires differentiation.

It is noteworthy that successful germination of seeds and the development of roots of the 1st order require the penetration resistance not to exceed 10 kgf/sq.cm, or even 5–7 kgf/sq.cm for small-seeded crops (such as sugar beets) [9, 10]. Taking the abovementioned into consideration, it is possible to recommend obligatory pre-sowing tillage, if the penetration resistance of the sowing layer exceeds 10 kgf/sq.cm, deep pre-sowing cultivation, if the layer, deeper than 7–12 cm, has the penetration resistance index, exceeding 20 kgf/sq.cm, and at least one deep tillage during the crop rotation (according to the precise agriculture technology), if the penetration resistance of the tillage pan exceeds 40 kgf/sq.cm.

**Crop yield data and their correlation with soil physical properties.** The range of fluctuations for crop yield (in terms of crop units) on the investigated allotments (dt/ha) is as follows: Romaniv – 20.2–54.3; Kolky – 1.4–20.2; Vediltsy – 9.0–32.2; Korotych – 10.7–29.8; Kommunar – 19.4–43.3; Donetsk – 31.6–60.3. A considerable range was also preserved in the after-effect on the fields, where similar observations were performed (Romaniv, Korotych, Kommunar). It is remarkable that a considerable range of fluctuations was noted both on the fields with more favorable fertility indices (Kommunar) and on the field with the worst agronomic parameters (Kolky). The negative correlation of the investigated indices and the yield was rather high (Table 4).

It is most likely that yield mottling reflects both the heterogeneity of soil physical properties and a number of other factors of natural and especially economic origin. A considerable contribution into mottling is also made by poor quality of sowing, administration of fertilizers, work at seedlings, and harvest. Thus, the matter of solving the task of decreasing the mottling of field fertility should be started with increasing the quality of performing agrotechnical works.

Using the data obtained to plan precise tillage. Reliable spatial heterogeneity of lumpiness, bulk density and penetration resistance, established on the example of the investigated fields, provides for the substantiation of their precise tillage. For this reason let us consider the norms of distinguishing the contours with different parameters of physical properties and their area. The latter may be used as directions for tillage differentiation in the investigated fields proper.

**Standards and area for soil agrotechnological groups.** A relevant argument in favor of precise agriculture is the data on the ratio of zones with favorable, less favorable and unfavorable agrophysical conditions

<table>
<thead>
<tr>
<th>Soil index</th>
<th>Depth, cm</th>
<th>Coefficient correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content of aggregates, %</td>
<td>0–10</td>
<td>–0.57</td>
</tr>
<tr>
<td>Bulk density</td>
<td>0–5</td>
<td>–0.68</td>
</tr>
<tr>
<td></td>
<td>10–15</td>
<td>–0.70</td>
</tr>
<tr>
<td></td>
<td>20–25</td>
<td>–0.60</td>
</tr>
<tr>
<td></td>
<td>30–35</td>
<td>–0.48</td>
</tr>
<tr>
<td>Penetration resistance</td>
<td>0–10</td>
<td>–0.79</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>–0.7</td>
</tr>
<tr>
<td></td>
<td>20–30</td>
<td>–0.0</td>
</tr>
<tr>
<td></td>
<td>30–40</td>
<td>–0.64</td>
</tr>
</tbody>
</table>

*The data on yields and soil physical properties of different objects are united into one selection.

<table>
<thead>
<tr>
<th>Object</th>
<th>No tillage</th>
<th>Minimal</th>
<th>Standard for the zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vediltsy</td>
<td>10</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Romaniv</td>
<td>60</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Kolky</td>
<td>25</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Korotych</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Kommunar</td>
<td>70</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Donetsk</td>
<td>75</td>
<td>22</td>
<td>3</td>
</tr>
</tbody>
</table>

*Standards to select the tillage method are demonstrated in Table 6.
on the investigated fields (Table 5). According to these data, the field is divided into specific contours – no tillage, minimal tillage and traditional tillage. The higher the share of sites with favorable parameters of balanced bulk density on the field in the pre-sowing period or prior to the main tillage is, the more relevant (and more cost-efficient) the precise tillage becomes.

While analyzing the data of Table 5, noteworthy is considerable mottling in the ratio of the areas of agro-technological groups in the soils of different natural zones and of different genesis. Natural fertility of soils in Polissia, Forest-Steppe and Steppe has considerable differences. It was rather surprising to find considerable areas of the sites with favorable properties on Polissia objects of Romaniv and Vediltsy. This considerable reserve of minimization (up to complete refusal) of pre-sowing and other work is unfortunately not realized yet, as Polissia is known for low popula-

Table 6. Preliminary standards of estimating physical properties of the cultivated soil layer to substantiate the intensity of tillage practice*

<table>
<thead>
<tr>
<th>Index</th>
<th>Qualitative estimate of the tilled soil layer</th>
<th>Recommendations on the intensity of pre-sowing tillage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of aggregates in the sowing layer, %:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>Favorable</td>
<td>No tillage needed</td>
</tr>
<tr>
<td>5–15</td>
<td>Satisfactory</td>
<td>Moderate tillage</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>Unsatisfactory</td>
<td>Intense</td>
</tr>
<tr>
<td>Bulk density in the sowing layer, g/ccm:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1.2</td>
<td>Favorable</td>
<td>No tillage needed</td>
</tr>
<tr>
<td>1.2–1.3</td>
<td>Satisfactory</td>
<td>Moderate tillage</td>
</tr>
<tr>
<td>&gt; 1.3</td>
<td>Unsatisfactory</td>
<td>Intense</td>
</tr>
<tr>
<td>Penetration resistance in plow plan, kgf/sq.cm:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>Favorable</td>
<td>No tillage needed</td>
</tr>
<tr>
<td>20–40</td>
<td>Satisfactory</td>
<td>No tillage needed</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>Unsatisfactory</td>
<td>Intense</td>
</tr>
</tbody>
</table>

*The standards are applicable to soils of medium and heavy loamy soil texture.
rity of minimal and even more zero or similar tillage technologies. At the same time it testifies in favor of wide application of precise technologies in the mentioned zone.

The fields with chernozem soils almost do not have any sites, requiring enhanced technology of crop cultivation. Instead, there are large areas of soils in the fields, referred by us to the agrotechnological groups with favorable soil properties. Thus, it is possible to state the presence of prerequisites for the development of precise agriculture both in the Forest-Steppe and in the Steppe. However, based on the ratio of areas on the investigated objects, the content and direction of agrotechnologies are different.

Based on 2-D diagrams and standards, land allotments are subdivided into separate sites, forming a maximally justified rectangular form, most suitable for the work of modern tillage machines. Fig. 7 presents the example of field division with the isolation of sites for tillage practice, different in its intensity, – intense, minimal, zero.

Unfortunately, at present there are no technical means, capable of accepting the standards regarding the differentiation of tillage methods in the space of a land allotment. However, there are attempts of elaborating such instruments [11, 12]. The intensity of tillage is automatically selected in the combined rotor-type machine for pre-sowing tillage and sowing, depending on the bulk density prior to the tillage. The higher the density, the higher the speed of rotor spinning is and the better the degree of soil tillage in the sowing layer is.

**Prospects of precise tillage in Ukraine.** Precise tillage is based on the information about spatial heterogeneity of soil physical properties of the field, the main one being the structural composition (especially the content of the lumpy fraction in the sowing layer), bulk density and penetration resistance. After the geostatistical processing this information is transformed into working sites for the administration of differentiated agrotechnological methods. If the information about spatial heterogeneity of the soil cover is enriched with the data about the administration of geoinformational technologies and new technical means and contains actual data on the external economic situation, it is gradually transformed into a conceptionally new agricultural strategy.

The expressed spatial heterogeneity of soil physical properties in all the investigated fields, located in Polissia, Forest-Steppe and Steppe, brings definitive evidence in favor of the promising prospects of the development of precise soil tillage in Ukraine. The most vivid heterogeneity is manifested in the sowing layer regarding the content of the lumpy fraction, which makes the pre-sowing tillage a very relevant object for precise tillage. The precise basic tillage is also a promising approach, as the fields in all natural zones are different in their bulk density, and some fields (especially in the chernozem zone) with the parameters, close to the requirements of plants, may have zero tillage. It is the Forest-Steppe where the efficiency of precise agriculture may be the highest, although it is also promising in other natural zones as well.

**CONCLUSIONS**

The lumpiness, bulk density and penetration resistance of the main soils of Polissia, Forest-Steppe and Steppe of Ukraine as reliable indices of their differentiated (precise) tillage were studied. The reliability of indices is proven by the presence of their rather high correlation with the crop yield.

The heterogeneity of soil physical properties, diagnosed using the statistical and geostatistical criteria, is characterized as moderate and enhanced, which creates favorable prospects for the development of precise agriculture in Ukraine.

Depending on the range of fluctuations for soil physical properties and the standards of their quality estimation the investigated fields were divided into agrotechnological groups for differentiated (precise) pre-sowing treatment. The ratio of qualitatively different contours fluctuates depending on the natural zones, but the differentiation in each of them is perceived to be useful and cost-efficient, as it presupposes partial and even complete refusal from pre-sowing tillage and deep basic tillage.

**ACKNOWLEDGMENT**

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Просторова неоднорідність фізичних властивостей грунтів України

В. В. Медведев, І. В. Пліско

e-mail: vmedvedev@ukr.net

Національний науковий центр «Інститут грунтоznавства і агрохімії імені О. Н. Соколовського» НААН України

Вул. Чайковська, 4, Харків, Україна, 61024

Мета. Дослідити структурний склад (брзлистість), щільність будови та твердість у грунтах Полісся, Лісостепу і Степу. Методи. Ділянки для дослідження на полі віділено методом накладання регульованої сітки. Вимірювання, проведені перед збиранням врожаю, свідчать про рівноважний стан фізичних властивостей грунтів. Дани обробляли геостатистичним методом. Результати. Отримано основні параметри просторової неоднорідності (коекфіцієнти варіації, гістограми, автокореляційна функція, двовимірна гістограма, здійсненням 2-D- і 3-D-діаграми тощо). Неоднорідність фізичних властивостей, виявлена для всіх грунтів, характеризується помірними і підвищеними значеннями. За результатами обробки даних досліджено поля розділено на три агротехнологічні групи відповідно до якісних параметрів їхніх фізичних властивостей.

Висновки. Для кожного групі сформовано рекомендації щодо передпосівного або основного обробітку різної інтенсивності – без обробітку (там, де параметри наближені до вимог висівної культури), з помірним обробітком зонального типу (де параметри наближені до модальних величин) і з обробкою підвищеної інтенсивності (параметри незадовільні, потрібно виконати більш інтенсивне передпосівне розпушування).

Ключові слова: просторова неоднорідність почв, фізичні властивості, точна обробка.

Пространственная неоднородность физических свойств почв Украины

В. В. Медведев, И. В. Плиско

e-mail: vmedvedev@ukr.net

Научный научный центр «Институт почвоведения и агрохимии имени А. Н. Соколовского» НААН Украины

ул. Чайковская, 4, Харьков, Украина, 61024

Цель. Исследовать структурный состав (глинистость), плотность сложения и твердость в почвах Полесья, Лесостепи и Степи. Методы. Делянки для исследования выделены на поле методом наложения регулярной сети. Измерения, проведенные перед уборкой урожая, свидетельствуют о равновесном состоянии физических свойств почв. Данные обрабатывали геостатистическим методом. Результаты. Получены основные параметры пространственной неоднородности (коэффициенты вариации, гистограммы, автокорреляционная функция, вариограммы, 2-D- и 3-D-диаграммы и др.). Неоднородность физических свойств, выявленная во всех почвах, характеризуется умеренными и повышенными значениями. По результатам обработки данных исследованные поля разделены на три агротехнологические группы в соответствии с качественными параметрами их физических свойств.

Выводы. Для каждой группы сформулированы рекомендации по предпосевной или основной обработке различной интенсивности — без обработки (там, где параметры близки к требованиям высеваемой культуры), с умеренной обработкой зонального типа (где параметры близки к модальным величинам) и с обработкой повышенной интенсивности (параметры неудовлетворительны и требуется более интенсивное предпосевное рыхление).

Ключевые слова: пространственная неоднородность почв, физические свойства, точная обработка.

REFERENCES


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INTRODUCTION

Sweet pepper is one of the most important vegetables, enriching human ratio with necessary vitamins, microelements, dietary fibers, and wholesome sugars. The representatives of Capsicum genus are widely used in food canning, pharmaceutical and other branches of industry [1].

Capsicum is a crop of tropic and subtropic regions of South America. It covers about 30 species, including five main cultivated ones, namely, C. annuum L., C. frutescens L., C. chinense Jacq., C. baccatum L. and C. pubescens Ruiz and Pavon [2].

According to the biological criteria, pepper is a perennial self-pollinating plant, but in Ukraine’s conditions it is a one-year plant, referred to optionally self-pollinating plants. Depending on weather conditions, transpollination may reach 40–60 %. Pepper flowers may be pollinated both with its own pollen, and with the pollen of another plant, brought by an insect. Limp and bent flowers are usually pollinated with the pollen of the same plant. Hot pepper is more likely to have cross-pollination, compared to sweet pepper [3].

Most varieties of Capsicum are diploid (2n = 2x = 24), but there are several varieties with 2n = 2x = 32.
Capsicum has a large genome, the size of which fluctuates in the range of 7.65 thousand bp for C. annuum to 9.72 thousand bp for C. pubescens, and the average value is approximately 8.42 thousand bp. [4].

The genes of Capsicum have been studied since 1912, when Weber started his studies on the inheritance of several traits [5]. The works in Capsicum genetics have been conducted by scientists of many countries: in England – Aktins and Sherard, in India – Deshpande, in Japan – Ikeno, in the United States – Halstead and Dale [5, 6]. In particular, the scientists paid much attention to the reproductive system, including genetic, cytoplasmic, functional male sterility, as well as female sterility and genes of fertility restoration [7].

The aim of the current work was to describe and systematize known genes of the reproductive system of Capsicum genus plants and to suggest their usage in the creation of new varieties and hybrids of sweet pepper in the conditions of Ukraine’s protected soils.

MATERIALS AND METHODS

Plant breeding experiments with sweet pepper in the conditions of protected soils have been conducted by us since 2001 on the basis of the Research and Education Center of Covered Soil at the Scientific and Research Production Agricultural Complex Pushchavodytsia. The work was started with the study of the world collection of varieties and hybrids of sweet pepper of different countries. Over 400 specimens have been analyzed during the years of the studies. About 1,000 specimens of the lines of our own selection and 1,000 of new hybrids F₁, selected by us, were also used in the studies.

The experiments were conducted according to common methods, described in [3, 8, 9] by the following trends: the creation of initial material for the selection of varieties and heterosis plant breeding, the obtaining of new varieties and hybrids F₁ for the conditions of plastic and glass greenhouses. Soil was used as a substrate in plastic greenhouses and gravel – in glass greenhouses. The plants were grown with drop irrigation and optimal supply of organo-mineral fertilizers. The scheme of planting was as follows: 70 × 35 cm – in plastic greenhouses, and 100 × 60 with a tape – in glass greenhouses. The plants were formed into one stalk. The area of the experimental plot was 5 sq.m.

The variety testing of the best new hybrids F₁ was conducted with three repeats. The best hybrids F₁ of domestic and foreign selection were used as standards. About 25–30 hybrids F₁ were tested in the breeding ground of the main variety testing every year.

The evaluation of the collection and selection material involved the analysis of the morphological traits of the plant, the size, form and color of the pepper, its location, quality indices, and resistance to diseases.

Special attention was paid to the manifestation of marker genes in different generations and their association with economically valuable traits of sweet pepper. The combination capability of selection lines was studied in test crossing. The selection was conducted by Pedigree’s method.

RESULTS AND DISCUSSION

The preconceptual work in planning the studies and forming the initial material for the selection and creation of hybrids involved the study of the availability of marker genes in sweet pepper, which could be used in the estimation and selection of hybrid populations and heterosis plant breeding. Considerable attention was paid to the genes, regulating the reproductive systems, in particular, genetic and cytoplasmic male sterility and the genes of fertility restoration, genes of male fertility, genes of female sterility and genes, responsible for flowering.

The analysis of literature data demonstrated that there are genes, which can be used as markers in the selection process. In our opinion, genes of functional male sterility, genetic male sterility, cytoplasmic male sterility, fertility restoration and genes, responsible for flowering processes are of special relevance.

Genetic and functional male sterility. The first scientists, who studied genetic male sterility, were Shifriss and Frankel, who discovered the first gene of male sterility ms-1 in the spontaneous sterile mutant “All Big” in 1969 [10] (Table 1). Many scientists worked at the study of genes of male sterility. Table 1 presents the list of known genes of genetic and functional male sterility.

Cytoplasmic male sterility (CMS). CMS is widely used in modern heterosis selection of sweet pepper. It is a special type of deviation from the normal development of pollen, when pollen sterility is caused not by deviations in meiosis or spermogenesis, but by the pathological vacuolization of cytoplasm of pollen grains in the process of development. CMS is conditioned by the specificities of cytoplasm, not by the presence of the gene of pollen abortiveness in the homozygous state (genetic sterility). CMS is inherited only in the maternal line. The main criterion of cytoplasmic inheritance is the result of reciprocal
### Table 1. The genes of genetic and functional male sterility

<table>
<thead>
<tr>
<th>Name (synonym)</th>
<th>Name and characteristic of trait manifestation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dms</strong> ds</td>
<td>Dominant genetic male sterility; mutation of ms-5</td>
<td>[12, 13]</td>
</tr>
<tr>
<td><strong>fi-1; (mutant-1, fi)</strong></td>
<td>Filiform; filiform leaves, irregular flowering; male sterility</td>
<td>[13]</td>
</tr>
<tr>
<td><strong>fi-2</strong></td>
<td>Filiform, similar to fi-1; narrow cotyledons and leaves (3–4 mm); filiform petals; the pistil does not grow together with stamens, except for the ones, which originate from small-fruited varieties; incomplete male sterility</td>
<td>[14, 15]</td>
</tr>
<tr>
<td><strong>fms</strong></td>
<td>Functional male sterility; degenerated corolla; withering of stamens leads to underdevelopment of anthers and stigma; the anthers are closed with a long calyx; the mutant form of “Fudijian”</td>
<td>[16]</td>
</tr>
<tr>
<td><strong>ms-1</strong></td>
<td>Genetic male sterility; the anthers are small and wrinkled, without the pollen grains; gene ms-1 may be associated with one of the genes, influencing the pigmentation; the mutant form of “All Big”</td>
<td>[10, 18, 19]</td>
</tr>
<tr>
<td><strong>ms-2</strong></td>
<td>Genetic male sterility; wrinkled anthers, releasing many abortive (incapable of germination) pollen grains; the mutant form of “California Wonder”</td>
<td>[20]</td>
</tr>
<tr>
<td><strong>ms-3</strong></td>
<td>Genetic male sterility; wrinkled anthers, in some cases only a small number of fertile and sterile grains are formed; radiation-induced mutant of “Pasardijska Kapia 794”</td>
<td>[21]</td>
</tr>
<tr>
<td><strong>ms-4</strong></td>
<td>Genetic male sterility; anthers are not completely reduced, they contain a small number of fertile and sterile grains; radiation-induced mutant of “Kapia 794”</td>
<td>[22, 23]</td>
</tr>
<tr>
<td><strong>ms-6, ms-7</strong></td>
<td>Genetic male sterility; wrinkled anthers of reduced size; sometimes only a small amount of fertile pollen is formed; all ms-1–ms-8 are non-allelic; radiation-induced mutant of “Zlaten Medal”</td>
<td>[19, 24]</td>
</tr>
<tr>
<td><strong>ms-8</strong></td>
<td>Genetic male sterility; wrinkled anthers of reduced size; sometimes only a small amount of fertile pollen is formed; radiation-induced mutant of “Zlaten Medal”; located on the lower chromosome arm P9</td>
<td>[19, 24, 25]</td>
</tr>
<tr>
<td><strong>ms-9; (mr-9)</strong></td>
<td>Genetic male sterility; gamma-radiation-induced male sterility</td>
<td>[12, 26]</td>
</tr>
<tr>
<td><strong>ms-10; (mc-509)</strong></td>
<td>Genetic male sterility; the mutant with male sterility, induced by ethylmethane sulfonate (EMS); ms-10 was revealed to be allelic to the isolated allele msk in Korea</td>
<td>[12, 26, 27]</td>
</tr>
<tr>
<td><strong>ms-11; (mc-705)</strong></td>
<td>Genetic male sterility; the mutant with male sterility, induced by EMS</td>
<td>[12, 26]</td>
</tr>
<tr>
<td><strong>ms-12</strong></td>
<td>Genetic male sterility; small and wrinkled anthers without any pollen grains; postmeiotic opening of microspores; non-allelic to ms-1 and ms-2; allelism to ms-alleles of Daskalov is unknown; the mutant of “Gambo”</td>
<td>[28]</td>
</tr>
<tr>
<td><strong>ms-13; (ms)</strong></td>
<td>Genetic male sterility; complete pollen sterility; postmeiotic opening of microspores; the mutant of “CA452-1”</td>
<td>[29]</td>
</tr>
<tr>
<td><strong>ms-14</strong></td>
<td>Genetic male sterility; the androecium transforms into a petal-like structure; the mutant of “Kalyanpur selection”</td>
<td>[30]</td>
</tr>
<tr>
<td><strong>ms-15; (ms)</strong></td>
<td>Genetic male sterility; the anthers are decreased by 50 %, of dark blue color; postmeiotic opening of microspores during the formation of male gametes; originated from “CA-960”</td>
<td>[31]</td>
</tr>
<tr>
<td><strong>msc-1</strong></td>
<td>Genetic male sterility; the spontaneous mutant was discovered in China; allelism to ms-1–ms-15 and smk is unknown</td>
<td>[32, 33]</td>
</tr>
<tr>
<td><strong>msc-2</strong></td>
<td>Genetic male sterility; the spontaneous mutant of “Ying Ge Bai Er” was discovered in China; allelism to ms-1–ms-15, smc-1 and smk is unknown</td>
<td>[34, 35]</td>
</tr>
<tr>
<td><strong>msk</strong></td>
<td>Genetic male sterility; the spontaneous mutant was discovered in Korea for Capsicum annuum L.</td>
<td>[28, 36]</td>
</tr>
</tbody>
</table>
crossings. The genes, controlling CMS, are described in Table 2.

While using CMS in the selection work of creating hybrid seeds, the obligatory stage is the fertility res-
toration (Table 3), conditioned by the participation of dominant allele genes. Thus, depending on the function, fulfilled by the pollinator regarding the sterile plant – fertility restoration or sterility securing – the hybrid progeny obtained will be either fertile or sterile.

One of these genes is \( R_f \) [19, 36, 38]. If a pollinator turns out to be incomplete restorer of fertility, the hybrid progeny will have both sterile and fertile plants with different degrees of fertility \( pr \) [19, 36, 48–50].

