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

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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.

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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 bodyweight, 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.

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. In Japan (2004, Okayama University) Fe content varied from 24.6 to 63.3 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, Fibar, 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 1 water (18 Mohm), obtained in the purifica-

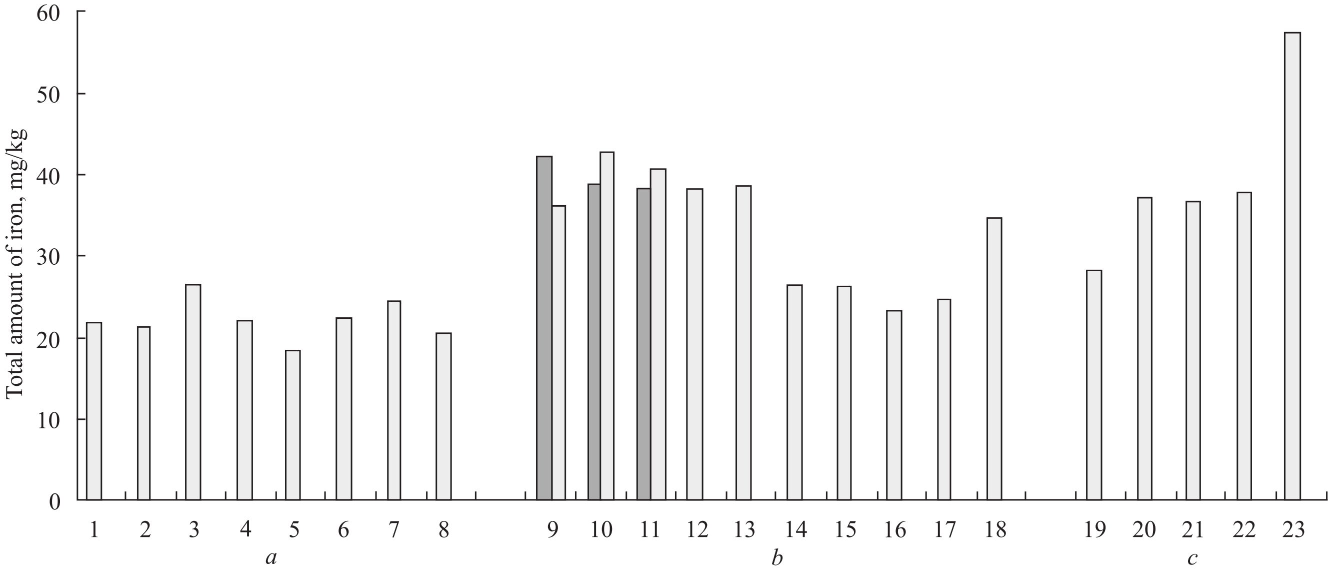

Fig. 1. Iron content in barley caryopsis: *a* – of white color; *b* – of brown or blue color; *c* – of black color (1 – Alberte; 2 – Fibar; 3 – Hilose; 4 – SL-2019Wx; 5 – SL-2023Wx; 6 – SL-2028Wx; 7 – SL-432/33; 8 – SL-432/36; 9 – Achilles-Odesa, 2013, 2014; 10 – Achilles-Dnipropetrovsk, 2013, 2014; 11 – Achilles-Kyiv, 2013, 2014; 12 – Lophy-1; 13 – SL-2043; 14 – SL-250/21; 15 – SL-2083; 16 – SL-430/57; 17 – SL-430/60; 18 – k-3977 (local from Mongolia); 19 – k-18703 (local from Ethiopia); 20 – UA-0800338 (Arabische); 21 – UA-0804248 (Abyssinian 1105); 22 – UA-0802460 (Negra Manfredi); 23 – UA-0801394 (Sulto-1))