### Table 4. The genes of male fertility

<table>
<thead>
<tr>
<th>Name (synonym)</th>
<th>Name and characteristic of trait manifestation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camf1</td>
<td>Capsicum annuum male fertile gene; a specific gene of anthers; plays a relevant role in the development of pollen in <em>C. annuum</em>; was discovered only in anthers of the fertile line of “114B” pepper; belongs to the group of “early” genes</td>
<td>[54]</td>
</tr>
<tr>
<td>CaMF2</td>
<td>Capsicum annuum male fertile gene; a specific gene of anthers; plays a relevant role in the development of pollen in <em>C. annuum</em>; was discovered in the buds of the fertile line of “114B” pepper; belongs to the group of “early” genes</td>
<td>[53]</td>
</tr>
<tr>
<td>CaMF3</td>
<td>Capsicum annuum male fertile gene; a specific gene of anthers, which is revealed only in the buds at the late stage of development and in open flowers of the fertile line of “114B” pepper; belongs to the group of “late” genes; was discovered in the buds of the male fertile line “114B”</td>
<td>[55]</td>
</tr>
<tr>
<td>fl</td>
<td>Folded leaf; upward folded leaves, which creates the boat-like form; is manifested in young plants; partial fertility; originated from “PC 1”</td>
<td>[51]</td>
</tr>
</tbody>
</table>

### Table 5. The genes of female sterility

<table>
<thead>
<tr>
<th>Name (synonym)</th>
<th>Name and characteristic of trait manifestation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfs</td>
<td>Conditional female sterile; strong plants with compact habit; normally developed flowers contain fertile pollen grains; obtained from M2 of population of cv. “Borjana”</td>
<td>[56]</td>
</tr>
<tr>
<td>fcf; (fc)</td>
<td>Fasciflora; female sterility, plants with pollen, fertile for 27–55 %, seedless fruit</td>
<td>[12]</td>
</tr>
<tr>
<td>fems</td>
<td>Female and male sterility; wrinkled pollen does not contain starch; many small seedless fruit; the mutant of line “4526”</td>
<td>[57]</td>
</tr>
<tr>
<td>flf</td>
<td>Flowerless plant; intense vegetative growth, obtained from “PC1”</td>
<td>[51]</td>
</tr>
<tr>
<td>fs</td>
<td>Female sterile; complete female sterility without any other previous phenotype manifestations; adult plants have weak multiflowered periphery; the mutant of PI 159276</td>
<td>[58]</td>
</tr>
<tr>
<td>Pf</td>
<td>Parthenocarpic fruit; no seeds; reduced size of fruit; the eggs start degenerating on the second day after the opening of the flower and degenerate completely after five days; pollen fertility is greatly reduced</td>
<td>[59]</td>
</tr>
<tr>
<td>sel-1</td>
<td>Seedless; normal pollen is formed, but eggs are poorly formed (deformed) and non-functional</td>
<td>[60]</td>
</tr>
<tr>
<td>sel-2</td>
<td>Seedless; deformed placenta (embryo sac); pericarp is thick and carnous; flowering takes place much later</td>
<td>[61]</td>
</tr>
<tr>
<td>sl-1; (sl)</td>
<td>Styleless; absence of normal style or stigma; incomplete female sterility</td>
<td>[62]</td>
</tr>
<tr>
<td>sl-2</td>
<td>Styleless; flowers are without a style or stigma, the ovary is not damaged, without the formation of fruit; female sterility; isolated from “Kalyanpur Red”</td>
<td>[63]</td>
</tr>
<tr>
<td>sp</td>
<td>Spinach; limited development of the stalk, many large leaves are formed into heavy body in close proximity to the surface of the soil; buds are completely blocked; mutant form PI 159280</td>
<td>[58]</td>
</tr>
</tbody>
</table>
Male fertility. There were previous reports on one recessive gene, controlling male fertility [7, 51, 52]. At present three more genes of male fertility have been determined [53–55] (Table 4).

Female sterility. Female sterility of sweet pepper is conditioned by different pathologies of female reproductive organs. The genes, controlling this trait, are presented in Table 5.

The genes of the reproductive system of Capsicum genus also include the ones, responsible for flowering: its presence, time of occurrence, its course, etc. Two recessive genes were listed in the previous lists of genes [7, 52], namely, ef, nf. We added two dominant genes, discovered by Cohen et al. and Tan Shu et al. to this list (Table 6).

The genetics of Capsicum has been studied for almost a century, the selection of this genus has been greatly improved due to the study of genetic and cytoplasmic male sterility, male fertility and its restoration, female sterility and genetic control over flowering.

The application of marker genes allowed accelerating the selection process for sweet pepper and reducing the volumes of selection breeding grounds. The application of marker genes of phenotype traits (the form of a fruit, the color of a fruit in technical and biological ripeness, growth type, pericarp thickness) in the maternal line allowed controlling the degree of hybridism, which facilitates the system of hybrid reproduction.

For instance, while selecting genotypes with genes dm, dt, sp, ef, short, early-ripening forms were obtained with determinate plants, necessary while growing in temporary covers and plastic greenhouses for early harvest.

Usually the selection of phenotypes (marker genome P, cone-like fruit) was aimed at the formation of early

Table 6. The genes, responsible for flowering

<table>
<thead>
<tr>
<th>Name (synonym)</th>
<th>Name and characteristic of trait manifestation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cas, Ca-AN</td>
<td>Flowering related genes; responsible for the activity of flower meristems; epistatic to other genes, controlling the transition to flowering with the corresponding composition of flowers</td>
<td>[64]</td>
</tr>
<tr>
<td>ef</td>
<td>Early flowering; flowering occurs 20–25 days earlier than for “PC 1”; there are a few seeds formed; obtained from “PC 1”</td>
<td>[51]</td>
</tr>
<tr>
<td>nf</td>
<td>Nonflowering; no flowers are formed during the cultivation season</td>
<td>[65]</td>
</tr>
<tr>
<td>Nle</td>
<td>Participates in the regulation of flowering period in the population of Capsicum; was discovered in P2-chromosome in 2012, confirmed in 2014</td>
<td>[64, 66]</td>
</tr>
</tbody>
</table>

Table 7. New varieties and hybrids F1, introduced into the State register of varieties of plants, suitable for growing in Ukraine

<table>
<thead>
<tr>
<th>Name of variety, hybrid</th>
<th>Trait</th>
<th>Fruit color in technical ripeness</th>
<th>Fruit color in biological ripeness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth type</td>
<td>Fruit form</td>
<td>sw2</td>
</tr>
<tr>
<td>Soniachny – early-ripening, cone-like fruit, light color/red</td>
<td>Dt</td>
<td>P</td>
<td>sw2</td>
</tr>
<tr>
<td>Vatag – early-ripening, cube-like fruit, dark green/red</td>
<td>Dt</td>
<td>fb</td>
<td>sw3</td>
</tr>
<tr>
<td>Dobirny – early-ripening, cube-like fruit, dark green/red</td>
<td>Dt</td>
<td>fb</td>
<td>sw3</td>
</tr>
<tr>
<td>Lysko – early-ripening, cube-like fruit, light yellowish-green/yellow</td>
<td>Dt</td>
<td>fb</td>
<td>sw2</td>
</tr>
<tr>
<td>Aborygen – early-ripening, tomato-like fruit, dark green/red</td>
<td>dt</td>
<td>O</td>
<td>sw3</td>
</tr>
<tr>
<td>Advokat – early-ripening, tomato-like fruit, light green/yellow-orange</td>
<td>dt</td>
<td>O</td>
<td>sw2</td>
</tr>
<tr>
<td>F1 Anika – middle early, cube-like fruit, dark green/red</td>
<td>Dt</td>
<td>fb</td>
<td>sw3</td>
</tr>
</tbody>
</table>
The genes of reproductive system of *Capsicum* genus and the search for ways...

**CONCLUSIONS**

The study of genes of *Capsicum* genus is going on. In particular, important tasks are the determination of allelic relations between similar genes, obtaining of new gene mutants, study of homologous genes of other varieties, determination of the location of loci, responsible for certain traits, within chromosomes, genome mapping, creation of molecular markers of genes and defining the functions of genes.

The results of studies obtained may be efficiently used to create new genotypes of sweet pepper. Special attention should be paid to determining features of plant habit, taking into consideration the requirements of technologies of open and protected soil; size, form, color of fruit, marking of biochemical processes, responsible for quality traits of fruit and their resistance to biotic and abiotic factors.

There is a need for intensification of the study of marker genes, promoting the manifestation of heterosis, including genes of sterility, securing and restoration of sterility, as well as for the determination of interrelations of such genes and economically valuable traits. There is an evident requirement for the application of marker genes while planting hybrids of the first generation, which will allow visual control of hybridism degree and purity of initial forms at all the stages. The use of molecular markers with the purpose of obtaining an increased number of substances of dietary, protecting and healing value in sweet pepper seems to be a promising trend.

The application of a number of marker genes allowed us to create commercial varieties and hybrids, successfully grown in conditions of plastic and glass greenhouses. Noteworthy are the following varieties: Soniachny – early-ripening, yellow-red color of ripe fruit; Lysko – early-ripening, yellow color of ripe fruit; Dobirny – middle ripening, large cube-like dark red fruit in the ripeness phase; hybrid F₁ Anika – middle early with large fruit, dark red in the phase of biological ripeness.

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**Гени репродуктивної системи роду *Capsicum* та пошуки шляхів їхнього використання за умов захищеного грунту України**

В. А. Кравченко ¹, Т. А. Степенко ²

e-mail: st4teen@gmail.com

¹ Національна академія аграрних наук України
² Національний університет біоресурсів та природокористування України

Вул. Героїв Оборони, 15, Київ, Україна, 03041

Перець (рід *Capsicum*) є однією з найважливіших ово-чевих і прямих культур у світі. Роботи з вивчення генетич-ки перцю виконуються постійно. Станом відомої нові генів, генетичні маркери, досконалише виявляють раніше відкриті. **Мета.** Метою цієї роботи було описати та систематизувати існуючі на сьогодні генів репродуктив-ної системи рослини роду *Capsicum* і вивчити питання щодо використання ресурсних мутантних генів у селекційному процесі при створенні нових сортів і гібридів перцю солодкого. **Методи.** Для класифікації ре-продуктивних генів у селекційних дослідженнях вико-римано світову колекцію перцю солодкого, існуючі сорти і гібриди різних країн, власний селекційний ма-теріал. Експерименти здійснювали за умов плівкових і скляних теплиць згідно зі сучасними методиками. **Результати.** Отримані дані систематизовано в такі групи генів: функціональної і генетичної стерильності, цитоплазматичної чоловічої стерильності, відновлення фертильності, жіночої фертильності та відповідальні за процеси цвітіння. Частину описаних генів можна
використовувати в селекції на гетерозис та при створенні оригінального вихідного матеріалу. Наведено приклади застосування рецесивних мутантних генів при розробці нових сортів і гібридів перцю солодкого, а також їхні короткі характеристики. Нові сорти і гібриді沸 перцю солодкого занесено до Державного реєстру сортів рослин, придатних до використання в Україні.

Висновки. Охарактеризовані гени можна застосовувати в генетичних і селекційних дослідженнях. За участі рецесивних мутантних генів створено низку нових комерційних сортів і гібридів перцю солодкого.

Ключові слова: перець, ген, селекційний процес, репродуктивна система, стерильність, фертильність.

Гени репродуктивної системи рода Capsicum і пояснення їх використання в умовах захищеної групи України

В. А. Кравченко 1, Т. А. Степенко 2

e-mail: st4teen@gmail.com

1 Національна академія аграрних наук України
Ул. Васильківська, 37, Київ, Україна, 03022
2 Національний університет біоресурсів і природопольового розвитку
Ул. Героїв Обороної, 15, Київ, Україна, 03041

Перець (род Capsicum) – одна з важливіших овоців і пряних культур в світі. Роботи по визначенню генетики цього виду ведуться неперервно. Станом визначено нові гени, генетичні маркери, що досконало визначають раніше класифікацію. Цель. Цілью даної роботи було описати і систематизувати статеві і генетичні характеристики на сьогодення генів репродуктивної системи роду Capsicum і прояснити питання відповідної застосування рецесивних мутантних генов в селекційному процесі при створенні нових сортів і гібридів перцю солодкого. Методи. Для класифікації репродуктивних генов в селекційних першій відмінності з іншими видами використовувалися міжвидові колекції перцю солодкого, що включають сорта і гібриди різних станів, особливості для селекції на репродуктивність. Експерименти виконувалися під впливом погодних і екологічних умов. Результати. Описані генові використовувалися в селекційних дослідженнях на гетерозис і при створенні оригінального висококачісного матеріалу. Наведені приклади виділення рецесивних мутантних генов при розробці нових сортів і гібридів перцю солодкого, а також їхні короткі характеристики. Нові сорти і гібриді沸 перцю солодкого занесені до Державного реєстру сортів рослин, придатних до використання в Україні. Висновки. Охарактеризовані гени можна застосовувати в генетичних і селекційних дослідженнях. С участю рецесивних мутантних генів створено низку нових комерційних сортів і гібридів перцю солодкого.

Ключові слова: перець, ген, селекційний процес, репродуктивна система, стерильність, фертильність.

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INTRODUCTION

When harvesting the sugar beet we always tried to avoid those problems that could break the beet roots in the process of digging them from the soil, disrupt their outer surface or cause their total damage, as well. If mentioned problems persist then they are accompanied by a significant loss of the harvest.

In particular, during the excavation of beet root from the soil by vibrations we can, with a given degree of accuracy, represent it as a resilient rod with one end fixed in the elastic medium and the rod is exposed to vibrations that are arising from working tool. What is more and very significant, the soil surrounding the roots, is also an elastic medium.

Fundamental analytical study of transverse vibrations of the body of the root was performed and published in [1]. Here the sugar beet root crop was simulated as a body of conical shape with one point fixed at the bottom and that has elastic properties. In this case, the transverse vibrations of the root body are described by differential equation with partial derivatives of the fourth order. The solution of this equation made it possible to elaborate the theory of longitudinal vibrations of a solid elastic body with one fixed end in the elastic medium. The example of such a body may be found in a sugar beet root in soil, the latter being elastic medium.

THEORY OF LONGITUDINAL VIBRATIONS OF A CONICAL ELASTIC BODY IN AN ELASTIC MEDIUM

V. M. Bulgakov 1, V. V. Adamchuk 2, I. V. Holovach 1, D. Orszaghova 3

1 National University of Life and Environmental Sciences of Ukraine
15, Heroyiv Oborony Str., Kyiv, Ukraine, 03041

2 National Scientific Centre, Institute for Agricultural Engineering and Electrification
11, Vokzalna Str., Glevakha-1, Vasylkiv District, Kyiv Region, Ukraine, 08631

3 Slovak University of Agriculture in Nitra
2, Trieda Andreja Hlinku, 949 76 Nitra, Slovakia

e-mail: vb Bulgakov@meta.ua; adamchukvV@mail.ru

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Aim. To elaborate the theory of longitudinal vibrations of a solid elastic body with one fixed end in the elastic medium. The example of such a body may be found in a sugar beet root in soil, the latter being elastic medium.

Methods. The principle of stationary action of Ostrogradsky-Hamilton and the Ritz method were applied in the work.

Results. The Ritz method was applied to obtain the Ritz frequency equation for the oscillating process under investigation. The analytic expressions were defined to determine the first and second eigenfrequencies of vibration and the amplitude of constrained vibrations of any of its cross-sections. The values of the first and second eigenfrequencies of the elastic body under investigation with specific geometric and physical parameters were found. The dependency diagrams for the first and second eigenfrequencies on the coefficient of elastic contraction of soil as the elastic medium, and the dependency diagrams for the amplitude of constrained oscillations of the mentioned body on the coefficient of elastic deformation of soil and the distance of the cross-section of the body from the conditional point of fixation were drawn. The dependency diagrams for the amplitude of constrained oscillations of the elastic body on the change in the amplitude and the frequency of perturbing force were obtained.

Conclusions. The impossibility of resonance occurrence was substantiated as the frequency of the perturbing force cannot equal the frequency of eigenvibrations of the elastic body due to technological and technical reasons. It was proven that the breaking of the elastic body is impossible with longitudinal deformations due to the shortness of the amplitude of longitudinal vibrations of the mentioned body.

Keywords: solid elastic body, elastic medium, functional of Ostrogradsky-Hamilton, longitudinal vibrations, eigenforms, eigenfrequencies, Ritz method.

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possible to determine the natural frequencies of free transverse vibrations of a root crop body. Directly, the process of extracting sugar beet roots from the soil, in this paper, is investigated further via composition of additional equations of kinetostatics, which allowed, with a certain degree of accuracy, find the conditions for the complete extraction of the root from the soil.

However, from constructional and technological point of view, the extraction of beet root from the soil in the good quality level, with the usage of transverse vibrations, proved impracticable. And that has stimulated the use of devices that provide the transfer of vibrations through the beet roots in the longitudinal vertical plane.

Fundamentally new formulation of the theory of vibrating excavation of beet roots from the soil when applying perturbing forces, namely in a longitudinal vertical plane, was published in papers [2−4]. The case of free and forced transverse vibrations of the body of the root when matching directions of perturbing forces with the direction of translational motion of a vibrating digging up the working body is published in the works [5−8] and is of interest both from theoretical and practical points of view.

To consider the more general problem about longitudinal vibrations of a continuous elastic conical body, fixed in an elastic medium.

MATERIALS AND METHODS

The problem is solved on the basis presented in publications [9−12], means, on the general theory of vibrations of straight rods with variable cross section. Also was used the methodology of research of mechanical systems, that is widely presented in [13, 14].

RESULTS AND DISCUSSION

We will consider such case when vibrational motion to the said body will be exerted in a longitudinal vertical plane. This position will corresponds to a situation in which vibrational forces of the harvesting machine will be applied on the both sides of the root (which is initially in the undisturbed soil) and during its extraction from the soil.

For the study of oscillations of holonomic systems with infinite number of degrees of freedom apply the principle of stationary action of Ostrogradskij-Hamilton [9]. In the theory of longitudinal, torsional and transverse vibrations of straight rods are used functionals Ostrogradsky-Hamilton, which in the most general form looks like (see [9]):

\[
S = \int_0^L \left[ t, x, y, \frac{\partial y}{\partial t}, \frac{\partial y}{\partial x}, \frac{\partial^2 y}{\partial t^2}, \frac{\partial^2 y}{\partial t \partial x}, \frac{\partial^2 y}{\partial x^2} \right] dx dt,
\]

(1)

where \( L = T – \Pi \) means Lagrange function; \( T \) is the kinetic energy of the system; \( \Pi \) is the potential energy of the system.

Using the principle of Ostrogradsky-Hamilton, explore the longitudinal vibrations of an elastic solid body, occurring under the action of vertical perturbing force, which varies according to a harmonic law, such as the following:

\[
Q_{df}(t) = H \sin \omega t,
\]

(2)

where \( H \) is the amplitude of the perturbing force; \( \omega \) is the frequency of the perturbing force.

As we can see from the composed equivalent scheme (Fig. 1), the continuous elastic body is a crop root that has a conical shape (the angle at the vertex equal to \( 2\gamma \)), and the upper part is slightly higher than the soil surface), is modeled as a rod of variable cross-section with a fixed bottom end (point \( O \)). At the center of gravity, which is indicated by the point \( C \), is applied force \( \overline{G} \)– body weight. Total length of the body is \( h \). The connection of the body (the crop root) with the soil is determined by the total soil reaction \( \overline{R} \), which is located down along the x-axis.

The above mentioned perturbing force \( \overline{Q}_{df} \) is applied to the body directly from its two sides, so in the diagram it is represented by two components \( \overline{Q}_1 \) and \( \overline{Q}_2 \). These forces are applied at a distance from the origin (point \( O \)) and they cause vibrations of the body (the root) in a longitudinal vertical plane, which disrupts its connection with the soil and create conditions of the extraction.

We form the Ostrogradskij-Hamilton’s functional \( S \) for the vibrational process, which is being explored. With this aim we will introduce the necessary notations:

\( F(x) \) – the area of the cross-section of the body at any point, which is at a distance \( x \) from the lower end (m²);

\( E \) – Young’s modulus for the material of the body (N/m²); \( \gamma(x, t) \) – longitudinal displacement of any cross-section of the body at the time \( t \) (m); \( Q(x, t) \) – the intensity of the external longitudinal load directed along the axis of the body (N/m); \( \mu(x) \) – momentum of the body (kg/m).

According to [9] the functional of Ostrogradskij-Hamilton for longitudinal vibrations of straight bars is as follows:
We will find the expression of all the quantities in the functional (3). Considering that the body has a conical shape, we find that its cross-sectional area at a point which is located at any distance $x$ from the point $O$, is equal to:

$$F(x) = \pi x^2 \tan^2 \gamma.$$  \hspace{1cm} (4)

Evidently, the momentum of the body can be determined using the following expression

$$\mu(x) = \rho \cdot \pi x^2 \tan^2 \gamma,$$  \hspace{1cm} (5)

where $\rho$ is the density of the body ($\text{kg/m}^3$).

Since the quantity $Q(x, t)$ which is the part of the functional (3), is the intensity of the distributed load (measured in $\text{N/m}$), the perturbing force should have a dimension of the intensity of the load. Using the first-order impulsive function $\sigma_1(x)$ [9] we can determine the intensity of a concentrated load, and thus incorporate concentrated forces in the load (distributed along the length).

If $Q_{df}(t)$ is a concentrated perturbing force applied at the point $x_1$ and is measured in $\text{N}$ (Newton), then the function:

$$Q_{df}(x, t) = Q_{df}(t) \cdot \sigma_1(x-x_1),$$  \hspace{1cm} (6)

is measured in $\text{N/m}$ and expresses the intensity of the concentrated load at the point $x_1$.

Function $\sigma_1(x-x_1)$ will be equal zero for all $x$, except $x = x_1$, where it goes to infinity.

If the perturbing force varies according to equation (2), then in accordance with the equation (6) can be written:

$$Q_{df}(x, t) = H \sin \omega t \cdot \sigma_1(x-x_i).$$  \hspace{1cm} (7)

Since continuous elastic body is connected with the soil, which is also an elastic medium, then during the action of disturbing force (2) arises the force of soil resistance against displacement of the body which is doing vibrations. This force also affects the process of natural vibrations of the body in the soil, especially in the beginning of the oscillatory process, while body’s connections with the soil are not broken yet.

It is obvious that the power of resistance of the soil (for the whole body) represents a distributed load on the area of the contact of the body with the soil, and therefore we can determine its intensity as the force of the soil resistance against the displacement of a unit length of the body.

Let $c$ is the coefficient of the elastic soil deformation (ratio of the first coefficient of Winkler to the area of contact ($\text{N/m}^3$)). Then the intensity $P(x, t)$ of the resistance of the soil against the body displacement at a point $x$ will be equal to:

$$P(x, t) = 2\pi c x \cdot \tan \gamma \cdot y(x, t).$$  \hspace{1cm} (8)

Thus, we will have this relation for the longitudinal external load:

$$Q(x, t) = Q_{df}(x, t) - P(x, t).$$

Considering expressions (4), (5), (7) and (8), the functional of Ostrogradskij-Hamilton (3) will have the form:

$$S = \frac{1}{2} \int_{t_0}^{t_f} \left[ \rho \pi x^4 \tan^2 \gamma \left( \frac{\partial y}{\partial t} \right)^2 - E \pi x^2 \tan^2 \gamma \left( \frac{\partial \gamma}{\partial x} \right)^2 + H \sin \omega t \cdot \sigma_1(x-x_i) y(x, t) - 2\pi c x \cdot \tan \gamma \cdot y(x, t) \right] dx dt.$$  \hspace{1cm} (9)

To find the eigenform and frequencies of longitudinal vibrations of the body in the soil we apply the method...
of Ritz [9]. According to this method, we will search harmonic longitudinal vibrations of the body in this form:

\[ y(x, t) = \phi(x)\sin(pt + \alpha), \]

(10)

where \( \phi(x) \) is eigenform of major vibrations; \( p \) is eigenfrequency of major vibration.

Because eigenforms and eigenfrequencies are associated with the free vibrations of the system, it is necessary to identify in the functional (9) that part which describes exactly the free vibrations of the system. It is clear that it will be the functional in following form:

\[
S = \frac{1}{2} \int_0^b \int_0^t \left[ \rho \pi x^2 \gamma^2 \phi(x) \frac{\partial y}{\partial t}^2 - E \pi x^2 \gamma^2 \left( \frac{\partial \phi}{\partial x} \right)^2 - 2 \pi cx \gamma \phi(x) y(x, t) \right] \, dx \, dt.
\]

(11)

Substituting expression (10) in the functional (11), we obtain:

\[
S = \frac{1}{2} \int_0^b \int_0^t \left[ \rho \pi x^2 \gamma^2 \phi(x) \left( \frac{\partial \phi}{\partial x} \right)^2 - E \pi x^2 \gamma^2 \phi(x)^2 - 2 \pi cx \gamma \phi(x) \phi'(x)^2 \right] \, dx \, dt.
\]

(12)

Integrating the expression (12) with respect to \( t \) within one period, we have:

\[
S = \frac{\pi}{2 \rho} \int_0^b \left[ \rho \pi x^2 \gamma^2 \phi(x) \phi'(x)^2 - E \pi x^2 \gamma^2 \phi'(x)^2 - \pi cx \gamma \phi(x) \phi'(x) \right] \, dx.
\]

(13)

According to the method of Ritz, values of the functional (13) are considered on a set of linear combinations of functions, i.e. expressions of the following form:

\[
\phi(x) = \sum_{i=1}^n a_i \psi_i(x),
\]

(14)

where \( a_i \) are parameters corresponding to definition; \( \psi_i(x) \) - basis functions, which are specially selected and are known, they satisfy the geometric boundary conditions of the problem.

Thus, by substituting the expression (14) into expression (13), after appropriate adjustments we obtain:

\[
S = \frac{\pi}{2 \rho} \int_0^b \left[ \rho \pi x^2 \gamma^2 \sum_{i=1}^n \psi_i(x) \psi_i'(x) a_i a_i - E \pi x^2 \gamma^2 \sum_{i=1}^n \psi_i(x) \psi_i'(x) \right] \, dx.
\]

(15)

Next, we introduce the following notations:

\[
\int_0^b \rho \pi x^2 \gamma^2 \psi_i(x) \psi_i'(x) \, dx = T_{a_i},
\]

\[
\int_0^b E \pi x^2 \gamma^2 \psi_i(x) \psi_i'(x) \, dx = U_{a_i},
\]

\[
\int_0^b 2 \pi cx \gamma \psi_i(x) \psi_i'(x) \, dx = C_{a_i},
\]

\[
(i, k = 1, 2, \ldots, n).
\]

Substituting (16) into (15) gives the functional in the form of the function with parameters \( a_1, a_2, \ldots, a_n \):

\[
S(a_1, a_2, \ldots, a_n) = \frac{\pi}{2 \rho} \sum_{i=1}^n T_{a_i} a_i a_i - \frac{\pi}{2 \rho} \sum_{i=1}^n U_{a_i} a_i a_i - \frac{\pi}{2 \rho} \sum_{i=1}^n C_{a_i} a_i a_i.
\]

(17)

We investigate the extreme of the functional (17). To do this, we will differentiate the expression (17) according to parameters \( a_i \), \( i = 1, 2, \ldots, n \) and obtained partial derivatives should be equal to zero. As a result, we obtain the system of linear homogeneous equations with the unknowns \( a_1, a_2, \ldots, a_n \), from which (in turn) we find the equation for the frequencies of Ritz for longitudinal vibrations of a continuous elastic body, fixed in the soil:

\[
p^2 T_{11} - U_{11} - C_{11} = 0, \quad p^2 T_{12} - U_{12} - C_{12} = 0, \quad \ldots, \quad p^2 T_{1n} - U_{1n} - C_{1n} = 0, \quad \ldots, \quad p^2 T_{nn} - U_{nn} - C_{nn} = 0.
\]

(18)

In practice, usually only the lower frequencies are determined, mostly the first and second one, which significantly affect the considered technological process.

To determine the first (main) frequency of natural vibrations, the equation (18) takes the following form:

\[
p^2 T_{11} - U_{11} - C_{11} = 0.
\]

(19)

As a result of the solution of equation (19) we obtain an analytical expression for finding the first frequency:

\[
p_1 = \sqrt{\frac{0.505 E \gamma + 2.207 c h}{0.917 h \sqrt{\rho \gamma}}}
\]

(20)

For the calculation of the first eigenfrequency \( p_1 \) according to the expression (20) we take (in accordance with [15]) for the sugar beet \( h = 250 \) mm, \( \gamma = 14^\circ \), \( E = 18.4 \cdot 10^6 \) N/m², \( \rho = 750 \text{ kg/m}^3 \). According to [1] we...
take the coefficient $c$ of the elastic deformation of the soil $c = 2 \cdot 10^5 \text{N/m}^3$.

From the calculation we obtain: $p_1 = 496.4 \text{ c}^{-1}$ or $p_1 = 79 \text{ Hz}$, which (with a high degree of accuracy) is consistent with the experimental data given in [16], according to which $p_1$ is in the range 75–120 Hz, and confirms similar data obtained theoretically in [17].

For the determining the first and second frequencies the equation (18) takes the form:

$$
\begin{bmatrix}
p^2 T_{11} - U_{11} - C_{11} \\
p^2 T_{12} - U_{12} - C_{12} \\
p^2 T_{21} - U_{21} - C_{21} \\
p^2 T_{22} - U_{22} - C_{22}
\end{bmatrix} = 0.
$$

(21)

Solving the equation (21) in the program Mathcad with the same parameters as in the previous case, there is obtained graphical dependence between the first, respectively the second natural frequency of the body of the root and the value of the coefficient of elastic deformation of the soil (see Fig. 2, 3).

As we can see from the graph (Fig. 2), with changing values of the coefficient $c$ (coefficient of elastic deformation of the soil) within the range $(0–2) \times 10^5 \text{N/m}^3$, the values of the first angular frequency $p_1$ increases monotonically in the range 480–587 $\text{s}^{-1}$, or the frequencies are in the range 76.4–93.4 Hz.

From the graph on the Fig. 3 can be seen that with changing values of the coefficient $c$ (coefficient of elastic deformation of the soil) within the range $(0–2) \times 10^6 \text{N/m}^3$, the second eigenfrequency of free vibrations varies in a small range: $p_2$ is from 3318 to 3344, or the frequencies are in the range 528–532 Hz.

Now we turn to the study of forced vibrations of a continuous elastic body. Purely forced vibrations will occur in accordance with the law:

$$
y(x, t) = \varphi(x) \sin \omega t,
$$

(22)

where $\varphi(x)$ is the form of forced vibrations.

To determine the form of forced oscillations of the body, we substitute the expression (22) in the functional (9), we obtain the following functional:

$$
S_1 = \frac{1}{2} \int_0^L \left\{ \rho \pi x^2 \gamma^2 \varphi(x)^2 \cos^2 \omega t - E \pi x^2 \gamma |\varphi(x)|^2 \sin \omega t + H \sigma(x-x') \varphi(x) \sin \omega t - 2 \pi c x \gamma \varphi(x) \sin \omega t \right\} dx dt.
$$

(23)

Integrating the expression (23) with respect to $t$ within one period $T = 2\pi/\omega$, we have:

\begin{center}
Fig. 2. The dependence between the first eigenfrequency of the longitudinal vibrations of the root and the coefficient $c$ of elastic deformation of the soil ($p_01$ – first angular frequency, $p_{011}$ – first frequency, $c_{11}$ – coefficient of elastic deformation)
\end{center}

\begin{center}
Fig. 3. The dependence between the second eigenfrequency of the longitudinal vibrations of the root and the coefficient $c$ of elastic deformation of the soil ($p_02$ – second angular frequency, $p_{022}$ – second frequency, $c_{11}$ – coefficient of elastic deformation)
\end{center}

$$
S_2 = \frac{\pi}{2\omega} \int_0^L \left\{ \rho \pi x^2 \gamma^2 \varphi(x)^2 \omega^2 - E \pi x^2 \gamma |\varphi(x)|^2 \right\} + H \sigma(x-x') \varphi(x) - 2\pi c x \gamma \varphi(x) \omega dx dt.
$$

(24)

According to the method of Ritz, we consider the value of the functional (24) on the set of linear combinations of the following form:

$$
\varphi(x) = \alpha \psi(x),
$$

(25)

where $\alpha$ is the parameter corresponding to definition; $\psi(x)$ – basis function.
By substituting the expression (25) into functional (24) we obtain:

\[ S_2 = \frac{\pi}{2a_0} \int_0^h \rho \pi x^2 \tan^2 \gamma \alpha' \psi'(x) \omega' - \pi \pi x^2 \tan^2 \gamma \alpha' \psi'(x) \alpha' \omega' \right] dx + H \sigma_0 (x - x_1) \alpha \psi(x) - 2 \pi c x \tan \gamma \alpha' \psi'(x) \right] dx. \tag{26} \]

We introduce the following notations:

\[
\int_0^h \rho \pi x^2 \tan^2 \gamma \psi'(x) \omega' \right] dx = T, \tag{27}
\int_0^h E \pi x^2 \tan^2 \gamma \psi'(x) \omega' \right] dx = U, \tag{28}
\int_0^h 2 \pi c x \tan \gamma \psi'(x) \omega' \right] dx = M, \tag{29}
\int_0^h H \sigma_0 (x - x_1) \alpha \psi(x) \omega' \right] dx = L. \tag{30}
\]

Substituting expressions (27)–(30) into (26) we obtain:

\[ S_2 = \frac{\pi}{2a_0} [\omega^2 Ta^2 - (U + M) \alpha^2 + La]. \tag{31} \]

Thus, on a set of functions (25) the functional (26) becomes a function with the independent variable \( \alpha \), which has the form (31).

The necessary condition for stationarity of the functional (31) (i.e., the existence of an extreme) is that the first derivative equals zero, namely:

\[ \frac{\partial S_2}{\partial \alpha} \cdot \delta \alpha = 0, \tag{32} \]

which results in the following equation:

\[ 2 \omega^2 Ta - 2(U + M) \alpha + L = 0, \tag{33} \]

from which we can find the required value of the parameter \( \alpha \). It will be:

\[ \alpha = \frac{L}{2(U + M - \omega^2 T)}. \tag{34} \]

We take as the basis function \( \psi(t) \) the form of forced longitudinal vibrations of the spike with constant cross-section with one end strongly fixed, which arise under the influence of the longitudinal harmonic force with frequency \( \omega \), applied at the point \( x = x_1 \).

According to [9], the form of forced vibrations of mentioned spike is as follows:

\[ \psi(x) = D_2 \sin ax \quad \text{for} \quad x < x_1, \tag{35} \]

\[ \psi(x) = D_2 \cos a(h - x) \quad \text{for} \quad x > x_1, \tag{36} \]

where

\[ D_2 = \frac{1}{aE} \frac{\cos a(h - x_1)}{\cos ah}, \tag{37} \]

\[ D_2 = \frac{1}{aE} \frac{\sin ax_1}{\cos ah}, \tag{38} \]

\[ a = \omega \sqrt{\frac{\mu}{E}}, \tag{39} \]

\[ \mu - \text{momentum of the spike; } F - \text{cross-sectional area of the spike; } E - \text{Young's modulus for the material of the cone-shaped elastic body; } h - \text{length of the elastic conical body; } \omega - \text{the frequency of forced vibrations of elastic conical body.} \]

It is easy to check that the boundary conditions for basis functions (35) and (36) are satisfied and therefore, taken basis functions satisfy the requirements of the Ritz method.

To determine the parameter \( a \) we calculate the parameters \( T, U, M \) and \( L \).

The result is:

\[ T = \rho \pi t \tan^2 \gamma \left\{ D_0 \left( \frac{x_1^3}{6} + \frac{x^2 \sin 2ax_1}{6} - \frac{x \cos 2ax_1}{4a} \right) + \frac{\sin 2ax_1}{8a} \right\} + D_1 \left( h^2 - \frac{x_1^3}{6} + \frac{x^2 \sin (2ah - 2ax_1)}{4a} \right) + \frac{h}{4a} \frac{x \cos (2ah - 2ax_1)}{8a} \sin (2ah - 2ax_1) \right\}, \tag{40} \]

\[ U = E \pi t \tan^2 \gamma \left\{ D_2 \frac{x^2 \sin ax_1}{4a} + D_3 \frac{x \sin 2ax_1}{6} + D_4 \left( \frac{x^2 \sin (2ah - 2ax_1)}{4a} \right) \right\} + \frac{x_1 \cos 2ax_1}{4a} - \frac{\sin 2ax_1}{6} - \frac{\sin (2ah - 2ax_1)}{4a} \right\}, \tag{41} \]

\[ M = 2 \pi c D_1 \tan \gamma \left\{ \frac{x_1^2}{4} \frac{x \sin 2ax_1}{4a} + \frac{1 - \cos 2ax_1}{8a^2} \right\} + \frac{+ 2 \pi c D_3 \tan \gamma \left[ \frac{1 - \cos 2a(h - x_1)}{8a^2} - \frac{(h - x_1)^2}{4} + \frac{h(h - x_1)^2}{2} \right] + \frac{x_1 \sin 2a(h - x_1)}{4a} \right\}, \tag{42} \]

\[ L = HD_1 \sin ax_1. \tag{43} \]

Substituting expressions (40)–(43) into the expression (34), we obtain the necessary value of the pa-
THE THEORY OF LONGITUDINAL VIBRATIONS OF A CONICAL ELASTIC BODY IN AN ELASTIC MEDIUM

Considering the expressions (25), (35) and (36), we obtain expressions for the form of forced oscillations of a continuous elastic body fixed in the soil. They have the following form:

\[ \varphi(x) = \alpha \cdot D_1 \sin(ax) \text{, for } x \leq x_1, \]
\[ \varphi(x) = \alpha \cdot D_2 \cos(a(h-x)) \text{, for } x > x_1, \]  
(44)

where \( \alpha \) is defined according to (34).

Substituting the expression (44) into (22), we finally obtain the law for forced vibrations of a continuous elastic body fixed in the soil:

\[ y(x, t) = D_1 \alpha \sin ax \sin \omega t \text{, for } x \leq x_1, \]
\[ y(x, t) = D_2 \alpha \cos a(h-x) \sin \omega t \text{, for } x > x_1. \]  
(45)

On the basis of the results of theoretical investigations of forced vibrations of a continuous elastic body (root of sugar beet) fixed in the soil, we made the specific computation of the amplitude of these vibrations.

In the program Mathcad it was realized the calculation of the dependence of the amplitude of forced longitudinal vibrations of the conical body in relation to the \( c \) – coefficient of elastic deformation of the soil and to the distance \( x \) (distance between the cross-section from the conditional point of fixation \( x_1 \), Hz)

at the frequency of the perturbing force \( \nu = 10 \text{ Hz } \) and \( \nu = 20 \text{ Hz } \) and at the amplitude of this force \( H = 500 \text{ N. } \)

According to the calculated results there were created graphs (Fig. 4).

As can be seen from the graphs, with increasing of the coefficient \( c \) (elastic deformation of the surrounding soil) the amplitude of forced vibrations of the root decreases, with increasing of the distance between the cross-section of root and the conditional point of fixation for \( x < x_1 \) the amplitude of the opposite – increases, while for \( x > x_1 \) – almost unchanged.

We calculated also the correlation between the amplitude of forced longitudinal vibrations of the mentioned elastic body and the amplitude of the perturbing force at the frequency \( \nu = 20 \text{ Hz } \) for the same parameters of the body, as above. The calculation was realized using the program Mathcad where the perturbing force was changing in the range 100–600 \( N \) for different cross-sections of the body. The graphs shown in Fig. 5 are the results of this calculation.

As can be seen from graphs, with an increase in the amplitude of the perturbing force the amplitude of forced longitudinal vibrations of the body of the root increases linearly. While, below the point of fixation \( x < 0.15 \text{ m } \) with the increase in the distance of the

\[ \text{Fig. 4. The dependence of the amplitude of forced longitudinal vibrations of the conical body of root fixed in the soil in relation to the coefficient } c \text{ (coefficient of elastic deformation of the surrounding soil) and to the distance } x \text{ (distance between the cross-section from the conditional point of fixation } x_1, \text{ Hz).} \]
with the soil during their harvesting by vibrational method.

### CONCLUSIONS

On the basis of the use of the variation principle of Ostrogradskij-Hamilton we obtained equations for calculation of the natural frequencies of any order for longitudinal vibrations of a continuous elastic body with one fixed end. Thus, there were obtained the analytical expressions for finding the first and second eigenfrequency and also expressions for finding the amplitude of forced vibrations for any cross-section of a continuous elastic body with respect to its balance position. Given theoretical analyses open up opportunities for study the process of disruption of connections of crop roots

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**Fig. 5.** Dependence between the amplitude of forced longitudinal vibrations of the elastic body of root and the amplitude of the perturbing force \( x < x_1, \nu = 20 \text{ Hz} \)

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root cross-section from the conditional point of fixation \( O_1 \) the amplitude also increases. Thus, for \( x = 0.07 \text{ m} \) the range of the amplitude is 0.13–0.8 mm; for \( x = 0.1 \text{ m} \) in the range 0.19–1.14 mm; for \( x = 0.15 \text{ m} \) (point of fixation) – in the range 0.28–1.7 mm. However, above the point of fixation \( (x > 0.15 \text{ m}) \) with the increase in the distance of the cross-section of the root from the conditional point of fixation \( O_1 \), the amplitude remains almost unchanged.

Because the first eigenfrequency of the considered example of a sugar beet as an elastic conical body is not less than 75 Hz, and the frequency of the perturbing force by for technological and technical reasons cannot be greater than 20 Hz, the resonance case is not possible, in fact. In addition, the calculated value of the amplitude of forced longitudinal vibrations of a root crop body, which is in the range 0.13–1.7 mm shows that the rupture of the root at its longitudinal deformation is also impossible.

### CONCLUSIONS

On the basis of the use of the variation principle of Ostrogradskij-Hamilton we obtained equations for calculation of the natural frequencies of any order for longitudinal vibrations of a continuous elastic body with one fixed end. Thus, there were obtained the analytical expressions for finding the first and second eigenfrequency and also expressions for finding the amplitude of forced vibrations for any cross-section of a continuous elastic body with respect to its balance position. Given theoretical analyses open up opportunities for study the process of disruption of connections of crop roots
Теория продольных колебаний конусообразного упругого тела в упругой среде

В. М. Булгаков 1, В. В. Адамчук 2,
Н. В. Головач 3, Д. Орсагова 3

1 Национальный университет биоресурсов и природопользования Украины
Ул. Героев Обороны, 15, Киев, Украина, 03041
e-mail: vbulgakov@meta.ua

2 Национальный научный центр “Институт механизации и электрификации сельского хозяйства”
НААН Украины
Ул. Вокзальная, 11, Глеваха-1, Васильковский р-н, Киевская обл., Украина, 08631
e-mail: adamchukvv@mail.ru

3 Славацкий аграрный университет в Нитре

Цель. Создать теорию продольных колебаний сплошного упругого тела в упругой среде с одним закрепленным концом. Примером такого тела может быть расположенный в почве корнеплод сахарной свеклы, причем почва, в которой он находится, также является упругой средой. Методы. Применены принцип стационарного действия Остроградского–Гамильтона и метод Ритца. Результаты. При помощи метода Ритца получено уравнение частот Ритца для рассматриваемого колебательного процесса. Выписаны аналитические выражения для определения первой и второй собственных частот колебаний тела и амплитуды вынужденных колебаний любого его поперечного сечения. Найдены значения первой и второй собственных частот рассматриваемого упругого тела с конкретными геометрическими и физическими параметрами. Составлены графики зависимости первой и второй собственных частот от коэффициента упругой деформации почвы как упругой среды, а также графики зависимости амплитуды вынужденных колебаний указанного тела от коэффициента с упругой деформации почвы и расстояния поперечного сечения тела от условной точки закрепления. Получены графики зависимости амплитуды вынужденных колебаний упругого тела от изменения амплитуды и частоты возмущающей силы. Выводы. Обоснована невозможность наступления резонанса, поскольку частота возмущающей силы не может быть равна частоте собственных колебаний упругого тела по технологическим и техническим причинам. Доказано, что из-за малости амплитуды продольных колебаний упругого тела его разрыв при продольных деформациях невозможен.

Ключевые слова: сплошное упругое тело, упругая среда, функционал Остроградского–Гамильтона, продольные колебания, собственные формы и частоты, метод Ритца.

REFERENCES


INTRODUCTION

It is commonly known that the main aim of plant quarantine is protection of state plant resources from introduction, spread and damage of quarantine pests, the list of which is formed on the basis of pest risk analysis (PRA). The aim of PRA is not just to define the status of the analyzed pests for a certain area (which allows characterizing them as regulated quarantine, regulated non-quarantine or non-regulated pests), but also to select proper phytosanitary measures to prevent the introduction of these pests into new territories or to localize and eradicate existing outbreaks.

It is rather time and effort consuming to conduct full-scale PRA, the algorithm and methodology of which is stipulated in full detail in international [1, 2] and regional standards [3] and national guidances [4]. PRA is conditionally divided into four stages: initiation, pest risk assessment, pest risk management and official registration of PRA process in a protocol format. Here the second stage is the most critical, as it is aimed at assessing the probability of introduction and establishment of adventives species in new territories, free from the latter, which is primarily conditioned by the presence of host plants and the suitability of ecoclimatic conditions [5–7].
There are different approaches to solve this task, in particular, there are suggestions of defining the area of potential establishment of adventive pests using the statistical data of FAOSTAT, EUROSTAT, JRC or SEAMLESS regarding the availability of host plant, and their distribution in the PRA area [8–11]. Instead, it is recommended to assess the climatic suitability in the PRA area via the degree of its correspondence to ecoclimatic conditions in the area of current distribution of the pest analyzed – by the sum of effective temperatures, precipitation, hydrothermal coefficient, etc. Complex models [12, 13] and software were designed to solve the mentioned tasks, for instance, CAPRA program [8, 14] or CLIMEX program [15].

The application of the listed instruments envisages processing of a considerable amount of disaggregated data and obtaining the final assessment only at the end of a multi-stage algorithm. But it is also possible to assess the probability of establishment of adventive pests in a new territory via more convenient and accelerated procedure, using other software – Agro Atlas [16], MapInfo v.11.0 [17] and Idrisi Taiga [18] which combines geoinformational technologies and the biogeographical data and allows conducting ecological and geographical analysis and simulating potential spatial distribution of an organism using precise determination of a current area of the species and agroclimatic conditions of its establishment. This was set as the aim of our studies.