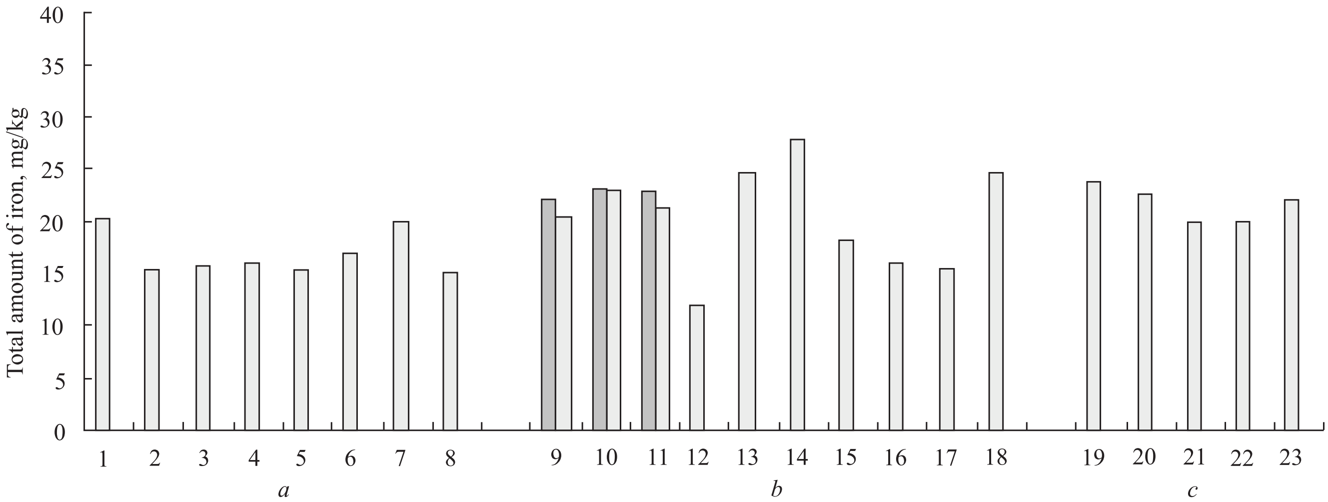

Fig. 2. Zinc content in barley caryopsis: *a* – of white color; *b* – of brown or blue color; *c* – of black color (1 – Alberte; 2 – Fibar; 3 – Hilose; 4 – SL-2019Wx; 5 – SL-2023Wx; 6 – SL-2028Wx; 7 – SL-432/33; 8 – SL-432/36; 9 – Achilles-Odesa, 2013, 2014; 10 – Achilles-Dnipropetrovsk, 2013, 2014; 11 – Achilles-Kyiv, 2013, 2014; 12 – Lophy-1; 13 – SL-2043; 14 – SL-250/21; 15 – SL-2083; 16 – SL-430/57; 17 – SL-430/60; 18 – k-3977 (local from Mongolia); 19 – k-18703 (local from Ethiopia); 20 – UA-0800338 (Arabische); 21 – UA-0804248 (Abyssinian 1105); 22 – UA-0802460 (Negra Manfredi); 23 – UA-0801394 (Sulto-1))

tion system Scholar-UV Nex Up 1000 (Human Corporation, Korea). Fluka Multielement standard solution 5 for ICP served as calibration standard [15].

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 hukk-less barley breeding lenes were studied for TE content, most valuable for human he-alth – iron, zinc, manganese and selenium. The data on the detection of iron content in barley caryopsis are presented in Fig. 1.