MATERIALS AND METHODS

The availability of economically relevant agricultural crops, grown in Ukraine, which are host plants for adventive plant pests, was determined using Agro Atlas software, which allows determining spatial location of over 500 cultivated plant species, their wild relatives and weeds [14].

Such software as MapInfo v.11.0, Agro Atlas and Idrisi Taiga [16–18] which allow designing compositions of scan-line and vector layers using computer-aided procedures, was applied to determine:

- environmental factors in the area of current pest distribution, which limit the spread of the species;
- quantitative ecological amplitude of the species regarding each limiting factor, comparing similar indices from the area of the adventive species and ecological maps by the methods of overlapping layers-maps and data extraction;
- ecologically suitable territories on the map of Ukraine for the distribution of the species in terms of each limiting factor (reclassification);
- potential area of establishment of the adventive species in the PRA area (Ukraine), favourable for its existence, via the combination of ecologically suitable territories into a unified map.

RESULTS AND DISCUSSION

The harmonization of phytosanitary legislation of Ukraine with EU requirements envisages the practical introduction of periodic reviews of the lists of regulated pests, including the ones due to the introduction of changes to the corresponding lists of the European and Mediterranean Plant Protection Organization (EPPO) – List A1 (the list of pests recommended for regulation, absent in the EPPO member countries) and List A2 (the list of pests recommended for regulation, present but not widely distributed in the EPPO region, and which are officially controlled).

Although this norm was reflected in the Law of Ukraine “On Plant Quarantine”, the latest review of the list of regulated pests of Ukraine was in 2010, whereas EU lists have been edited dozens of times during 2010–2016. In particular, such changes were noted for the status of fruit crop pests: lemon tree borer Oemona hirta (Coleoptera: Cerambycidae) and false codling moth Thaumatotibia leucotreta (Lepidoptera: Tortricidae), which were transferred from the EPPO Alert list to A1 and A2 Lists, respectively, after the pest risk analysis conducted for EPPO countries.

As fruit production is one of the relevant branches of Ukrainian agriculture, there is a need for pest risk analysis and for the determination of the status of these pests in the territory of this country. The possibility of assessing the probability of establishment of adventive species in a new territory was elaborated in the framework of the analysis, using software – Agro Atlas, MapInfo v.11.0 and Idrisi Taiga.

O. hirta – lemon tree borer, is a pest of citrus fruit trees, wide-spread in New Zealand [19, 20]. In 1983 and 2010 it was intercepted in plants for planting of Wisteria, imported to Great Britain from New Zealand. In 2010, this species was included in the EPPO Alert list on the initiative of Great Britain, and in 2013 it was transferred to List A1 (the list of pests recommended for regulation, absent in the EPPO region) according to PRA results. Express-PRA, performed by the experts of the UK National Plant Protection Organization, and a subsequent full-scale PRA conducted by EPPO ex-
perts demonstrated that this pest poses a threat for the EPPO region.

The main host plants for this pest are citrus fruit trees (lemon, tangerine and grapefruit). In addition, it may infest about 200 species of plants from 81 families. Most infested plants are trees and shrubs, but the host list also includes vines and lianas as well as tall-growing perennial grasses with a tall stalk and a considerable number of ornamental plants. In New Zealand *O. hirta* is mainly viewed as a pest of citrus fruit trees. However, considerable damage to apple-tree gardens, vineyards and persimmon was registered in the 90s of the last century.

In general, this pest may infest over 40 species of fruit plants, including apple-tree, pear-tree, cherry-tree, plum-tree, peach-tree, grapes, walnut tree, persimmon, almond-tree, blueberry as well as many foresters and ornamental species of trees (poplar, birch, acacia, alder, elm, chestnut, pine, eucalyptus, wisteria, hibiscus, genista, hawthorn, spindle, lilac, rose). Many of these plants are grown in Ukraine (Table 1).

Spatial location of the majority of these plants in Ukraine may be determined using Agro Atlas software (Fig. 1).

Therefore, it was determined that the host-plant range on the whole territory of Ukraine may provide sufficient resources for the development of *O. hirta*.

While determining the possibility of establishment of this pest in Ukraine using MapInfo and Idrisi Taiga software, the following climatic factors, which may limit the pest development, were defined: the indices of average annual air temperature, average temperature of the warmest month (June) and the sum of active temperatures exceeding 10 °C. The results of determining Ukrainian territories, ecologically suitable for the development of *O. hirta* regarding each limiting factor are presented in Fig. 2.

It was determined that some climatic indices of the Ukrainian territory are partially satisfactory for the de-

<table>
<thead>
<tr>
<th>Fruit trees</th>
<th>Shrub</th>
<th>Species of ornamental and forest trees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple (<em>Malus</em>)</td>
<td>Grapes (<em>Vitis vinifera</em>)</td>
<td>Poplar (<em>Populus</em>)</td>
</tr>
<tr>
<td>Pear (<em>Pyrus</em>)</td>
<td>Hawthorn (<em>Crataegus</em>)</td>
<td>Acacia (<em>Acacia</em>)</td>
</tr>
<tr>
<td>Cherry (<em>Prunus cerasus</em>)</td>
<td>Spindle (<em>Euonymus</em>)</td>
<td>Alder (<em>Alnus</em>)</td>
</tr>
<tr>
<td>Plum (<em>Prunus domestica</em>)</td>
<td>Lilac (<em>Syringa vulgaris</em>)</td>
<td>Pine (<em>Pinus</em>)</td>
</tr>
<tr>
<td>Peach (<em>Prunus persica</em>)</td>
<td>Rose (<em>Rosa</em>)</td>
<td>Chestnut (<em>Castanea</em>)</td>
</tr>
<tr>
<td>Nectarine (<em>Prunus persica var. nucipersica</em>)</td>
<td>Wisteria (<em>Wisteria</em>)</td>
<td>Birch (<em>Betula</em>)</td>
</tr>
<tr>
<td>Walnut (<em>Juglans</em>)</td>
<td>Hibiscus (<em>Hibiscus</em>)</td>
<td>Elm (<em>Ulmus</em>)</td>
</tr>
<tr>
<td>Almond (<em>Prunus dulcis</em>)</td>
<td>Blueberry (<em>Vaccinium</em>)</td>
<td>Sycamore (<em>Platanus</em>)</td>
</tr>
<tr>
<td>Apricot (<em>Prunus armeniaca</em>)</td>
<td>Gooseberry (<em>Ribes uva crispa</em>)</td>
<td>Oak (<em>Quercus</em>)</td>
</tr>
</tbody>
</table>
development of *O. hirta* in different regions. However, judging by the combination of climatic factors the general area of establishment is considerably smaller and covers only the southern part of Odessa region, an inconsiderable part of south-western Mykolayiv region, the south-western territory of Kherson region and almost the entire territory of the Crimean Peninsula.

*T. leucotreta* (*Lepidoptera:Tortricidae*) – false codling moth, is a pest of fruit and field crops. The current area of its distribution covers a considerable part of the African continent [21]. In 1984, this pest was first found outside its area, in the planting of macadamia nuts in Israel, and in 2003 – on cotton and castor plant. In 2009, the pest was registered in a greenhouse in the Netherlands, and later – in Great Britain and Sweden. From 2001 to 2010 there were over 50 cases of interception of the pest in citrus fruits, imported from South Africa to EPPO countries [22]. As to EPPO region, this pest has been found at present on inconsiderable local territories, where cotton and castor plants are grown in Israel.

Table 2. Agricultural crops in Ukraine endangered by false codling moth

<table>
<thead>
<tr>
<th>Host plants of the pest</th>
<th>English name</th>
<th>Family</th>
<th>Crops grown in Ukraine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Capsicum</em> spp.</td>
<td>Pepper</td>
<td>Solanaceae</td>
<td>Pepper</td>
</tr>
<tr>
<td><em>Citrus reticulata</em> &amp; hybrids</td>
<td>Mandarin orange</td>
<td>Rutaceae</td>
<td></td>
</tr>
<tr>
<td><em>Citrus sinensis</em> &amp; hybrids</td>
<td>Orange</td>
<td>Rutaceae</td>
<td></td>
</tr>
<tr>
<td><em>Citrus paradisi</em></td>
<td>Grapefruit</td>
<td>Rutaceae</td>
<td></td>
</tr>
<tr>
<td><em>Gossypium</em> spp.</td>
<td>Cotto</td>
<td>Malvaceae</td>
<td></td>
</tr>
<tr>
<td><em>Litchi chinensis</em></td>
<td>Litchi, Litchee</td>
<td>Sapindaceae</td>
<td></td>
</tr>
<tr>
<td><em>Macadamia</em> spp.</td>
<td>Mango</td>
<td>Anacardiaceae</td>
<td></td>
</tr>
<tr>
<td><em>Mangifera indica</em></td>
<td>Peach</td>
<td>Rosaceae</td>
<td></td>
</tr>
<tr>
<td><em>Prunus persica</em></td>
<td>Nectarine</td>
<td>Rosaceae</td>
<td>Nectarine</td>
</tr>
<tr>
<td><em>Prunus persica</em> var. nucipersica</td>
<td>Avocado</td>
<td>Rosaceae</td>
<td>Peach</td>
</tr>
<tr>
<td><em>Persea americana</em></td>
<td>Beans</td>
<td>Fabaceae</td>
<td>Kidney beans</td>
</tr>
<tr>
<td><em>Phaseolus vulgaris</em></td>
<td>Apricot</td>
<td>Rosaceae</td>
<td>Apricot</td>
</tr>
<tr>
<td><em>Prunus armeniaca</em></td>
<td>Plum</td>
<td>Rosaceae</td>
<td>Plum</td>
</tr>
<tr>
<td><em>Prunus domestica</em></td>
<td>Cherry</td>
<td>Rosaceae</td>
<td>Cherry</td>
</tr>
<tr>
<td><em>Prunus cerasus</em></td>
<td>Guava</td>
<td>Myrtaceae</td>
<td></td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td>Pomegranate</td>
<td>Lythraceae</td>
<td></td>
</tr>
<tr>
<td><em>Punica granatum</em></td>
<td>Oak</td>
<td>Fagaceae</td>
<td>Oak</td>
</tr>
<tr>
<td><em>Quercus robur</em></td>
<td>Castor oil plant</td>
<td>Euphorbiaceae</td>
<td>Castor plant</td>
</tr>
<tr>
<td><em>Ricinus communis</em></td>
<td>Rose</td>
<td>Rosaceae</td>
<td>Wild rose</td>
</tr>
<tr>
<td><em>Rosa</em> sp.</td>
<td>Sorghum</td>
<td>Poaceae</td>
<td>Sorghum</td>
</tr>
<tr>
<td><em>Sorghum</em> sp.</td>
<td>Eggplant</td>
<td>Solanaceae</td>
<td>Eggplant</td>
</tr>
<tr>
<td><em>Solanum melongena</em></td>
<td>Tomato</td>
<td>Solanaceae</td>
<td>Tomato</td>
</tr>
<tr>
<td><em>Solanum lycopersicum</em></td>
<td>Grape</td>
<td>Vitaceae</td>
<td>Grape vine</td>
</tr>
<tr>
<td><em>Vitis vinifera</em></td>
<td>Maize</td>
<td>Poaceae</td>
<td>Corn</td>
</tr>
</tbody>
</table>

Fig. 2. Ecologically suitable territories for the development of *Oemona hirta* in Ukraine according to the indices of average annual air temperature (a); average temperature of the warmest month – June (b); the sum of active temperatures exceeding 10 °C (c); potential area of pest establishment in Ukraine (d)
The studies demonstrated that the potential area of *T. leucotreta* in Ukraine may include inconsiderable territories of the Black Sea coast, Tarkhankut Peninsula and Kerch Peninsula in the Crimea, the conditions of which are suitable for the development of false codling moth only during summer months.

**CONCLUSIONS**

The application of MapInfo v.11.0, AgroAtlas and Idrisi Taiga software is efficient to determine the probability of establishment of adventive plant pests in new, pest-free territories, and to define the borders of their potential area of distribution, which allows recommending this software for pest risk assessment, especially when the quick procedure is required.

It was determined that a potential area of establishment of *O. hirta* in Ukraine is the southern part of Odessa region, an inconsiderable part of south-western Mykolayiv region, south-western territory of Kherson region and almost the entire territory of the Crimean Peninsula. A potential area for *T. leucotreta* in Ukraine is an inconsiderable territory of the Black Sea coast, Tarkhankut Peninsula and Kerch Peninsula in the Crimea.

**Pрактична методологія аналізу ймовірності акліматизації адвентивних фітофаґів**

Ю. Е. Клечковський 1, Л. Г. Тітова 1, О. В. Палагіна 1, Л. А. Пилипенко 2, А. Д. Орлінський 3

1 Дослідна станція карантину винограду і плодових культур Інституту захисту рослин НААН
2 Інститут захисту рослин НААН
3 Секретаріат Європейської та Середземноморської організації захисту рослин

Бульв. Ришара Ленуара, 21, Париж, Франція, 75011
e-mail: titova_l@mail.ru; liliya.pylypenko@gmail.com; Orlinski@eppo.int

**Мета.** Розробити практичну методологію оцінки ймовірності акліматизації адвентивних шкідливих організмів в Україні (за межами їхніх існуючих ареалів) за використання спеціалізованих комп’ютерних програм.

**Методи.** Міжнародні стандарти з аналізу фітосанітарного ризику ISPM № 2, ISPM № 11, ISPM № 21, PM 5/1(1), PM 5/2(2), PM 5/3(5), PM 5/5(1) і програмне комп’ютерне забезпечення Agro Atlas (Afonin et al., 2008), MapInfo v.11.0 (Pitney Bowes) та Idrisi Taiga (Clarklabs).

**Результати.** Досліджено наявну кормову ба-
Практическая методология анализа вероятности акклиматизации в Украине вредных вредителей по применению компьютерных программ Agro Atlas, MapInfo v.11.0 и Idrisi Taiga. Установлено, что потенциальной зоной акклиматизации O. hirta является южная часть Одесской области, незначительная часть юго-запада Николаевской области, юго-западная территория Херсонской области и почти вся территория Крымского полуострова. Потенциальным ареалом для T. leucotreta служат незначительные территории побережья Черного моря – полуостровов Тарханкут и Керченский в Крыму.

Ключевые слова: практическая методология, анализ фитосанитарного риска, акклиматизация, Oemona hirta, Thaumatotibia leucotreta.

REFERENCES
12. Jeschke JM, Strayer DL. Usefulness of bioclimatic mo-

Цель. Разработать практическую методологию оценки вероятности акклиматизации вредных вредителей и использовать специализированные компьютерные программы. Методы. Международные стандарты по проведению анализа фитосанитарного риска ISPM № 2, ISPM № 11 ISPM № 21 PM 5/1(1), PM 5/2(2), PM 5/3(5), PM 5/5(1) и программное компьютерное обеспечение Agro Atlas (Afonin et al., 2008), MapInfo v.11.0 (Pitney Bowes) и Idrisi Taiga (Clarklabs).

Результаты. Исследована имеющаяся кормовая база (растения-хозяев) и соответствие экологических условий Украины (как зоны анализа фитосанитарного риска) показателям современного ареала на примере вредителей Oemona hirta (лимонного усача) и Thaumatotibia leucotreta (ложной яблоневой моли) и определены потенциальные зоны их акклиматизации в Украине. Выводы. Доказаны эффективность и удобство методов оценки риска акклиматизации в Украине вредных вредителей по применению компьютерных программ Agro Atlas, MapInfo v.11.0 и Idrisi Taiga. Установлено, что потенциальной зоной акклиматизации O. hirta является южная часть Одесской области, незначительная часть юго-запада Николаевской области, юго-западная территория Херсонской области и почти вся территория Крымского полуострова. Потенциальным ареалом для T. leucotreta служат незначительные территории побережья Черного моря – полуостровов Тарханкут и Керченский в Крыму.

Ключевые слова: практическая методология, анализ фитосанитарного риска, акклиматизация, Oemona hirta, Thaumatotibia leucotreta.

REFERENCES
12. Jeschke JM, Strayer DL. Usefulness of bioclimatic mo-


INTRODUCTION

At present bacterial plant diseases become ever more widespread and bring even more damage to agriculture. Along with such objective factors as the change in climatic conditions, the main reasons include some subjective factors: the absence of resistant cultivar, and almost complete unavailability of preparations, protecting from agents of bacteriosis [1].

One of the most relevant elements of the system of controlling the distribution of bacterial plant diseases is timely diagnostics and determination of a disease agent. It allows ensuring efficient protection of plants and mitigating the possibility of disease distribution.

As symptoms of many plant diseases of different etiology are similar, it is very important to isolate and identify the agent preferably in laboratories, sufficiently equipped for this purpose. It is impossible to determine the real reasons of plant diseases without the microbiological analysis and to plan and implement the corresponding complex of measures to limit the distribution of the agent and to reduce the loss of agricultural crop harvest [2].

The main requirements to the methods, applied for diagnostics and identification of phytopathogenic bacteria, are accuracy and reproducibility of the obtained results as well as minimal time, resources and labor expenditures.

Modern methods, applied to identify phytopathogenic bacteria, may be divided into microbiological, immunological, and molecular-biological ones. The application of microbiological methods is often sufficient for most laboratories to conduct routine analyses in detecting and diagnosing bacterial disease agents. In addition, their advantage is the possibility to obtain in-
formation about the biological specificities of the agent and the data, required to detect infection sources. However, the application of such methods is limited by a considerable duration of analyses and the requirement to use a great number of different media and reagents. To accelerate routine microbiological analyses, many companies offer commercial kits of known microbiological tests – so called test-systems [3]. The kits of bioMerieux company are some of the most commonly used test-systems.

Diagnostic API-systems were elaborated to identify a wide spectrum of microorganisms to be used in scientific and practical laboratories [4]. However, they are mostly used to identify bacteria, pathogenic and conditionally pathogenic for humans, and those of practical value for humans.

The aim of present work was to validate the suitability of commercial API 20E test-system (bioMerieux) for the identification and characterization of facultative gram-negative phytopathogenic bacterial isolates.

**MATERIALS AND METHODS**

API 20E test-system (bioMerieux), containing test-plates and a set of reagents, was used to identify gram-negative facultative anaerobic rods. When used with the corresponding software of the manufacturer, this system allows identifying bacteria of *Enterobacteriaceae* family and some other gram-negative bacteria.

The possibility of using API 20E test-systems to identify phytopathogenic bacteria was analyzed taking into consideration the availability of diagnostically relevant tests for these bacterial species in the test-system and the data of the properties of bacteria, kept in the collection of live cultures of the Department of Phytopathogenic Bacteria of the D. K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine.

The following bacterial strains were used in the work:

- *Erwinia amylovora* (Burril 1882) Winslow et al. 1920 type strain UKM B-1095 (ATCC 15580, NCPPB 683);
- *Pectobacterium carotovorum* (Jones 1901) Hauben et al. 1999 type strain UKM B-1075 (ATCC 15713, NCPPB 312);

Cultural-physiological and biochemical properties of phytopathogenic bacteria were studied by conventional microbiological methods [2, 5]. API 20E test-system was applied according to the manufacturer’s protocol (bioMerieux, France). The results were registered after the cultivation for 24 h at 28 °C and compared against the data, obtained using classic methods.

**RESULTS AND DISCUSSION**

All species of *Erwinia* genus and species of *Pectobacterium* genus, which have been rather recently reclassified from *Erwinia* genus and are most frequent agents of soft rot of plants, belong to phytopathogenic gram-negative facultative anaerobic bacteria. The strains of *Pantoea agglomerans*, previously also referred to *Erwinia* genus, are now referred to the group of gram-negative facultatively anaerobic bacteria. *P. agglomerans* is present on the surface of healthy plants as a representative of epiphytic microflora and may induce the infectious process as a facultative pathogen. The representatives of the mentioned species were used to estimate the reasonability of applying API 20E test-system to identify phytopathogenic bacteria (Table 1).

The diagnostics of bacteriosis agent also requires the determination of pathogenic properties of the isolates and the confirmation of their participation in pathogenesis after artificial infection of plants.

Recently such commercial test-kits, as API 20E, API 20Ne, Microbact NE, BIOLOG GN, have been used to study biochemical and physiological properties of microorganisms more frequently [3].

---

### Table 1. Cultural and physiological properties of *E. amylovora*, *P. carotovorum*, *P. agglomerans*

<table>
<thead>
<tr>
<th>Test</th>
<th><em>E. amylovora</em> UKM B-1095</th>
<th><em>P. carotovorum</em> UKM B-1075</th>
<th><em>P. agglomerans</em> P324</th>
</tr>
</thead>
<tbody>
<tr>
<td>The form of cells</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Gram's staining</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Motility</td>
<td>Mobile</td>
<td>Mobile</td>
<td>Mobile</td>
</tr>
<tr>
<td>Pigment formation</td>
<td>−</td>
<td>−</td>
<td>Yellow</td>
</tr>
<tr>
<td>Fermentation of glucose:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aerobic</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>anaerobic</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pectinase</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Oxidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Note. (−) – negative sign; (+) – positive sign.
APPLICATION OF COMMERCIAL TEST-SYSTEMS TO IDENTIFY GRAM-NEGATIVE

Test-systems are easy to use; they ensure faster and more accurate identification of a larger number of bacterial strains.

API 20E test-system, designed to identify bacteria of Enterobacteriaceae family, is based on 21 standardized biochemical tests. This system is a plate (a strip) of 20 microwells, containing dehydrated substrates (Figure). The cell suspension of the investigated microorganism in physiological solution and required reagents are introduced to each well in sterile conditions in accordance to the manufacturer’s instructions. The registration of results is performed 24 h later, noting the change in the color of the medium in accordance with the table of registering the results.

The microbiological tests, presented in API 20E test-system, include such diagnostically relevant tests for Erwinia, Pectobacterium, Pantoea species as tests for the presence of arginine dihydrolase, phenylalanine deaminase, urease, utilization of citrates, mannitol, rhamnose [6].

The advantage of API 20E system is the velocity of conducting rather a large number of biochemical tests as well as their standardization which ensures the possibility of comparing results, obtained in dif-

The general layout of API 20E test-system

<table>
<thead>
<tr>
<th>Test/enzyme</th>
<th>E. amylovora UKM B-1095 Using API 20E</th>
<th>By conventional methods</th>
<th>E. amylovora according to the literature data [6]</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-galactosidase</td>
<td>−</td>
<td>n/d</td>
<td>a/d</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>−</td>
<td>n/d</td>
<td>−</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>−</td>
<td>n/d</td>
<td>−</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>−</td>
<td>n/d</td>
<td>−</td>
</tr>
<tr>
<td>Utilization of citrates</td>
<td>−</td>
<td>−</td>
<td>a/d</td>
</tr>
<tr>
<td>Formation of H₂S</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tryptophane deaminase</td>
<td>−</td>
<td>n/d</td>
<td>−</td>
</tr>
<tr>
<td>Formation of indole</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Formation of acetoin (reaction of Voges-Proskauer)</td>
<td>+</td>
<td>+</td>
<td>a/d</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of D-glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>of D-mannitol</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>of inositol</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>of D-sorbite</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>of L-rhamnose</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>of D-sucrose</td>
<td>+</td>
<td>+</td>
<td>a/d</td>
</tr>
<tr>
<td>of D-melibiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>of amygdaline</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>of L-arabinose</td>
<td>−</td>
<td>−</td>
<td>var.</td>
</tr>
<tr>
<td>Reduction of nitrates</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Note. Here and in Tables 3, 4 (−) – negative sign; (+) – positive sign; var. – variable feature; a/d – absent data; n/d – not defined.
ferent laboratories. API-systems are widely used to characterize and identify microorganisms of different groups [4].

Tables 2–4 present the results of studying the properties of *E. amylovora* UKM B-1095, *P. carotovorum* UKM B-1075 and *P. agglomerans* P324 using the mentioned test-system. In most cases they corresponded to the characteristics of these bacterial species, mentioned in the literature. There was also an observed agreement between the results, obtained by conventional methods and using API 20E test-system.

It should be noted that some authors indicate the inconsistency of results, obtained while using conventional microbiological methods and API test-systems [3]. We observed some differences while studying the ability of strains *E. amylovora* UKM B-1095 and *P. agglomerans* P324 to dilute gelatine (Table 2, 4). According to the literature data, these bacterial species are capable of diluting gelatine, however, contrary results were registered while using API 20E test-system. The use of the conventional method to study this feature demonstrated that *E. amylovora* UKM B-1095 and *P. agglomerans* P324 dilute gelatine, but it occurs during long-lasting cultivation.

The software, provided by the manufacturer of API 20E test-system, was used to confirm that *P. agglomerans* P324 belongs to *P. agglomerans* species with rather a high similarity level. The strain *P. carotovorum* UKM B-1075 was also defined as *P. agglomerans*, but with 40 % similarity level.

API 20E test-system is primarily designed for clinical use; it contains tests, relevant for the identification of gram-negative bacteria of clinical and dietary origin. However, recently its application has been spread to other spheres of microbiology as well [4, 7].

### Table 3. Physiological and biochemical properties of *Pectobacterium carotovorum*

<table>
<thead>
<tr>
<th>Test/enzyme</th>
<th><em>P. carotovorum</em> UKM B-1075</th>
<th><em>P. carotovorum</em> according to the literature data [6]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Using API 20E</td>
<td>By conventional methods</td>
</tr>
<tr>
<td><strong>β-galactosidase</strong></td>
<td>+</td>
<td>n/d</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>−</td>
<td>n/d</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>−</td>
<td>n/d</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>−</td>
<td>n/d</td>
</tr>
<tr>
<td>Utilization of citrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Formation of H₂S</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tryptophane deaminase</td>
<td>−</td>
<td>n/d</td>
</tr>
<tr>
<td>Formation of indole</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Formation of acetoin (reaction of Voges-Proskauer)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Utilization:</strong></td>
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<td></td>
</tr>
<tr>
<td>of D-glucose</td>
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<td>of D-mannitol</td>
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<td>of inositol</td>
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<td>of D-sorbitol</td>
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<tr>
<td>of L-rhamnose</td>
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<td>of D-sucrose</td>
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<td>of D-melibiose</td>
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<tr>
<td>of amygdaline</td>
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<td>+</td>
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<tr>
<td>of L-arabinose</td>
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<td>+</td>
</tr>
<tr>
<td>Reduction of nitrates</td>
<td>−</td>
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</tbody>
</table>
There are rare literature data, testifying to successful application of API 20E test-system to study the properties of bacteria, associated with plants. For instance, API 20E test-system was used to characterize and identify strains of *Erwinia amylovora*, isolated from chokeberry tree and wild strawberry [8]. Also test-systems API 20Ne and API 20E were used to study bacteria, isolated from grass and previously referred to *Pseudomonas graminis* species. A high similarity level was obtained for these strains (98.5 %) and *P. chlororaphis* [9]. Vantomme *et al.* [10] studied the possibilities of API 20E test-system for express-diagnostics and comparison of *E. amylovora* strains, isolated in different countries, and demonstrated the efficiency of using this test-system to identify *E. amylovora* [10].

The impossibility to identify phytopathogenic bacteria with high similarity level, using the test-system and software to it, is related to the absence of the data about phytopathogenic and plant-associated gram-negative facultative anaerobic bacteria in the database. However, the application of this test-system to determine the bacteriosis agent provides for fast obtaining of results, promoting the identification of bacteria.

**CONCLUSIONS**

It was shown that the results of tests, obtained for the investigated species of *Erwinia amylovora*, *Pectobacterium carotovorum* and *Pantoea agglomerans* using API 20E test-system and conventional microbiological methods, coincided. The application of API 20E test-system (bioMerieux) ensures fast obtaining of important data, which may be used to identify phytopathogenic bacteria of *Erwinia, Pectobacterium, Pantoea* species.

**Table 4. Physiological and biochemical properties of *Pantoea agglomerans***

<table>
<thead>
<tr>
<th>Test/enzyme</th>
<th><em>P. agglomerans</em> P324</th>
<th></th>
<th><em>P. agglomerans</em> according to the literature data [6]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Using API 20E</td>
<td>By conventional methods</td>
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<tr>
<td>β-galactosidase</td>
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<td>n/d</td>
<td>a/d</td>
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<tr>
<td>Arginine dihydrolase</td>
<td>–</td>
<td>n/d</td>
<td>–</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>–</td>
<td>n/d</td>
<td>–</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>–</td>
<td>n/d</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of citrates</td>
<td>+</td>
<td>+</td>
<td>a/d</td>
</tr>
<tr>
<td>Formation of H₂S</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tryptophane deaminase</td>
<td>+</td>
<td>n/d</td>
<td>+</td>
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<tr>
<td>Formation of indole</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>a/d</td>
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<tr>
<td>Gelatinase</td>
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<td>Utilization:</td>
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<td>of D-glucose</td>
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<tr>
<td>of D-mannitol</td>
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<td>+</td>
</tr>
<tr>
<td>of inositol</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>of D-sorbit</td>
<td>+</td>
<td>+</td>
<td>var.</td>
</tr>
<tr>
<td>of L-rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>of D-sucrose</td>
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<td>of D-melibiose</td>
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<td>of amygdaline</td>
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<tr>
<td>of L-arabinose</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Reduction of nitrates</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>
Заставування комерційних тест-систем для ідентифікації грам-негативних факультативно анаеробних фітопатогенних бактерій

В. П. Патика, Л. М. Бущенко, Л. А. Пасичник

Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України

Вул. Академіка Заболотного, 154, Київ, Україна, 03143
e-mail: patykavolodymyr@gmail.com

Мета. Оцінити можливість використання комерційної API-системи для ідентифікації вивченняластостей факультативно анаеробних грам-негативних бактерій.

Методи. Загальноприйняті методи, тест-система API 20Е (Biomerieux) за інструкцією виробника.

Результати. Набір мікробіологічних тестів API 20Е містить діагностично значущі тести для характеристики факультативно анаеробних фітопатогенних бактерій видів Erwinia amylovora, Pectobacterium carotovorum і Pantoea agglomerans. Властивості бактерій E. amylovora, P. carotovorum, P. agglomerans, визначені з використанням тест-системи і класичними методами, збігаються з даними літератури для цих видів. Із заставуванням програмного забезпечення виробника тест-системи, з достатньо високим рівнем подібності вдалося ідентифікувати бактерії виду P. agglomerans. Хоча не всі види факультативно анаеробних фітопатогенних бактерій можна ідентифікувати за допомогою тест-системи API 20Е, її заставування, без сумніву, дозволяє отримати достовірні і важливі для ідентифікації бактерій дані щодо їхніх фізіології-біохімічних властивостей протягом 24 год.

Висновки. Результати тестів, одержані для досліджених видів за використання тест-системи API 20Е і класичними мікробіологічними методами, збігаються. Заставування тест-системи API 20Е (Biomerieux) дозволяє швидко отримати важливі дані, які можна використати для ідентифікації фітопатогенних бактерій родів Erwinia, Pectobacterium, Pantoea.

Ключові слова: фітопатогенні бактерії, бактеріальні хвороби рослин, властивості бактерій, діагностика, тест-системи.

REFERENCES
INTRODUCTION

The problem of providing food for the humanity is extremely complicated [1]; as for Ukraine, a country with powerful grain production, it is also a strategic task related to food and economic safety of the state. Key trace elements (TE), playing a relevant role in many vital processes of human activity, are redox-system components, first and foremost, – iron, zinc, manganese and selenium. TE malnutrition is deemed to be hidden hunger.

According to WHO statistics, there are over 2 billion people with anemia in the world, each third child and almost each pregnant woman and nursing mother suffer from iron-deficient anemia of a different degree [2].

Iron content is only about 35 mg/kg of human body-weight, yet its biological relevance is hard to overestimate. This TE is a universal component of a living cell, participating in many exchange processes. Iron-containing enzymes are involved in the synthesis of thyroid gland hormones and immunity maintenance. Iron is a part of hemoglobin – the main protein of red blood cells – erythrocytes. Hemoglobin consists of two parts: a large protein molecule – globin, and a haem, built into the latter, with an iron ion in the center. Iron interacts with air oxygen and participates in its transportation to all the cells of the organism. In addition, iron is a component of myoglobin – a protein, creating oxygen reserves in muscles, which is a part of over 70 different enzymes.

QUANTIFICATION OF TRACE ELEMENTS Fe, Zn, Mn, Se IN HULL-LESS BARLEY GRAIN

S. S. Polishchuk 1, E. K. Kyrdohlo 1, L. M. Mykhalska 2, B. V. Morgun 2,3, S. Yu. Pokhylko 3, O. I. Rybalka 2,3, V. V. Schwartau 2

1 Institute of Selection and Genetics – National Center of Seed and Cultivar Investigation, NAAS of Ukraine
3, Ovidiopolska Road, Odesa, Ukraine, 65036
2 Institute of Plant Physiology and Genetics, NAS of Ukraine
31/17, Vasylkivska Str., Kyiv, Ukraine, 03022
3 Institute of Cell Biology and Genetic Engineering, NAS of Ukraine
148, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143
e-mail: molgen@icbge.org.ua
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Aim. To explore the content of trace elements (TE), most valuable for human health – iron, zinc, manganese and selenium – in cultivars and breeding lines of hull-less barley, and to check the impact of abiotic environmental factors on TE accumulation in the grain of Achilles cultivar. Methods. Trace elements content was measured using ICP-MS Agilent 7700x. Results. The content of vital TE was determined in 26 samples of hull-less barley grain. The increased concentration of TE was observed in the samples with brown, blue and black caryopsis. The absence of dependence between abiotic environmental factors and TE accumulation was demonstrated using Achilles cultivar, presented by six different repeats. The correlation between the content of protein and that of iron in grain was established. Conclusions. White grain barley cultivars and lines are inferior in the content of Fe, Zn, Mn, and Se comparing to genotypes with brown, blue or black grain. The presence or absence of a film on a hull-less barley grain has almost no impact on the amount of TE, which, as expected, are located in the aleurone layer and the germ of caryopsis. It was proven that the content of Fe, Zn, Mn in the Achilles grain almost did not change regardless of abiotic environmental factors. A high correlation, \( r = 0.87, P > 0.05–0.001 \), was found between the content of protein and Fe in grain.

Keywords: barley, microelements, iron, zinc, manganese, selenium.
DOI: 10.15407/agrisp3.01.049
Iron deficiency disrupts protective and adaptive forces of the organism and metabolism [2–7].

To prevent iron-deficient anemia, nutrition specialists suggest eating products of plant and animal origin: pomegranate juice, apples, laminaria, beets, buckwheat, beef meat, liver and tongue, but it is still not enough. The average physiological human need of iron is 2–4 mg a day [2].

Recently a number of developed countries (USA, UK, Sweden, the Netherlands) adopted national programs of iron-deficiency prevention via enriching bread, fruit juices, baby’s milk formulas with inorganic iron salts, but this measure did not yield any substantial results. Moreover, due to adverse effects it is prohibited to use inorganic iron salts to enrich food products in France, Belgium, Germany and other countries. At present the problem of anemia has not been solved in any country of the world, thus it is one of the most urgent tasks of biochemistry, selection and physiology of nutrition.

In 2001 polarized Zeeman AAS method was first used in the Bioresources Center (BCCAM) in the USA to investigate Fe content in 135 collection samples of barley cultivars. The variability by this feature was in the range of 21.0 to 83.0 mg/kg for 274 cultivar samples [3]. Since that time there has been intensive selection of barley by this trait. The mentioned work is in progress in many laboratories of the world [8].

The average daily human requirement of zinc is 15 mg. Zinc is found in plant products (black current, radish, beet, apples, green fruit, figs) and products of animal origin (meat of rabbits, turkey meat, liver, sea fish). Zinc content in these products is 0.7–30 mg/kg. Zinc deficiency may result in difficult delivery or cause a miscarriage. Zinc facilitates clearing the organism from dangerous toxins and prevents the accumulation of heavy metals, protects skin from inflammations and keeps hair healthy. According to the results of the studies, recently performed in the leading laboratories of the USA, Canada and Japan, zinc content in the barley grain varies in the range of 18–40 mg/kg [3–7, 9–12].

Manganese participates in many fermentation processes of a human organism: in synthesis and exchange of neuromediators of the nervous system, stabilization of cellular membrane structure, in the metabolism of the thyroid gland; it enhances hypoglycemic effect of insulin, increases the intensity of fat utilization, counteracts fatty degeneration of liver, is involved in the regulation of the exchange of vitamins of groups B and E, and fully functional reproductive function, etc [4–7]. The average daily human requirement of manganese is 0.2–0.3 mg per 1 kg of bodyweight. Manganese is present in many plant products. Pepper, soy, oats, salad, kidney beans, peas, rice, barley, raspberry and chocolate, the richest in this TE, contain 30–60 mg of manganese per 1 kg of fresh weight [13].

The relevance of selenium for fully functional human activity became known at the end of the previous century. Selenium protects erythrocytes and cell membranes from harmful impact of free radicals, improves immunity, blocks the development of malignant tumors, takes an active part in metabolism of thyroid gland hormones, especially in old age. Selenium deficiency in mother’s milk is the main reason of infant mortality, especially for male babies [4–7, 14]. The daily intake of selenium by the organism fluctuates depending on age and gender and is 50–70 μg on average.

The highest amount of selenium in plant products is in wheat millrace ~ 0.7 mg/kg, in hull-less barley ~ 0.7 mg/kg, in sunflower seeds ~ 0.5 mg/kg, in sesame seeds ~ 0.3 mg/kg [5]. The selection of cultivars with increased level of selenium in hull-less barley grain is also an urgent task of modern science.

MATERIALS AND METHODS

20 hull-less barley breeding lines were selected for the investigation of the content of relevant TE. The breeding lines were divided into three groups depending on the caryopsis color. White grain was attributed to such cultivars and lines as Alberte, Fihar, Hilose, SL-2019Wx, SL-2023Wx, SL-2028Wx, SL-432/33, SL-432/36. The following cultivars and lines have either brown or blue grain: Achilles, Lophy-1, SL-2043, SL-250/21, SL-2083, SL-430/57, SL-430/60, k-3977 (local from Mongolia). Black grain was registered for the following cultivars and lines: k-18703 (local from Ethiopia), UA-0800338 (Arabische), UA-0804248 (Abyssinian 1105), UA-0802460 (Negra Manfredi), UA-0801394 (Sulto-1). Achilles cultivar samples originated from three different regions of Ukraine: Odesa, Dnipropetrovsk and Kyiv regions (harvests of 2013 and 2014).

The content of elements in the samples of soil, grain and plant material was determined using (ICP-MS) 7700x (Agilent Technologies, USA) and ICP-MS Mass Hunter WorkStation after the digestion of samples (0.400 g) in ICP-grade hydrogen nitrate in the microwave digestion system Milestone Start D. All solutions were obtained using class I water (18 Mohm), obtained in the purific-
QUANTIFICATION OF TRACE ELEMENTS Fe, Zn, Mn, Se IN HULL-LESS BARLEY GRAIN

The results were processed using ICP-MS Mass Hunter Software. Statistical analysis was carried out in MS Excel 2014.

RESULTS AND DISCUSSION

For the first time in Ukraine 20 hull-less barley breeding lines were studied for TE content, most valuable for human health – iron, zinc, manganese and selenium. The data on the detection of iron content in barley caryopsis are presented in Fig. 1.
The highest iron content was observed in caryopsis of brown, blue and black color, with the cultivar UA-0801394 (Sulto-1) being the most well-distinguished among 26 samples studied, showing iron content at 57 mg/kg. Iron content of six samples of Achilles cultivar differed in the place and years of cultivar cultivation from 36 to 42 mg/kg. However, this was rather insignificant difference comparing to white caryopsis samples, in which iron content was 21–22 mg/kg on average.

The highest content of zinc was found in barley samples, the caryopsis color of which was brown, blue and black, although the difference was not as significant as that for the previous TE (Fig. 2). The highest amount of zinc was registered for SL-250/21 hull-less barley breeding line – 27.9 mg/kg (see Fig. 2, column 14), whereas the lowest amount – in Lophy-1 hull-less barley breeding line – 12 mg/kg (see Fig. 2, column 12). For six samples of Achilles cultivar no considerable difference was observed, which indicated insignificant impact of climatic conditions and resource possibilities of soil on zinc accumulation in grain of this barley cultivar.

Fig. 3 presents a diagram which shows manganese content, determined in 26 samples of barley caryopsis. In this case, the highest amount of manganese was found in hull-less barley breeding lines with the black caryopsis, and the lowest amount – in white caryopsis. The top cultivars of manganese accumulation were UA-0800338 (Arabische) – 16.5 mg/kg, UA-0802460 (Negra Manfredi) – 16.2 mg/kg and UA-0801394 (Sulto-1) – 16 mg/kg. The lowest amount of manganese was found in SL-2023Wx – 7.2 mg/kg, which was 2.3 times less than that for top cultivars. Achilles cultivar did not demonstrate any special differences in manganese content in six studied samples.

As for selenium content, only two samples (SL-2043 and SL-2083) had the increased amount of it – 1.7 mg/kg in each, which was almost the triple amount for the average index in barley. Also these lines have brown caryopsis.

A similar correlation between TE content and grain color was observed for winter wheat cultivars, includ-
ing Chornobrova (black grain) – Fe – 29.1 mg/kg, Zn – 20.6 mg/kg, Mn – 29.5 mg/kg; Kuyalnyk (red grain) – Fe – 17.6 mg/kg, Zn – 8.6 mg/kg, Mn – 26.7 mg/kg; Biliava (white grain) – Fe – 14.1 mg/kg, Zn – 7.6 mg/kg, Mn – 17.9 mg/kg.

The high correlation between the content of protein and that of iron in hull-less barley grain was established: \( r = 0.87, P > 0.05−0.001 \). This marker could be utilized in the barley breeding program (Fig. 4).

CONCLUSIONS

It was established that white grain cultivars and lines are inferior in the content of Fe, Zn, Mn, and Se to genotypes with brown, blue or black grain. A similar correlation between TE content and grain color was determined among winter wheat cultivars as well. The presence or absence of a film on a barley grain has almost no impact on the amount of TEs, which, as expected, are located in the aleurone layer and the germ of caryopsis. It was demonstrated that the content of Fe, Zn, and Mn in Achilles cultivar has almost no changes depending on abiotic environmental factors. A high correlation, \( r = 0.87, P > 0.05−0.001 \), was found between the content of protein and Fe in grain.

ACKNOWLEDGMENTS

The work was performed in the framework of projects of the National Academy of Sciences of Ukraine “Elaboration of Systems of Molecular Markers for Selection of Useful Traits in Cereals” and “Molecular Biotechnology of Highly Efficient Application of Nutrients for Winter Wheat Cultivars”. The analytic study was conducted with support of EU project “Health and Ecological Program around the Chornobyl Exclusion Zone”.

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Визначення вмісту мікроелементів Fe, Zn, Mn, Se у зерні ячменю

С. С. Поліщук1, Є. К. Кирдогло1, Л. Н. Михальська2,
Б. В. Моргун2, 3, С. Ю. Похілько1,
О. І. Рибалка2, 3, В. В. Швартав2

1 Селекційно-генетичний інститут – Національний центр семеноведення та сортовивчення НААН України
Овідиопольська дорога, 3, Одеса, Україна, 65036

2 Інститут фізіології рослин і генетики НАН України
Вул. Васильківська, 31/17, Київ, Україна, 03022

3 Інститут клітинної біології та генетичної інженерії НАН України
Вул. Академіка Заболотного, 148, Київ, Україна, 03143
e-mail: molgen@icbge.org.ua

Цель. Исследовать содержание наиболее ценных для здоровья человека микроэлементов (МЭ): железа, цинка, марганца, селена в сортах и селекционных линиях ячменя, а также проверить влияние абиотических факторов среды на количество МЭ в зерне сорта Ахиллес.

Методы. Содержание МЭ измеряли на масс-спектрометре с индуктивно связанной плазмой (ICP-MS) «Agilent 7700х».

Результаты. Исследованы 26 образцов ячменя для выявления содержания ценных МЭ. Большее количество МЭ наблюдалось в сортах с коричневыми, синими и черными зерновками. Сорт Ахиллес, представленный шестью различными повторностями, продемонстрировал отсутствие зависимости между абиотическими факторами среды и накоплением МЭ.
Определена корреляционная зависимость между содержанием белка и железа в зерне. **Выводы.** По содержанию Fe, Zn, Mn, Se белозерные сорта и линии уступают генотипам с коричневым, синим или черным зерном. Наличие или отсутствие пленки в зерне ячменя почти не влияет на количество МЭ, которые, как и ожидалось, содержатся в алейроновом слое и зародыше зерновки. Доказано, что содержание Fe, Zn, Mn в зерне сорта Ахиллес в зависимости от абиотических факторов среды почти не меняется.

**Ключевые слова:** ячмень, микроэлементы, железо, цинк, марганец, селен.

REFERENCES

INTRODUCTION

Corn is one of the most productive cereals of the universal purpose, the yield rate of which exceeds that of many crops in conditions of adequate moisture supply. At the same time it is characterized by rather high drought resistance and on condition of the optimization of the main agrotechnical measures it is capable of forming stable productivity even without any irrigation. The most efficient agents, influencing the rate of grain productivity of corn, are its hybrid content, the application of irrigation, mineral fertilizers, microfertilizers and growth stimulants [1–3].

Due to the necessity of improving technological ways of cultivating corn hybrids and determining the adaptiveness of certain genotypes to soil-ecological and technological conditions of cultivation in the south of Ukraine, the comparative studies with eight new corn hybrids were carried out both with and without irrigation. When hybrids were grown under irrigation conditions, the scheme of the experiment involved the application of microfertilizers and growth stimulants, as their application is known to be one of the most important ways of increasing the yield by 15–20 % and improving the quality of products [4–6].

The aim of the work was to determine efficient microfertilizers and growth stimulants taking into consideration biological specificities of new maize hybrids of different FAO groups under irrigation conditions in the south of Ukraine and to trace their impact on the formation of grain productivity of plants.
MATERIALS AND METHODS

The following methods were used: the field method – to analyze the interaction of the investigated object and both experimental and environmental factors with further registration of the yield volume and biometric measurements; the laboratory method – to determine soil humidity, humidity content in grain and quality indices of grain; the statistical method – to estimate the reliability of the results obtained; and the computational method – in economic and energetic estimation of the employed cultivation techniques.