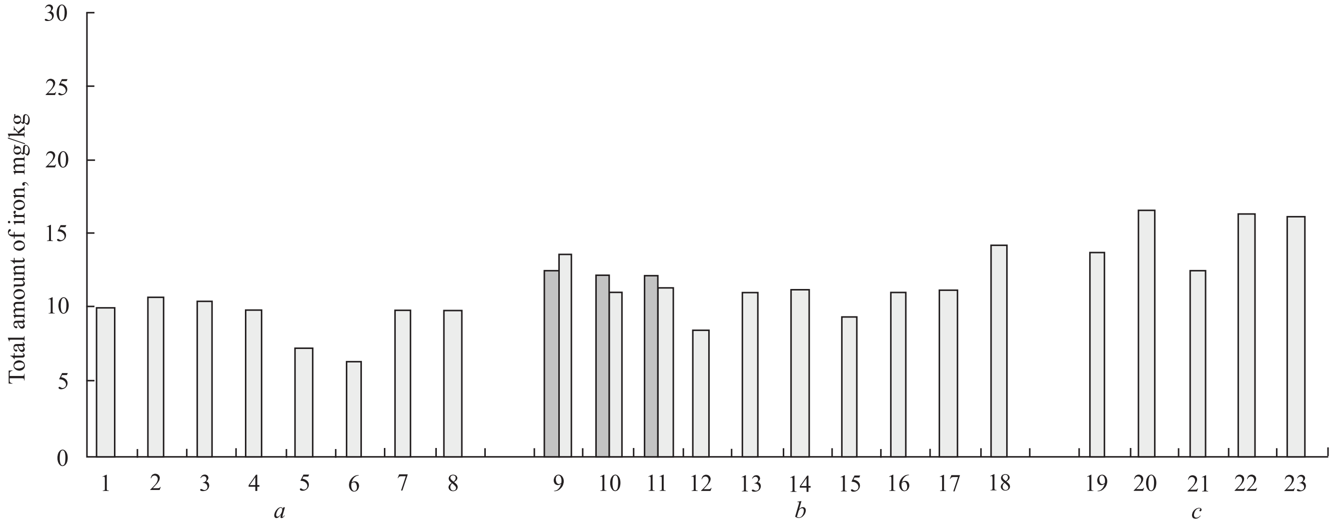

Fig. 3. Manganese content in barley caryopsis: *a* – of white color; *b* – of brown or blue color; *c* – of black color (1 – Alberte; 2 – Fibar; 3 – Hilose; 4 – SL-2019Wx; 5 – SL-2023Wx; 6 – SL-2028Wx; 7 – SL-432/33; 8 – SL-432/36; 9 – Achilles-Odesa, 2013, 2014; 10 – Achilles-Dnipropetrovsk, 2013, 2014; 11 – Achilles-Kyiv, 2013, 2014; 12 – Lophy-1; 13 – SL-2043; 14 – SL-250/21; 15 – SL-2083; 16 – SL-430/57; 17 – SL-430/60; 18 – k-3977 (local from Mongolia); 19 – k-18703 (local from Ethiopia); 20 – UA-0800338 (Arabische); 21 – UA-0804248 (Abyssinian 1105); 22 – UA-0802460 (Negra Manfredi); 23 – UA-0801394 (Sulto-1))

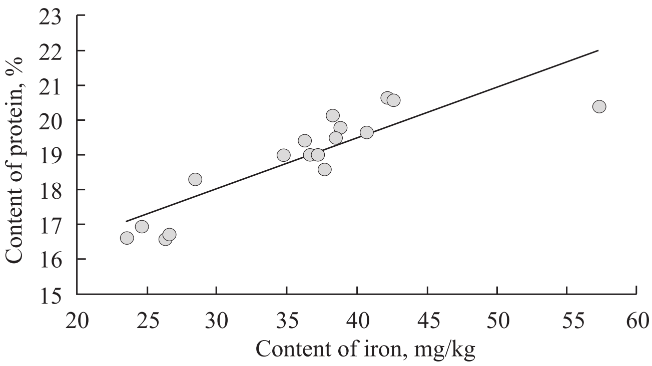

Fig. 4. The correlation between the content of protein and that of iron in barley grain

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, including 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.

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

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Мета. Дослідити вміст важливих для здоров'я людини мікроелементів (МЕ): заліза, цинку, марганцю, селену в сортах і селекційних лініях ячменю, а також перевірити вплив абіотичних факторів середовища на накопичення МЕ у зерні сорту Ахіллес. **Методи**. Визначення вмісту МЕ проводили на мас-спектрометрі з індуктивно зв'язаною плазмою (ICP-MS) «Agilent 7700x». **Результати**. Досліджено 26 зразків ячменю для виявлення вмісту життєво необхідних МЕ. Підвищену концентрацію МЕ спостерігали в зразках з коричневими, синіми та чорними зернівками. На сорті Ахіллес, представленому шістьма різними повторностями, продемонстровано відсутність залежності між абіотичними факторами середовища і накопиченням МЕ. Визначено кореляційну залежність між вмістом білка і заліза в зерні. **Висновки.** За вмістом Fe, Zn, Mn, Se білозерні сорти і лінії поступаються генотипам з коричневим, синім або чорним зерном. Наявність або відсутність плівки у зерні ячменю майже не впливає на кількість МЕ, котрі, як і очікувалось, містяться в алейроновому шарі і зародку зернівки. Доведено, що вміст Fe, Zn, Mn у зерні сорту Ахіллес залежно від абіотичних факторів середовища майже не змінюється. Між вмістом білка і Fe в зерні знайдено високу кореляційну залежність, $r = 0{,}87$, $P > 0{,}05−0{,}001$.

Ключові слова: ячмінь, мікроелементи, залізо, цинк, марганець, селен.

Определение содержания микроэлементов Fe, Zn, Mn, Se в зерне ячменя

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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 7700x». **Результаты.** Исследованы 26 образцов ячменя для выявления содержания ценных МЭ. Большее количество МЭ наблюдалось в образцах 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 в зерне сорта Ахиллес Fe, Zn, Mn в зависимости от абиотических факторов среды почти не меняется. Между содержанием белка и Fe в зерне наблюдается высокая корреляционная зависимость, r = 0,87, Рт> 0,05–0,001.

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

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