The studies were carried out in 2013–2015 on the experimental field of the Institute of Irrigated Agriculture NAAS of Ukraine, located in the south of Ukraine in the zone of the Ingulets irrigated area. The soil of the experimental plot is dark-chestnut, medium loam, weakly alkaline, the water table is deep.

It was a double-factor experiment: factor A – corn hybrids, different by FAO groups (DN Pvyvyka, Tendra, Batyrn 287 MB, Skadowskiy, Zbruch, Kak-hovskiy, DN Hetera, Arabat); factor B – microfertilizers and growth stimulants (no treatment; Sezam-Nano – seed treatment; Sezam-Nano – seed treatment + foliage sprinkling of HUMIN PLUS in the phase of 7–8 leaves; Sezam-Nano – seed treatment + foliage sprinkling with Grainactive-C in the phase of 7–8 leaves; HUMIN PLUS – seed treatment + foliage sprinkling in the phase of 7–8 leaves; Nanomix – seed treatment + foliage sprinkling in the phase of 7–8 leaves). The experiments were repeated four times with the location of variants by the method of randomized split plots. The area of the plot for sowing was 70 sq.m., the area for registration – 50 sq.m.

The abovementioned corn hybrids were grown for grain. The agrotechnology of cultivating corn, used in the experiments, was common for the southern zone of Ukraine, except for the investigated factors. Soy was the precursor plant. The irrigation was performed by the sprinkler method.

According to the scheme of the experiment, prior to sowing the seeds were treated with the solutions of preparations and sprinkled during the vegetation period in the phase of 7–8 leaves.

Mineral fertilizers were introduced in the estimated dose for pre-sowing cultivation. The dose was determined using the method of optimal parameters according to the difference between the yield removal and the actual content of nutrients in soil. Depending on the actual content of nutrients in soil it was $N_{220}P_{18}K_0$.

In optimal terms corn hybrids were sown in the first decade of May when the soil temperature at the depth of sowing the seeds was 12–14 °C.

The sowing, carrying out of the experiments, selection of soil and plant samples, their preparation for the analysis were conducted according to the methodological instructions for the experiment.

RESULTS AND DISCUSSION

Corn belongs to drought-resistant crops (mesophytes). However, the deficiency of moisture in the soil is a serious factor, limiting the obtaining of high yield of maize grain. The extreme weather conditions, frequently observed in the Southern Steppe of Ukraine (hot dry winds, high temperature, deficiency of productive moisture) have negative impact on the growth and development of these plants and decrease the efficiency of the fertilizers introduced.

In our experiments the plantings of corn were irrigated by vegetative watering, keeping the humidity at the level of 75 % from the least moisture-retention capacity in the soil layer of 0–70 cm.

The efficiency of introducing energy- and resource-keeping technologies in the sphere of agro-industrial complex is greatly dependent on such an important factor as microfertilizers and plant growth stimulants, containing relevant microelements, phytohormones and growth activators. Their application allows decreasing the application of chemical preparations, in particular, plant protectors, enhancing the efficiency of a number of technological operations, improving the resistance of plants to unfavorable environmental factors and the activity of pathogens, enhancing both quantity and quality characteristics of the products [8].

The application of microfertilizers and growth stimulants on the fields of corn in 2013–2015 had positive impact on the growth and development of plants and, as a result, on the yield formation. For instance, regardless of the terms of hybrid maturity during the years of studies the introduction of microfertilizers and growth stimulants increased the grain yield of corn hybrids by 0.38–1.26 t/ha on average with the performance gain of 3.8–10.04 % (Table 1, 2). This may be explained by complete or partial provision of plants with required microelements and growth-stimulating substances with their distribution during crop
vegetation, especially in the critical periods of plant
development.

The data, presented in Tables 1 and 2, testify to
the tendency of grain yield gain for all the groups
of maturity of corn hybrids depending on the ap-
plication of microfertilizers and growth stimulants.

The yield of corn grain under irrigation conditions
without any treatment with preparations fl-
uctuated in the range of 9.57–12.54 t/ha on average for all the
groups of hybrid maturity in 2013–2015. The high-
est yield (13.8 t/ha) for the years of studies under ir-
rigation conditions was demonstrated by medium-late
Arabat hybrid with the complex application of growth
stimulants – seed treatment with Sezam-Nano and fer-
tilizing corn plants with Grainactive-C in the phase of
7–8 leaves which is exceeding the control by 1.26 t/ha.

A similar regularity was found for other hybrids as
well. Due to such treatment the yield gain of hybrids
was 0.94–1.26 t/ha on average. It is noteworthy that the
most evident response to the application of microferti-
lizers and growth stimulants under irrigation conditions
was determined for medium maturity and medium-late
hybrids.

The results of the studies in 2013–2015 demon-
strate that higher yield stability (both actual and po-
tential) under irrigation conditions was determined
for hybrids of medium maturity and medium-late
groups. The rate of yield drop depending on the
biotype was found to be minimal for the investigat-
ed hybrids, FAO 310–430, which testifies to some
advantages of the stability of high yield of medi-
Table 1. The yield of corn hybrid grain, FAO 180–290, depending on microfertilizers and growth stimulants, t/ha

<table>
<thead>
<tr>
<th>Hybrid (A)</th>
<th>Treatment with the preparation (B)</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>10.96</td>
<td>10.68</td>
<td>10.31</td>
<td>10.65</td>
</tr>
<tr>
<td></td>
<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>10.98</td>
<td>10.76</td>
<td>10.33</td>
<td>10.69</td>
</tr>
<tr>
<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
<td>11.18</td>
<td>10.98</td>
<td>10.51</td>
<td>10.89</td>
</tr>
<tr>
<td></td>
<td>5. HUMIN PLUS</td>
<td>10.82</td>
<td>10.74</td>
<td>10.27</td>
<td>10.61</td>
</tr>
<tr>
<td>Tendra (FAO 190)</td>
<td>1. No treatment</td>
<td>9.91</td>
<td>9.57</td>
<td>9.23</td>
<td>9.57</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>10.52</td>
<td>10.22</td>
<td>9.71</td>
<td>10.15</td>
</tr>
<tr>
<td></td>
<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>10.59</td>
<td>10.27</td>
<td>9.77</td>
<td>10.21</td>
</tr>
<tr>
<td></td>
<td>5. HUMIN PLUS</td>
<td>10.47</td>
<td>10.19</td>
<td>9.67</td>
<td>10.11</td>
</tr>
<tr>
<td>Baturyn 287 MB (FAO 240)</td>
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<td>10.45</td>
<td>10.25</td>
<td>10.05</td>
<td>10.25</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>11.14</td>
<td>10.96</td>
<td>10.60</td>
<td>10.90</td>
</tr>
<tr>
<td></td>
<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>11.25</td>
<td>11.01</td>
<td>10.65</td>
<td>10.97</td>
</tr>
<tr>
<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
<td>11.51</td>
<td>11.17</td>
<td>10.95</td>
<td>11.21</td>
</tr>
<tr>
<td></td>
<td>5. HUMIN PLUS</td>
<td>11.15</td>
<td>10.81</td>
<td>10.50</td>
<td>10.82</td>
</tr>
<tr>
<td></td>
<td>6. Nanomix</td>
<td>11.43</td>
<td>11.07</td>
<td>10.80</td>
<td>11.10</td>
</tr>
<tr>
<td>Skadovskyi (FAO 290)</td>
<td>1. No treatment</td>
<td>10.88</td>
<td>10.56</td>
<td>10.30</td>
<td>10.58</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>11.55</td>
<td>11.33</td>
<td>10.93</td>
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<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
<td>11.87</td>
<td>11.63</td>
<td>11.21</td>
<td>11.57</td>
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<td>11.35</td>
<td>10.90</td>
<td>11.28</td>
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<tr>
<td></td>
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<td>11.80</td>
<td>11.58</td>
<td>11.09</td>
<td>11.49</td>
</tr>
<tr>
<td>HIP_{655} t/ha</td>
<td>A =</td>
<td>0.33</td>
<td>0.41</td>
<td>0.37</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>B =</td>
<td>0.18</td>
<td>0.24</td>
<td>0.22</td>
<td>–</td>
</tr>
</tbody>
</table>
early-maturity corn hybrids under irrigation conditions.

The economic efficiency of the applied technology elements was determined with the purpose of objective substantiation of the most rational combination of measures in agriculture, namely hybrids of different maturity groups, microfertilizers and growth stimulants along with the use of scheduled cost of material and technical resources for the cultivation of corn for grain under irrigation conditions. The general norms of performance, prices for manual and automated labor were accepted pursuant to the recommended production standards [9]. Grain was used in the calculations as the main kind of products while determining the cost of gross production from 1 ha. It was determined that the cost of the products obtained while cultivating corn changes with the regularity, observed for the culture productivity (Table 3).

The estimation of economic efficiency revealed that in case of cultivating the investigated hybrids without growth stimulants and microfertilizers the production costs were 0.8–1.7% less compared to the ones, incurred with the introduction of the mentioned factor to technological ways of corn cultivation. The maximal costs (21,575–21,741 hryvnia/ha) were determined for the application of HUMIN PLUS preparation to medium-maturity and medium-late hybrids.

The highest net profit in the experiment was provided by medium-late hybrid Arabat, regardless of growth stimulants and microfertilizers. It was maximal for hy-

<table>
<thead>
<tr>
<th>Hybrid (A)</th>
<th>Processing with the preparation (B)</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zbruch (FAO 310)</td>
<td>1. No treatment</td>
<td>11.32</td>
<td>11.10</td>
<td>10.82</td>
<td>11.08</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>12.09</td>
<td>11.85</td>
<td>11.37</td>
<td>11.77</td>
</tr>
<tr>
<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
<td>12.60</td>
<td>12.32</td>
<td>11.68</td>
<td>12.20</td>
</tr>
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<td></td>
<td>5. HUMIN PLUS</td>
<td>12.03</td>
<td>11.77</td>
<td>11.36</td>
<td>11.72</td>
</tr>
<tr>
<td></td>
<td>6. Nanomix</td>
<td>12.50</td>
<td>12.16</td>
<td>11.49</td>
<td>12.05</td>
</tr>
<tr>
<td>Kakhovskyi (FAO 380)</td>
<td>1. No treatment</td>
<td>11.61</td>
<td>11.29</td>
<td>11.06</td>
<td>11.32</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>12.36</td>
<td>12.12</td>
<td>11.61</td>
<td>12.03</td>
</tr>
<tr>
<td></td>
<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>12.40</td>
<td>12.16</td>
<td>11.68</td>
<td>12.08</td>
</tr>
<tr>
<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
<td>12.86</td>
<td>12.60</td>
<td>11.95</td>
<td>12.47</td>
</tr>
<tr>
<td></td>
<td>5. HUMIN PLUS</td>
<td>12.26</td>
<td>12.00</td>
<td>11.50</td>
<td>11.92</td>
</tr>
<tr>
<td>DN Hetera (FAO 420)</td>
<td>1. No treatment</td>
<td>12.21</td>
<td>11.95</td>
<td>11.66</td>
<td>11.94</td>
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<td></td>
<td>2. Sezam-Nano</td>
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<td>12.72</td>
<td>12.07</td>
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<td>13.44</td>
<td>13.16</td>
<td>12.46</td>
<td>13.02</td>
</tr>
<tr>
<td>Arabat (FAO 430)</td>
<td>1. No treatment</td>
<td>12.74</td>
<td>12.52</td>
<td>12.36</td>
<td>12.54</td>
</tr>
<tr>
<td></td>
<td>5. HUMIN PLUS</td>
<td>13.51</td>
<td>13.27</td>
<td>12.88</td>
<td>13.22</td>
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<tr>
<td>HIP_{95}, t/ha</td>
<td>A =</td>
<td>0.33</td>
<td>0.41</td>
<td>0.37</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>B =</td>
<td>0.18</td>
<td>0.24</td>
<td>0.22</td>
<td>–</td>
</tr>
</tbody>
</table>
breds in case of using growth stimulants Sezam-Nano and Grainactive-C (Table 3).

Compared to the variants with no treatment, growth stimulants and microfertilizers increased the net profit for hybrids by 1.7–12.2 % on average.

In 2015 the highest profit level (18.351 UAH/ha) and the profitability of 84 % was obtained on the fields of hybrid Arabat on conditions of seed treatment with the growth stimulant Sezam-Nano and in the phase of 7–8 leaves – with Grainactive-C, which is 12.2 % higher than untreated variants. High profit was also provided by hybrids DN Hetera and Kakhovskyi.

Quite a different situation was observed while determining the profitability level of cultivating corn hybrids. In 2015 it was found to be in the range of 34.1–71.8 % for all the hybrids without the use of growth stimulants and microfertilizers. When treating with preparations, the profitability level increased and was 35.9–84.0 % on average by the variants of treatments. It should be noted that it was considerably higher when growing corn hybrids with longer vegetation period, and the cost of growing a unit of production was decreased.

**CONCLUSIONS**

To obtain corn grain yield at the level of 10–14 t/ha under irrigation conditions on dark-chestnut soil in the south of Ukraine the corn hybrids of medium maturity and medium-late groups Zbruch, Kakhovskyi, DN Hetera, Arabat are recommended to grow with application of innovation growth stimulants and microfertilizers.

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**Table 3.** The economic efficiency of growing corn hybrids, FAO 180–430, depending on microfertilizers and growth stimulants in 2015

<table>
<thead>
<tr>
<th>Hybrid maturity group (A)</th>
<th>Treatment with the preparation (B)</th>
<th>Yield, tons/ha</th>
<th>Cost of production, UAH/ha</th>
<th>Net profit, UAH/ha</th>
<th>Profitability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAO 180–190</td>
<td>1. No treatment</td>
<td>9.23</td>
<td>27690</td>
<td>7042</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>9.71</td>
<td>29130</td>
<td>8297</td>
<td>39.8</td>
</tr>
<tr>
<td></td>
<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>9.77</td>
<td>29310</td>
<td>8415</td>
<td>40.3</td>
</tr>
<tr>
<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
<td>9.96</td>
<td>29880</td>
<td>8961</td>
<td>42.8</td>
</tr>
<tr>
<td></td>
<td>5. HUMIN PLUS</td>
<td>9.67</td>
<td>29010</td>
<td>8199</td>
<td>39.4</td>
</tr>
<tr>
<td></td>
<td>6. Nanomix</td>
<td>9.86</td>
<td>29580</td>
<td>8692</td>
<td>41.6</td>
</tr>
<tr>
<td>FAO 240–290</td>
<td>1. No treatment</td>
<td>10.45</td>
<td>31350</td>
<td>10638</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>2. Sezam-Nano</td>
<td>11.14</td>
<td>33420</td>
<td>12523</td>
<td>59.9</td>
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<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>11.25</td>
<td>33750</td>
<td>12791</td>
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<td>4. Sezam-Nano + Grainactive-C</td>
<td>11.51</td>
<td>34530</td>
<td>13547</td>
<td>64.6</td>
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<td>5. HUMIN PLUS</td>
<td>11.15</td>
<td>33450</td>
<td>12575</td>
<td>60.2</td>
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<td>6. Nanomix</td>
<td>11.43</td>
<td>34290</td>
<td>13338</td>
<td>63.7</td>
</tr>
<tr>
<td>FAO 310–380</td>
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<td>33960</td>
<td>12548</td>
<td>58.6</td>
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<tr>
<td></td>
<td>2. Sezam-Nano</td>
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<td>36270</td>
<td>14673</td>
<td>67.9</td>
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<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>12.11</td>
<td>36330</td>
<td>14671</td>
<td>67.7</td>
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<tr>
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<td>4. Sezam-Nano + Grainactive-C</td>
<td>12.60</td>
<td>37800</td>
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<td>5. HUMIN PLUS</td>
<td>12.03</td>
<td>36090</td>
<td>14515</td>
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<td>39180</td>
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<td></td>
<td>3. Sezam-Nano + HUMIN PLUS</td>
<td>13.11</td>
<td>39330</td>
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<td></td>
<td>4. Sezam-Nano + Grainactive-C</td>
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<td>5. HUMIN PLUS</td>
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Продуктивність гібридів кукурудзи різних груп ФАО залежно від мікродобрив та стимуляторів росту за умов зрошування на півдні України

Ю. О. Лавриненко, Р. А. Вожегова, О. А. Гож

e-mail: izz.ua@ukr.net

Інститут зрошуваного землеробства НААН України
Сел. Наддніпрянське, Херсон, Україна, 73483

Мета. Встановити ефективні мікродобриви і стимулятори росту з урахуванням біологічних особливостей нових гібридів кукурудзи різних груп ФАО за умов зрошування півдня України та простежити їхній вплив на формування зернової продуктивності рослин.

Методи. Для вивчення взаємодії об’єкта дослідження з експериментальними факторами і чинниками природного середовища використано польовий метод, який передбачає реєстрацію об’єкту вISIBLE ту та вимірювання біометричних показників; для визначення вологості ґрунту, вмісту вологи в зерні та показників якості зерна застосовано лабораторний метод, а також статистичний – для оцінювання достовірності отриманих результатів і розрахунковий – для економічної та енергетичної оцінки застосованих прийомів вирощування.

Результати. Встановлено вплив мікродобрив і стимуляторів росту на формування врожайність і якості зерна гібридів кукурудзи різних груп стиглості, а також на економічну ефективність їхнього вирощування.

Висновки. За умов зрошування Південного Степу України на темно-каштанових ґрунтах для отримання врожайністі 10–14 т/га і рентабельності виробництва 50–84 % рекомендується використовувати такі гібриди: ранньостиглий ДН Пивиха, середньоранній Скадовський, середньостиглий Каховський і середньопізній Арабат. Вирощування повинно супроводжуватися комплексним застосуванням стимуляторів росту (обробкою насіння «Сизам-Нано» та підживленням у фазі 7–8 листків кукурудзи «Грейнктив-С»).

Ключові слова: гібриди кукурудзи, групи ФАО, мікродобрива і стимулятори росту, зрошування, врожайність зерна, економічна ефективність.

REFERENCES

INTRODUCTION

At present the condition of animal genetic resources in the sphere of food production and agriculture is the subject of monitoring of relevant institutions, including the Food and Agriculture Organization (FAO) of the United Nations Organization. Since 2000 the breeds with specified molecular and genetic characteristics have been added into the Global Databank of Farm Animal Genetic Resources. It may be explained by the fact that sometimes it is rather hard to have a clear distinction between breeds, as some of them have similar features, especially useful for economy [1]. Due to this the determination of specific characteristics of the breed requires the study of its genetic structure. At present this study involves a wide application of DNA markers known for different types of inheritance. First of all, these are microsatellite and single nucleotide polymorphisms (SNPs), localized on autosomes and inherited from both parents. Secondly, these are markers, localized on the part of Y-chromosome, which do not recombine with X-chromosome and therefore are inherited only in paternal line. Finally, these are mitochondrial DNA markers, inherited in maternal line. Comprehensive information, obtained using all types of DNA-markers, will allow obtaining the integral picture of breed genetic structure.

The prevailing majority of transboundary breeds of pigs, including Large White, have already been analyzed for their molecular and genetic characteristics including the application of amplified fragment length polymorphism (AFLP) [2], microsatellites [3] and mitochondrial DNA polymorphism [4].
There are no complete molecular and genetic characteristics for Ukrainian Large White pigs. Therefore, the aim of the work was to estimate the genetic structure of this breed using mitochondrial DNA markers.

**MATERIALS AND METHODS**

The samples of blood, sperm or hair of 369 Ukrainian Large White boars and breeding sows, bred at breeding farms of Ukraine, were used in the study.

DNA was isolated using ion-exchange resin, Chelex 100 [5]. The D-loop fragment between positions 15531 and 15959 of the pig mitochondrial genome with one monomorphic 15558W and five polymorphic sites TasI 15580T > C, 15616T > C, 15714T > C, 15758T > C, 15916A > T, was analyzed, and the presence or absence of site TasI in the abovementioned positions was studied to determine mitochondrial DNA haplotypes, indicated with Latin letters from A to P. The oligonucleotide primers MITPRO2F: catacaaatgtgacccaa and MITPROR: gtgagcatgggctgattagtc were designed and synthesized in Biometra GmbH (Germany). The set of reagents (Taq) was used to amplify D-loop fragment of the pig mitochondrial genome. The amplification was performed at 94 ºC (5 min), 30 amplification cycles, each including denaturation (95 ºC), 30 s) and elongation (72 ºC, 30 s). The final synthesis was performed at 72 ºC for 3 min. The PCR results were found to be positive, if the amplicon of 426 b.p. was obtained.

The aliquot of the PCR product (10 µl) was hydrolyzed using endonuclease TasI (Thermo Scientific™, Lithuania). When the restriction of amplicons using endonuclease TasI was completed, the fragments obtained were separated by electrophoresis in 8% polyacrylamide gel in 1 × TBE buffer. pUC19/MspI (HpaII) (Thermo Scientific™) was used as a molecular weight marker. The products of amplification and restriction were visualized by staining with ethidium bromide and photographing at the transilluminator in ultraviolet light.

**RESULTS AND DISCUSSION**

Seven mitochondrial DNA haplotypes of different frequency were revealed among Ukrainian Large White pigs. Haplotypes G (0.141), J1 (0.133) and N (0.591) were the most frequent; considerably less frequent – haplotypes A (0.003), B1 (0.019), C (0.092) and L (0.022) (Figure).

Mitochondrial DNA haplotypes have their specificities of inheritance, as the introgression of mitochondrial genomes in the population of domestic animals occurs only due to inclusion into female population (maternal type of inheritance). Taking into consideration the fact that mitochondrial DNA haplotypes are known for many subspecies of wild boars and breeds of domestic pigs, it is possible to determine their impact on the formation of haplogroup of breeding sows of a specific breed.

The method of multiplex PCR-AFLP, used in this work, allows determining only six SNP (Table) which is less than the number of nucleotide substitutes, found on the D-loop site. For instance, this was approximately the site, where Fang et al. found 28 SNP [7], Alves et al. – 34 [8] and Giuffra et al. – 27 [9].

The nomenclature of haplotypes, suggested in this work, is commensurate with the one, suggested by Fernández et al.* [8], Fang et al.** [7] and Marines et al.*** [10]. Haplotype A matches haplotypes (EH12, EH31)**, (H01–H07, H25)***, haplotype C – (H2–H10, H13, H14, H21, H25, H26)*, (EH7, EH25, EH28)***, (H08, H11–H15, H24, H26)***, G – (H1, H15, H16, H18, H27, H29)*, (EH29, H09)***, J1 – H24*, (AH7, AH19, AH31, AH33, AH34)**, (H18, H20)***, L – (H11, H12, H28)*, (EH15,EH30)**, H174P***, N – (AH4, AH30)***, (H21, H22, H23)***. Haplotype B1 was not found in subspecies of wild boar and breeds of domestic pigs.

Therefore, haplotypes, revealed among the Ukrainian Large White pigs, and the previously described ones are in good agreement. For instance, haplotype G was previously found in wild boars of Belgium, France, Italy, haplotype J1 – in wild boars of Japan, China, Meishan and Large White, N – in wild boars of Japan, China, Berkshire and Large White, haplotype L was previously found in wild boars of Belgium and Large White. The haplotypes A (0.003), previously described for French wild boar, Mandalica, Duroc, and C (0.092) – for wild boars of France, Belgium, Poland, Israel and Landrace, Welsh breeds, had insignificant frequency. Haplotype B1 was revealed in Myrgorod breed and Poltava Meat and Red White Belted breeds, bred on its basis [11].

There may be several explanations for the presence of haplotypes, not remarkable for purebred Large White, in the haplogroup of Ukrainian Large White pigs. First of all, it is a long period of breeding Ukrainian Large White pigs.
The export of pigs of this breed started in the territory of modern Ukraine at the end of the XIX century. At the beginning of the XX century their number increased due to agricultural exhibitions. At that time the breeding of Large White pigs, imported from Shchepkin’s farm, was conducted in Maynivka agricultural school (Chernihiv region). In 1910–1912 the pig breeding departments of Poltava and Nosovskaya agricultural research stations started their work in creating pedigree herds of Large White. During the World War I, followed by the civil war, the Large White pig population was destroyed almost completely. All the pedigree animals were gathered in Poltava and Nosovskaya agricultural research stations and amounted to 11 boars and 66 breeding sows. In 1927 the specialists of the Poltava agricultural research station created the first State Herd-book of Large White pigs. During 1928–1930 194 boars and 871 breeding sows were registered therein. Since 1930 the network of breeding state-owned farms and breeding collective farms was established in Ukraine. The breeding state-owned farms were mainly supplied the animals from breeding farms of the Moscow region, while the breeding collective farms received their pigs from the Poltava agricultural research station. In 1933 the Poltava and Starokonstantyniv (Khmelnytsk region) state breeding centers of Large White were organized.

During World War II almost all the animals were eliminated, except for the best animals of the Poltava agricultural research station and the breeding farm of Maynivka state-owned farm-Dedovo technical school. After the war these animals were brought back to Ukraine. In 1945–1955 the breeding farm of the Maynivka state-owned farm-technical school became the leading breeding center.

The polymorphism positions of D-loop fragment of the pig mitochondrial genome and sizes of restrictive fragments of endonuclease TasI

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Polymorphism positions of TasI</th>
<th>Size of restrictive fragments, b.p.</th>
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</thead>
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<tr>
<td></td>
<td>15553</td>
<td>15558</td>
</tr>
<tr>
<td>A</td>
<td>AATT</td>
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<tr>
<td>B1</td>
<td>AATT</td>
<td>W</td>
</tr>
<tr>
<td>C</td>
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<td>W</td>
</tr>
<tr>
<td>G</td>
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<td>W</td>
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<td>J1</td>
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<tr>
<td>L</td>
<td>AATT</td>
<td>W</td>
</tr>
<tr>
<td>N</td>
<td>AATT</td>
<td>W</td>
</tr>
</tbody>
</table>

By 1970 Large White pigs were bred in Ukraine in 11 breeding centers, 9 breeding farms and 48 breeding collective farms. The number of pedigree Large White boars was 98.3 % of the total number of boars of this breed, and that of breeding sows was 38.9 % [12]. Therefore, it is possible that haplotypes of meat pigs A and B1 were preserved among Ukrainian Large White pigs.


At present Large White breed has a dominating position in Ukraine, amounting to almost 70 % in comparison to other breeds. At this stage of industry de-
velopment the pedigree foundation is presented by 74 breeding centers and 60 pedigree sow farms. The genealogical structure of the breed includes intrabreed types, farm lines and families (maternal lines). Three main selection directions have been established for the breed: 1) aimed at the creation of ULW-1 intermediate maternal type Ukrainian Large White 1 with high maternal and growing-fattening features; 2) aimed at the creation of intermediate paternal type ULW-2 with improved meat qualities, and 3) complex selection ULW-3.

Due to extensive import of Large White from abroad for the last 30 years, the number of lines of Ukrainian selection reduced to 10% compared to the newly brought ones. The main countries, from which Large White pigs are imported, are Great Britain, Ireland, France and Hungary.

Some countries have developed their domestic Large White breeds, for instance, Hungary has Hungarian Large White with the haplotype C (according to the nomenclature of Marincs et al., haplotypes H08, H11–H15, H24, H26) [10]. In Ukraine these breeds are registered as pedigree animals of Large White. This may be another reason of the presence of the haplotype, not remarkable for Large White breed.

CONCLUSIONS

The mitochondrial DNA haplogroup of Ukrainian Large White pigs includes seven haplotypes. Three mitochondrial DNA haplotypes – A, BI, C – in the haplogroup of Ukrainian Large White pigs are not remarkable for pedigree British Large White. Pigs with haplotypes G, J1, N and L may be referred to representatives of maternal lines, which did not have introgression of mitochondrial genomes of other breeds.

SUPPORT

The work was fulfilled in the framework of the project of the National Academy of Agrarian Sciences of Ukraine “Developing and testing DNA-markers of intermediate maternal type of inheritance in current system of pig breeding”, state registration No. 030101V003257.

Генетична структура свиней великої білої породи України, оцінена за допомогою мітохондріальних ДНК-маркёров

К. Ф. Почерняєв

e-mail: pochernyaev@mail.ru

Інститут свіноводства і агропромислового виробництва НААН

Ул. Шведська могила, 1, Полтава, Україна, 63013

Цель. Изучить генетическую структуру крупной белой породы свиней Украины на основе полиморфизма длинных рестриктных фрагментов (ПДРФ) митохондриальной ДНК.

Методы. Использован метод мультисайтового ПДРФ-анализа, особенность которого состояла в изучении фрагмента D-петли между позициями 15531 и 15959 митохондриональну геному свиньи, где расположены один мономорфный 15558W и пять полиморфных сайтов Tasl 15580T > C, 15616T > C, 15714T > C, 15758T > C, 15916A > T. Наличие или отсутствие сайта Tasl в указанных выше позициях определяет митохондриональные гаплотипы, обозначенные латинскими символами.
буквами от A до P. Результаты. Среди свиней крупной белой породы Украины обнаружены семь митохондриальных гаплотипов, встречающихся с различной частотой. С наибольшей частотой встречались гаплотипы G (0,141), J1 (0,133) и N (0,591), ранее описанные у дикого кабана Бельгии, Франции, Италии (G); дикая свинья Японии, Китая, пород мейшан и крупной белой (J1) и дикого кабана Японии, Китая, пород беркшир и крупной белой (N). Частота встречаемости гаплотипа L (дикий кабан Бельгии и крупная белая порода свиней) составляет 0,022; гаплотипа A (дикий кабан Франции, породы мангалица, дюрой) – 0,003; C (дикий кабан Франции, Бельгии, Польши, Израиля и породы ландрас, уэльс) – 0,092. Гаплотип B1 (0,019) обнаружен у мировой породы и созданных на ее основе полтавской мясной и красной белопоясой пород мясных свиней. Выводы. Гаплогруппа митохондриальной ДНК крупной белой породы свиней Украины включает семь гаплотипов митохондриальной ДНК. К животным крупной белой породы Украины, в которых не произошла интрогрессия по материнской линии других пород, можно отнести свиней с гаплотипами G, J1, N и L.

Ключевые слова: крупная белая порода свиней, гаплотип, гаплогруппа, ПЦР, ПДРФ, митохондриальная ДНК.

REFERENCES


INTRODUCTION

Equine endurance races have become an important field of competition in recent years but unfortunately have received little attention from scientists [1, 2]. The training of endurance horses and athletic longevity is comparable to that of a human marathoner [3].

HEMATOLOGICAL CHANGES AND RESISTANCE OF ERYTHROCYTES OF CRIMEAN HORSES IN RESPONSE TO 32 KM RACES

A. V. Andriichuk 1, H. M. Tkachenko 2, I. V. Tkachova 1

1 Institute of Animal Science, National Academy of Agrarian Sciences of Ukraine
3, 7th Hvardys'koi armiyi Str., urban village Kulynychi, Kharkiv District, Kharkiv Region, Ukraine, 62404
2 Institute of Biology and Environmental Protection, Pomeranian University in Słupsk
22b, Arciszewski Str., Słupsk, Poland, 76-200
anastasia.pohlyad@gmail.com; biology.apsl@gmail.com

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Aim. The aim of the present study was to investigate the alterations of some hematological parameters (haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), as well as resistance of erythrocytes to urea and hydrogen peroxide in horses after 32 km endurance race. Methods. Seven horses from Crimea region (Bilohirsk, Crimean region) were involved in this study. Haematological parameters (haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW)) were determined with use of hematological and biochemical methods. Blood samples have been investigated by centrifugation at 3,000 g for 15 min. The peroxide and osmotic resistance of erythrocytes were determined spectrophotometrically at 540 nm by monitoring the rate of erythrocytes disintegration by hydrogen peroxide. Endurance horses used in this study are trained and conditioned to perform over long distances at moderate speeds. The prolonged exercises were used in endurance race. The walk about 3 km/h for 20 min, the trot about 7 km/h for 15 min, and the canter about 5 km/h for 15 min and the walk about 1 km were repeated for 1 h (phase I); rest in an outdoor paddock without access to water for 30 min. And phase II: the walk about 3 km/h for 20 min, the trot about 7 km/h for 15 min, the trot about 7 km/h for 15 min, and the canter about 5 km/h for 15 min and the walk about 1 km was repeated for 1 h. Results. The results of the present study showed that adequate endurance race of low intensity could improve oxygen-dependent respiratory function in horses from Crimean region. Furthermore, the non-significant increase of red blood cells indices in endurance horses indicates good athletic level after 32 km endurance ride. Statistically significant differences in the percentage of hemolyzed erythrocytes between pre- and post-ride period were observed and thereby signify an oxidative stress-dependent impairment of erythrocyte stability. Conclusions. The haematological changes caused by various physical efforts reflect changes in the functions of different systems and can be used for health control and diagnosis of diseases. It also allows evaluating the level of sport performance, the accuracy of training, and physiological condition of horses. Keywords: endurance ride, endurance horses, hematological parameters, resistance of erythrocytes, exercise. DOI: 10.15407/agrisp3.01.066
spread in Carpathians Hucul horses – bred in Poland and Ukraine or horses in Crimean Mountains [6]. The basis of Crimean horses breed was formed from the horses of Bashkir breed imported to the Crimea in the 60s of the last century. As a result of crosses under the influence of harsh conditions of maintenance of horses herd, breeds showed genotypic and phenotypic heterogeneity. The horses in Crimea Mountains are small, about 145 cm at the withers. They are wide in the body and deep-chested, with a thoracic circumference (girth) averaging about 180 cm; they have a large head and a short neck, low withers and a flat back. The legs are short with heavy bone; cannon bone diameter may reach 20 cm. The most common coat colours are bay, red, brown, chestnut, mouse grey. The mane and tail are thick and the coat is also thick. Today, horses in Crimean Mountains are widely used in endurance race and recreational riding [6].

Metabolic responses during endurance races result from a build-up of free radicals in the muscles leading to poor performance and serious repercussions on health status, significant changes in internal homeostasis, blood volume, mean arterial pressure and plasma tonicity. There are several neuroendocrine mechanisms involved in the acute and chronic defense of internal homeostasis, which act to ensure an adequate blood flow to the working muscles and other vital tissues, together with the provision of a proper fluid volume for sweating and thermoregulation [1, 5, 7]. The working muscles of endurance horses depend on aerobic metabolism of glycogen stores, blood fatty acids and volatile fatty acids from hindgut fermentation, heart size and capacity to deliver large volumes of blood to the tissues [2, 5]. Certain cardiovascular and haematological adaptations are necessary to guarantee the correct supply of oxygen and blood substrates to active muscles during exercises and the release of metabolites. These systems could act as limiting factors to the aerobic potential and, thereby, could limit the physical performance [8].

Endurance exercise in horses leads to variety of physiological changes, i.e. an increase of haematocrit (HCT), cardiac output, mean pulmonary arterial blood pressure, and the arterial hypoxemia [2, 5, 8]. In addition, muscle temperature increases significantly during an exercise; therefore, this also can affect on the reduction of erythrocyte resistance. Also acidosis, elevated level of catecholamines, dehydration, and compression of erythrocytes in capillaries within the contracting muscle are some important mechanisms that play a role in intravascular haemolysis during regular physical activity [9]. Intravascular hemolysis is one of the most emphasized mechanisms for destruction of erythrocytes during physical activity in horses [6, 10−12]. In endurance races, stress and fatigue are clearly expressed by changes in hematological and biochemical parameters of horses [2]. Additionally, blood haematological parameters can be good indicators of the response to treatment, the severity and the systemic effects of a disease, as well as horse welfare, health and fitness levels of horses [1, 2, 5, 7]. Therefore, the main goal of the present study was to investigate the alterations of some haematological parameters [haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW)], as well as resistance of erythrocytes to hemolytic agents in Crimean horses after 32 km endurance race.

**MATERIALS AND METHODS**

**Horses.** Seven horses in Crimean region (Bilohirsk, Crimean region, Ukraine) were involved in our study (Fig. 1). The basis of local horses in Crimea was formed from the Bashkir Curly Horse which were imported to the Crimea in the 60s of the last century. Equestrian tourism in Crimea was established mainly in the mid-90s, when the horses ended up in private ownership, and each owner at his discretion led the subsequent reproduction of livestock. Thus, a lot
of horses in Crimea were subjected to chaotic cross-breeding with stallions of various breeds: Akhal-Teke, Trakehner, Arab, Thoroughbreds, trotters, and other cold blood horses, including Bashkir Curly Horse. As a result of crosses under the influence of harsh conditions of herd maintenance, breeds showed genotypic and phenotypic heterogeneity. These horses come in a wide range of sizes from miniature to draft, and in different color. Their hoofs are almost perfectly round in shape. They also have stout round-bone; straight legs; flat knees; strong hocks; short back; round rump; powerful rounded shoulders; round chest, all of which contribute to their strength and endurance.

All horses participated in endurance race. Horses were subjected to herd maintenance with feeding (hay and oat) provided twice a day and water available _ad libitum_.

All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant. Owners allowed to provide supplemental feed and salts to their horses. Information about suplementations in horse diets with antioxidant compounds, such as vitamin E or selenium, was not available. Information about previous physical activities was not available. A comprehensive physical examination was performed on all horses. The physical examination included monitoring horses’ vital clinical signs (heart rhythm, respiratory rhythm and gut sounds). In addition, the hydration status, gait of the animal, and presence of any injuries, especially in the legs, girth, withers, and back, were recorded. Only horses that had normal clinical parameters were allowed to participate in the endurance race.

_Endurance race._ The prolonged exercises were used in endurance race. The walk about 3 km per h for 20 min, the trot about 7 km per h for 15 min, the canter about 5 km per h for 15 min, and the walk about 1 km was repeated for 1 h (phase I); the rest in an outdoor paddock without access to water for 30 min. Phase II consisted of the walk about 3 km per h for 20 min, the trot about 7 km per h for 15 min, the canter about 5 km per h for 15 min, and the walk about 1 km was repeated for 1 h.

_Blood samples._ Blood was drawn from jugular veins of the animals in the morning, 90 min after feeding, while the horses were in the stables (between 8:30 and 10 AM), and immediately after endurance race (between 11:00 AM and 2:00 PM). Blood was stored into tubes with K-EDTA and held on ice until centrifugation at 3,000g for 15 minutes. The plasma was removed. The erythrocytes’ suspension (one volume) was washed with five volumes of saline solution three times and centrifuged at 3,000 g for 15 minutes. Plasma aliquots were frozen and stored at –25 °C until analyzed.

_Hematological assays._ Routine haematological parameters (haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW)) – were measured and counted with an automated hematology analyzer (Abakus Junior Vet, Austria).

_Assays of osmotic resistance of erythrocytes._ The osmotic resistance of erythrocytes was measured spectrophotometrically at the wavelength of 540 nm as described by Kamysnykov (2004) [13]. The method is based on the determination of differences between osmotic resistance of erythrocytes to a mixture containing various concentration of sodium chloride and urea. Absorbance of mixture contained erythrocytes and 0.3 M urea solution was determined as 100 % hemolysis (standard). The level of hemolysis in every test tube (%) was calculated in respect to the absorbance of standard. Hemolysis of erythrocytes (%) in every test tube with different urea concentration was expressed as curve [13].

_Assay of resistance of erythrocytes to hydrogen peroxide._ The peroxide resistance of erythrocytes was determined spectrophotometrically at 540 nm by monitoring the rate of erythrocytes disintegration by hydrogen peroxide as described by Gzhegotskyi et al. [14]. The mixture contained 0.25 mL of washed erythrocytes, 0.08 mL of 4 mM phosphate buffer (pH 7.4) with 4 mM sodium azide for inhibition of catalase activity, and 0.17 mL of 30 μM hydrogen peroxide dissolved in phosphate buffer (pH 7.4). In the blank, hydrogen peroxide was substituted by phosphate buffer. Absorbance of mixture containing erythrocytes, distilled water and hydrogen peroxide was determined as 100 %. The peroxide resistance of erythrocytes (hemolysed erythrocytes fraction) was expressed in % [14].

_Statistical analysis._ Results are expressed as mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov test (p > 0.05). In order to find significant differences (significance level, p < 0.05) between states before and after riding, Wilcoxon signed-rank test was applied to the data [15].
HEMATOLOGICAL CHANGES AND RESISTANCE OF ERYTHROCYTES

All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Poland). In addition, the relationships between values of hematological indices of all individuals were evaluated using Spearman’s correlation analysis [15].

RESULTS AND DISCUSSION

In our study, all hematological parameters of horses in Crimea region were within the reference values. In the present study, post-ride values of red blood cell indices did not significantly change compared to pre-ride period (Fig. 2).

Exercises have variable effects on the erythrocyte indices depending on work intensity, fitness and training levels, environmental conditions and breed of horses [6, 8, 16, 17]. Predominantly, the increase in the value of RBC indices in horses is caused by a release of erythrocytes from the spleen, where about 50–60% of the general number of these blood cells are located [16]. Exercise increases sympathetic activity in horses and thus increases hematocrit. The number of cells released from the spleen in response to exercise is not “all-or-none,” but rather it is related to the extent of the increase in sympathetic activity that is related to exercise intensity. However, the increase of hematocrit could also be attributable to changes in plasma volume in relation to thermoregulatory processes, mainly by sweating and evaporation from the respiratory mucosa and to fluid shift derived from physical activity [18]. Adamu et al. (2012) reported significant increases in RBC, HGB and HCT (p < 0.0001) which could indicate metabolic crisis and poor performance in endurance horses [4]. However, numerous studies have shown that horses exposed to high altitude have significantly higher RBC, HGB and platelet corpuscular volume (PCV) values, compared to animals that live at less altitude [16, 19]. It is considered a compensatory mechanism for the lower content of oxygen in the atmospheric air, which is proportionally reduced to the altitude [19].

Given fact that horses from Crimea region were housed under altitude of the Crimean Mountains, our results are consistent with previous studies [19]. Moreover, one of reasons of the slight increase of red blood cells indexes in the blood of Crimean horses can be their inhabitation on the altitude. Long term hypoxic exposure and/or stress to altitude can lead to an increment of red blood cells, hemoglobin, density of capillary blood vessels, and myoglobin density in skeletal muscle [20, 21], resulting in enhancement of oxygen delivery capacity. In the cellular level, hypoxic exposure can accelerate the proliferation of mitochondria in the muscles [22], increase the buffering capacity for lactic acid [23], and subsequently enhance endurance capacity in the high altitude environments. In spite of this theoretical rationale, the majority of studies investigating athletes who returned to the sea level from high altitude training reported no changes or

Fig. 2. Values of red blood cell indices of Crimean horses during 32 km riding

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...even reduction of the level of physical performance. And few studies demonstrated an improvement of physical performance after the altitude training [24]. This statement can be meaningful in connection with evidence of Adamu and coauthors which have shown that there are no significant changes of red blood cells indices in post-ride period in endurance horses with good performance level [2, 4].

The resistance of erythrocytes to \( \text{H}_2\text{O}_2 \) exposure in Crimean horses before and after 32 km riding was presented in Fig. 3.

No significant changes in resistance of erythrocytes to \( \text{H}_2\text{O}_2 \) exposure in Crimean horses during 32 km riding were observed (Fig. 3).

Exercise-induced hemolysis has been confirmed under various conditions in stallions [12, 25], mares [12, 26] and mixed population [6, 27]. In our study, erythrocytes exposed to the different concentrations of urea after endurance race had a higher level of hemolysis compared to the pre-ride period (Fig. 4). Increase of hemolyzed erythrocytes percent by 20.58% (\( p = 0.013 \)), by 17.3% (\( p = 0.023 \)), by 16.75% (\( p = 0.006 \)), by 11.57% (\( p = 0.015 \)), and by 8.73% (\( p = 0.008 \)) in 0.12, 0.135, 0.15, 0.165, and 0.18 M urea solutions, respectively, was observed in horses after endurance race.

Erythrocytes appear much more vulnerable to oxidative damage during intense exercise because of their continuous exposure to high oxygen fluxes and their high concentrations of polyunsaturated fatty acids (PUFAs) and heme iron [9, 28, 29]. Our findings are consistent with data of Devi et al. (2009) which have shown that osmotic stress at 0.3% and 0.4% NaCl imposed hemolysis in animals exposed to altitude and thereby signifies an oxidative stress-dependent impairment of erythrocyte stability [30]. It has been shown that lipid peroxidation and oxidation of proteins by free radicals play a major role in many oxidative erythrocytes damage and cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids [28, 29]. Based on our results, it is possible to affirm that enhanced percent of hemolyzed erythrocytes in horses after endurance ride indicates to an impairment of erythrocyte stability induced by oxidative stress.

CONCLUSIONS

Adequate endurance race of low intensity could improve oxygen-induced hematological function in Crimean horses. Furthermore, the non-significant increase of red blood cells indices in endurance horses indicates good athletic level after 32 km endurance ride. Statistically significant differences in the percentage of hemolyzed erythrocytes between pre- and post-ride period were observed. It signifies an impairment of erythrocyte stability induced by oxidative stress. The haematological changes caused by various physical efforts reflect changes in the functions of different systems and can be used for health control and diagnosis of diseases. It also allows evaluating the level of sport performance, the accuracy of training, and physiological condition of horses.

ACKNOWLEDGEMENTS

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Одне з прикладних галузей конярства, які наразі активно розвиваються, є дистанційні пробіги та кінні туризм. В Україні, враховуючи регіональні ландшафтні особливості, дистанційні пробіги є найпоширенішими в Криму та на заході країни. До коней, що використовують у дистанційних пробігах, існують певні вимоги, але, на жаль, у наукових роботах небагато досліджень, пов’язаних з аналізом складу захудлених коней, а також гематологічних та біохімічних показників крові. 

Мета. Провідні зміни показників червоної крові та резистентність еритроцитів у коней, які брали участь у дистанційному пробігу на 32 км. Методи. Об’єктом досліджень слугували сім’ї коней кримського типу. Гематологічні показники (гематокрит (HCT), концентрація гемоглобіну (HGB), кількість еритроцитів (RBC), середня концентрація гемоглобіну в еритроцитах (MCHS), середній об’єм еритроцитів (MCV), середній вміст гемоглобіну в еритроциті (MCH), індекс анізоцитозу (RDW)) визначали з використанням гематологічних та біохімічних методів. Зразки крові досягали центрифугуванням при 3000 г протягом 15 хв. Пероксидну та осмотичну стійкість еритроцитів встановлювали спектрофотометрично при довжині хвили 540 нм за швидкістю розпаду еритроцитів під дією пероксиду водню. Результати. Не виявлено достовірних змін в показниках червоної крові у коней після пробігу на 32 км, що свідчить про хороший фізичний стан та адаптацію коней до довготривалої ізди. Натомість осмотична резистентність еритроцитів була достовірно нижчу після пробігу, що свідчить про порушення цілісності еритроцитарних мембран внаслідок фізичних навантажень. Висновки. Вивчення динаміки показників крові у поєднанні з біохімічними параметрами та стійкістю еритроцитів дає змогу оцінити процеси адаптації до фізичних навантажень на витривалість в організмі коней, а також проаналізувати рівень їхнього фізіологічного резерву.

Ключові слова: дистанційні кінні пробіги, коні, гематологічні показники, резистентність еритроцитів, фізичні навантаження.

REFERENCES
INTRODUCTION

Ketosis is one of the most widespread diseases of dairy cows characterized by disorders of carbohydrate, lipids and protein metabolism. It is associated with accumulation of ketone bodies, injury of central nervous and endocrine systems, liver, heart, kidneys and other organs [1]. Amino acids play extremely important role in pathogenesis of ketosis, since they participate in synthesis of majority of endogenous biologically active substances, structural proteins, enzymes, some of hormones, nitrogenous bases, and neurotransmitters [2]. Metabolism of these substances is regulated with different biochemical and physiological mechanisms in order to maintain relatively constant concentration of amino acids in blood and tissues. Therefore, concentration of free amino acids and their derivatives in physiological liquids and tissues may be a specific showing of homeostasis, and patterns of formation of amino acid pool in organism may be a reliable reflection of a state of metabolic balance. Investigation of changes in free amino acids plasma content gives opportunity to get an insight into the very core of different diseases or their treatment. This will provide great diagnostic and prognostic value of such approach.

At the present day many treatment regimens are available for cows with ketosis. The most widespread is the conventional scheme with administration of glucoplastic agents, for example propylene glycol in combination with intravenous injections of glucose solution [3]. However, the given scheme of medicamentous therapy is effective only in case of subclinical form of disease, when liver remains unaffected. Moreover, physiological insulin resistance was revealed in high yielding cows, which is associated with formation of lactating dominant during afterpartum period [4]. Thus, exogenous glucose can not be used to its full extent by organism, since its utilization depends on the insulin level. Additional administration of in-
Insulin would increase assimilation of injected glucose, but nevertheless its major part will be excreted with urine, due to decreased sensitivity of tissue receptors to insulin during this physiological period. Propylene glycol is considered to be a glucose precursor, since it is absorbed through rumen wall and enters liver, where it is included into tricarboxylic acid cycle. In order to decrease activity of ketogenesis, Krebs cycle should be supplied with starting compounds, because intensity of ketogenesis depends on activity of tricarboxylic acid cycle. Ketogenesis is an alternative pathway for utilization of acetyl CoA. However, in case of ketosis in dairy cows tricarboxylic acid cycle may be inhibited not only due to lack of propionates, but also due to deficit of cyanocobalamin and excess of ammonia. Summarizing all abovementioned, we may suggest that conventional regimen is obsolete and not enough effective.

Reasoning from this fact, the aim of our work was to investigate and to compare the plasma content of free amino acids in ketotic dairy cows, which were treated with conventional and proposed therapeutic regimens.

MATERIALS AND METHODS

Study was performed on high yielding dairy cows in their second to fourth lactation with milk yield above 8000 kg per previous lactation. Clinical examination was performed two weeks after calving using conventional methods. Sick animals \((n = 20)\) were revealed on the basis of clinical examination and express diagnosis for the presence of ketone bodies in urine. Sick animals were divided into two groups and treatment was administered until decurrence of ketonuria. Cows from the first study group \((n = 10)\) were administered conventional for the farm treatment regimen. In particular, animals were given propylene glycol in daily dose 400 ml per head, intravenous 20 % solution of glucose \((500 \text{ ml/head/day})\) and intramuscular insulin \((200 \text{ U/head/day})\). Animals in the second group \((n = 10)\) were fed with analogous to the first group dose of propylene glycol and were given agent “Remivital” intravenously in dose of 500 ml/head/day. In this case treatment lasted five days. Samples of blood were withdrawn from jugular vein before feeding, before initiation of the therapy and after its discontinuation (on the sixth day). Obtained results of laboratory investigations were compared with parameters of clinically healthy animals \((n = 10)\) with negative test for the presence of ketone bodies in urine, kept in analogous to study animals conditions. The plasma level of free amino acids was determined using amino acid analyzer Biotronik LC 6001 (Germany).

Agent “Remivital” was developed in the Institute of Animal Biology of the NAAS. It contains fructose, amino acids and B-group vitamins. Advantage of the given agent against conventional glucoplastic substances used in treatment of cows is correction of metabolism simultaneously due to the agent’s hepato-protective and antioxidant properties. In particular, this agent contains fructose, which in contrast to glucose is quickly consumed in organism irrespective of the level of insulin. Another compound of “Remivital” is L-carnitine, which exerts antioxidant properties, participates in fatty acids transport through mitochondrial membrane and is great factor in maintenance of coenzyme A level. L-ornithine induces synthesis of carbamoyl phosphate synthetase, a crucial enzyme in synthesis of urea in hepatocytes. L-asparagine serves as raw material for synthesis of other vital amino acids and aspartic acid, which in its turn is essential in synthesis of urea. L-lysine under deficit of carbohydrates can be metabolized with formation of glucose, which is important source of energy for organism. Nicotinamide and cyanocobalamin participate in metabolism of fatty acids and elimination of ketone bodies.

RESULTS AND DISCUSSION

Results of conducted research (table) showed increase of the level of ketogenic and decrease of concentration of glycogenic amino acids in plasma of dairy cows affected with ketosis. The main reason for this is deficit of metabolic energy after calving and activation of gluconeogenesis. Amino acids, which are involved into tricarboxylic acid cycle or are converted into pyruvate can directly be transformed into glucose under the low concentration of the latter at the beginning of lactation. Consequently, the carbohydrate residue of amino acids accounts for 15 to 35 % of gluconeogenesis [5].

After conducted treatment of sick cows, improvement of general condition and absence of ketonuria were established. Herewith the plasma level of ketogenic amino acids in cows decreased. In particular, the level of leucine decreased by 24.8 % \((p < 0.001)\) and the level of phenylalanine was lower by 12.5 % \((p < 0.001)\) in group of animals treated using conventional regimen. Levels of leucine and phenylalanine were lower by 35 % \((p < 0.001)\) and 16.9 % \((p < 0.001)\) respectively in group of animals, which were injected “Remivital”. Moreover, administration of “Remivital” caused significant decrease of the level of methionine (by 34.7 %; \(p < 0.001\)), tryptophan (by 27.4 %; \(p < 0.001\)) and tyrosine (by 23.2 %; \(p < 0.001\)). It is worthy of note that plasma level of some ketogenic amino acids decreased after injection of “Remivital” (by 14.1 %; \(p < 0.001\)), arginine (by 16.9 %; \(p < 0.001\)) and histidine (by 35.4 %; \(p < 0.001\)).
acids in animals from the first study group was significantly higher than corresponding level in healthy animals (table). Namely, the level of leucine was higher by 21.6 % \((p < 0.001)\), tryptophan by 43.3 % \((p < 0.001)\), phenylalanine by 13.3 % \((p < 0.001)\) and methionine by 35.3 % \((p < 0.001)\). The plasma level of ketogenic amino acids after administration of proposed treatment regimen was within the range of statistical error, except for the level of lysine (that was higher by 27 %; \(p < 0.001\), which is obviously related to its presence in the composition of “Remivital”. Lysine decreases serum level of triacylglycerols, enhances uptake of calcium from blood and improves its transport into bone tissue [6]. The content of other ketogenic amino acids was significantly lower after administration of alternative treatment in comparison with such in case of conventional therapy (table).

Significant increase of the glycogenic amino acids plasma concentration was revealed in cows from the first group after administration of antiketotic therapy (table). In particular, the level of asparagine was higher by 11.2 % \((p < 0.001)\), histidine by 33.3 % \((p < 0.001)\) and cysteine – 2.4 times \((p < 0.001)\); table). No significant changes of the level of alanine, arginine and proline were observed. Herewith the level of alanine was lower by 19.3 % \((p < 0.01)\), arginine by 15.5 % \((p < 0.001)\), asparagine by 21.2 % \((p < 0.001)\), histidine by 23.8 % \((p < 0.001)\), and cysteine by 30.3 % \((p < 0.001)\) in comparison with healthy animals. Increase in the plasma level of alanine by 39.6 % \((p < 0.001)\), arginine by 31.8 % \((p < 0.001)\), asparagine by 49.7 % \((p < 0.001)\), histidine by 51.4 % \((p < 0.001)\), proline by 29.7 % \((p < 0.01)\) and cysteine – 4.2 times \((p < 0.001)\) was established in animals from the second study

### Plasma content of free amino acids in dairy cows, \(n = 10\), \(\mu\)mol/l

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy animals</th>
<th>Group I</th>
<th>Group II</th>
<th>(p_&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>p &lt;</td>
<td>Before treatment</td>
</tr>
<tr>
<td>Alanine</td>
<td>153.5 ± 8.93</td>
<td>116.1 ± 2.27***</td>
<td>123.9 ± 3.90***</td>
<td>0.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>58.2 ± 1.15</td>
<td>46.4 ± 1.06***</td>
<td>49.2 ± 2.21***</td>
<td>0.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>26.4 ± 1.07</td>
<td>18.7 ± 0.38***</td>
<td>20.8 ± 0.46***</td>
<td>0.001</td>
</tr>
<tr>
<td>Valine</td>
<td>122.4 ± 2.71</td>
<td>100.1 ± 2.60***</td>
<td>153.3 ± 4.93***</td>
<td>0.001</td>
</tr>
<tr>
<td>Histidine</td>
<td>36.2 ± 1.40</td>
<td>20.7 ± 0.87***</td>
<td>27.6 ± 1.01***</td>
<td>0.001</td>
</tr>
<tr>
<td>Glycine</td>
<td>412.4 ± 14.34</td>
<td>309.9 ± 8.02***</td>
<td>331.7 ± 9.23***</td>
<td>0.1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>106.5 ± 3.45</td>
<td>202.4 ± 4.40***</td>
<td>134.5 ± 5.24***</td>
<td>0.001</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>69.4 ± 3.52</td>
<td>65.6 ± 3.18</td>
<td>101.4 ± 3.78***</td>
<td>0.001</td>
</tr>
<tr>
<td>Leucine</td>
<td>48.6 ± 1.15</td>
<td>78.6 ± 2.94***</td>
<td>59.1 ± 3.11***</td>
<td>0.001</td>
</tr>
<tr>
<td>Lysine</td>
<td>56.6 ± 3.99</td>
<td>59.3 ± 2.17</td>
<td>42.4 ± 6.10*</td>
<td>0.01</td>
</tr>
<tr>
<td>Methionine</td>
<td>17.3 ± 0.78</td>
<td>24.9 ± 0.83***</td>
<td>23.4 ± 0.57***</td>
<td>0.1</td>
</tr>
<tr>
<td>Ornithine</td>
<td>39.3 ± 2.91</td>
<td>25.2 ± 1.35***</td>
<td>31.4 ± 1.60*</td>
<td>0.01</td>
</tr>
<tr>
<td>Proline</td>
<td>131.7 ± 2.95</td>
<td>115.2 ± 7.50*</td>
<td>117.5 ± 7.44</td>
<td>1.0</td>
</tr>
<tr>
<td>Serine</td>
<td>55.1 ± 3.13</td>
<td>62.9 ± 2.94</td>
<td>49.6 ± 2.43</td>
<td>0.001</td>
</tr>
<tr>
<td>Taurine</td>
<td>17.5 ± 0.47</td>
<td>26.9 ± 1.63***</td>
<td>20.5 ± 0.80***</td>
<td>0.01</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>28.9 ± 1.26</td>
<td>33.5 ± 1.64***</td>
<td>30.3 ± 1.11</td>
<td>0.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>45.7 ± 2.07</td>
<td>38.3 ± 3.69</td>
<td>41.9 ± 1.91</td>
<td>0.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>38.8 ± 1.43</td>
<td>55.8 ± 2.38***</td>
<td>55.6 ± 3.41***</td>
<td>1.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>24.0 ± 0.58</td>
<td>31.1 ± 1.24***</td>
<td>27.2 ± 0.52***</td>
<td>0.001</td>
</tr>
<tr>
<td>Cysteine</td>
<td>6.6 ± 0.24</td>
<td>1.9 ± 0.25***</td>
<td>4.6 ± 0.37***</td>
<td>0.001</td>
</tr>
<tr>
<td>Citrulline</td>
<td>41.6 ± 2.99</td>
<td>76.6 ± 1.61***</td>
<td>55.9 ± 3.51***</td>
<td>0.001</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>6.3 ± 0.57</td>
<td>27.7 ± 0.82***</td>
<td>9.9 ± 1.48***</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note. Difference between healthy and sick animals before and after treatment: *\(p < 0.05\); **\(p < 0.01\); ***\(p < 0.001\); difference between I and II study groups after treatment: *\(p < 0.05\); **\(p < 0.01\); ***\(p < 0.001\).
group. Thus, obtained results give evidence of positive effect of administered medicamentous therapy on compensation of energy deficit. Activity of compensatory mechanisms directed towards stimulation of gluconeogenesis decreased after treatment. Significant increase in concentration of cysteine is apparently related to its involvement in detoxification processes [7].

Comparing obtained results for animals of the first and the second study groups, significantly higher level of alanine (by 32.8 %; \(p < 0.001\)), arginine (by 24.6 %; \(p < 0.001\)), asparagine (by 33.2 %; \(p < 0.001\)), histidine (by 18.5 %; \(p < 0.01\)), proline (by 24.8 %; \(p < 0.01\)) and cysteine (by 54.3 %; \(p < 0.001\)) were revealed after administration of “Remivital”.

Serine plays an important role in metabolism of fatty acids. Under the influence of serine-oximethyltransferase in the presence of tetrahydrofolic acid serine is converted to glycine. Simultaneously this reaction is the first in catabolism of serine and formation of glycine [8]. In the process of degradation in organism serine undergoes direct or indirect deamination with formation of pyruvate, which is subsequently included in tricarboxylic acid cycle. Glycine in its turn exhibits antioxidant, adrenoblocking and antitoxic activity. Moreover, glycine regulates the function of glutamate receptors [9]. As can be seen from the results presented in table, decrease of serine concentration in plasma was found after medicamentous therapy in cows from the first (by 21.1 %; \(p < 0.001\)) and the second (by 15.9 %; \(p < 0.01\)) study groups. In concurrence with this increase of glycine content was observed, particularly in the first study group its level was higher by 7 %, but in comparison with healthy cows the concentration was lower by 19.6 % (\(p < 0.001\)). In plasma of cows from the second study group after treatment the content of glycine increased almost by 50% (\(p < 0.001\)) and was somewhat higher than in healthy cows (table). On the basis of the foregoing material it might be suggested that administered schemes of therapy, primarily with agent “Remivital” stimulate antioxidant and antitoxic systems of organism defence.

It is widely known [9] that glycine and serine are often synthesized in organism from threonine. The latter as well as methionine is a lipotropic substance and participates in fight against the fat deposition in liver and is essential in synthesis of purines, which in their turn decompose urea, waste product of protein synthesis. As can be seen from table data, administered conventional scheme of therapy had no significant influence on the concentration of threonine in plasma. An increase in its level by 34.2 % (\(p < 0.01\)) was revealed after administration of “Remivital” and propylene glycol. Simultaneously we observed significant decrease in taurine concentration (table). After administration of conventional regimen the concentration decreased by 23.8 % (\(p < 0.01\)), and after using of proposed scheme the level was lower by 30.9 % (\(p < 0.001\)). It is important to mention that the plasma concentration of taurine in animals from the first study group was higher than in healthy animals by 17.1 % (\(p < 0.001\)), and in the second group it reached the level of clinically healthy animals. Taurine is a sulphonic acid, synthesized in human and animal organism from cysteine and it plays vital role in digestion and lipid assimilation, is one of the constituents of bile. Moreover, taurine is an important antioxidant [10].

It facilitates digestion and producing of bile in liver, enhances cholesterol degradation, improves the function of gall bladder by formation of taurocholate from bile acids, which increases cholesterol elimination in the bile. Taurine is a key component of bile acids, which helps to maintain optimal liver function and is necessary for elimination of toxic chemical substances and metabolic waste products [11]. In view of this it might be suggested that normalization of taurine concentration is an evidence of restoration of bile secretion.

Increase of the level of valine and isoleucine was revealed during investigation of the content of branched amino acids. The blood level of leucine decreased in cows from both study groups (table), which is considered to be positive effect of therapy since this amino acid is ketogenic. The plasma concentration of valine in the first study group increased by 53.1 % (\(p < 0.001\)), and in the second by 33.5 % (\(p < 0.001\); table). The content of isoleucine was higher by 54.6 % (\(p < 0.001\)) and 34.8 % (\(p < 0.001\)) respectively (table). These three amino acids predominantly disintegrate in muscle tissue and play an important role in energy metabolism, particularly in formation and deposition of glycogen [5]. It is considered that during muscle work they may be used for synthesis of intermediate compounds for tricarboxylic acid cycle and gluconeogenesis, i.e. serve as source of energy. It should be mentioned that the plasma content of valine in cows, which were given conventional regimen of treatment, was higher by 25.2 % (\(p < 0.001\)) in comparison with healthy animals (table). Furthermore, significantly higher level of isoleucine should be pointed out (table). For example, after administration of conventional regimen of treatment, the plasma concentra-
The effect of "Remivital" on plasma amino acid composition in dairy cows with ketosis

The concentration of isoleucine was higher by 24.6 % (p < 0.001) in comparison with animals which were treated with "Remivital" or by 46.1 % (p < 0.001) in comparison with healthy animals. High level of isoleucine on the one hand is linked to a compensation of energy deficit, but on the other hand, under the deficit of enzymes catalyzing isoleucine decarboxylation, leads to development of ketoacidosis [8].

Extremely important role in utilization of ammonia and lactate, which in significant quantities are present in organism of ketotic cows, belongs to ornithine and citrulline. As table data show, plasma content of ornithine was higher, and the level of citrulline was lower in cows after combined administration of “Remivital” and propylene glycol. In particular, concentration of ornithine increased by 90.2 % (p < 0.001). The main reason of elevation of ornithine concentration is exogenous supply of the substance with agent “Remivital”. L- ornithine stimulates the synthesis of carbamoyl phosphate synthetase, crucial enzyme for synthesis of urea in hepatocytes. The content of citrulline was 2-fold lower (p < 0.001). Citrulline also promotes elimination of ammonia and urea from organism. Besides of that arginine the main donor of nitrogen, which improves blood flow in muscles, is synthesized from citrulline [12]. It might be suggested that significant elevation of the arginine level and decrease of citrulline concentration is explained exactly by this fact. Use of conventional regimen was associated with an increased level of ornithine (by 24.6 %; p < 0.01) and decreased concentration of citrulline (by 27.0 %; p < 0.001) in comparison with pre-treatment parameters. However, attention should be given to significantly lower level of ornithine (by 35.5 %; p < 0.001) and higher concentration of citrulline (by 42.6 %; p < 0.001; table) in comparison with proposed therapeutic regimen.

Conducted determination of amino acids, markers of muscle protein catabolism (3-methylhistidine, glutamine) revealed significant decrease of their content after therapy both in the first and the second study groups of cows (table). After administration of conventional regimen the concentration of 3-methylhistidine decreased 2.8-fold (p < 0.001) and the level of glutamine was lower by 33.5 % (p < 0.001). However the level of 3-methylhistidine was higher by 57.1 % (p < 0.01), and glutamine by 26.3 % (p < 0.001) in comparison with healthy cows. After administration of conventional therapy four of ten cows had the level of 3-methylhistidine exceeding the upper physiological limit. Administration of proposed scheme of therapy was associated with a more than 4-fold (p < 0.001) and 1.8-fold (p < 0.001) decrease in the level of 3-methylhistidine and glutamine respectively. In the second study group eight of ten cows had the level of 3-methylhistidine within the physiological limits for healthy animals (table). In comparison with the first study group the concentrations of 3-methylhistidine and glutamine were lower by 27.3 % and 15.7 % (p < 0.01) respectively. Obtained results give evidence of less active protein catabolism in dairy cows after treatment due to liquidation of energy deficit [13].

Increase in the level of glycogenic amino acids and decrease of the concentration of ketogenic amino acids after treatment caused an increase of ratio of glycogenic to ketogenic amino acids, which is a sign of normalization of protein metabolism (Figure). In the first group an increase was by 24.4 % (p < 0.001), and in the second by 45 % (p < 0.001).
CONCLUSIONS

After 5-day-long medicamentous therapy given to animals in the first and the second study groups, following changes were observed: improvement of general condition, absence of ketouria, increase of the level of glycogenic and decrease of ketogenic amino acids. Conducted determination of amino acids, markers of muscle protein catabolism (3-methylhistidine, glutamine) revealed significant decrease of their content after conducted therapy; however in animals from the first group their concentration was significantly higher in comparison with cows from the second group and healthy cows. Increase in the plasma level of glycogenic amino acids and decrease of the plasma concentration of ketogenic amino acids after treatment caused an increase of ratio of glycogenic to ketogenic amino acids, which is a sign of normalization of protein metabolism. In the first group an increase was by 24.4 % (p < 0.001), and in the second by 45 % (p < 0.001). Administration of conventional regimen of therapy was associated with significantly higher level of ornithine and lower concentration of citrulline, which give evidence of problems in utilization of ammonia and lactate, and the high level of isoleucine contributes to aggravation of ketoacidosis.

Vлияние препарата “Ремивитал” на аминокислотный склад плазмы крови молочных коров, больных кетозом

М. Р. Симонов, В. В. Влізло

e-mail: msimonov@inenbiol.com.ua

Інститут біології тварин НААН

Ул. В. Стуса, 38, Львів, Україна, 79034

Мета. Дослідити амінокислотний склад плазми крові молочних корів, хворих на кетоз

Метод. Встановлено зниження рівня кетогенных амінокислот, зростання соотношення глікогенних амінокислот та амінокислот з розгалуженними ланцюгами. Відмічене значне зменшення концентрації 3-метилгістидину, що свідчить про послаблення катаболізму скорочувальних білків.

Висновки. Запропонована схема лікування дозволила ефективною порівняно з традиційною, оскільки при застосуванні останньої встановлено вірогідну відміну орнітіну та низький – цитруліну. Це дає підстави стверджувати, що існує проблема в утилізації амінокислот та лактату. Крім цього, використання традиційної терапії великі обсяги показників маркерів катаболізму скорочувальних білків лишеється високою на відміну від тварин після запропонованого лікування, а значний вміст ізозелініну сприяє посиленню кетоацидозу.

Ключові слова: корови, кетоз, лікування, препарат “Ремивітал”, амінокислоти.

REFERENCES


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