Influence of germination of wheat grain with selenium sources on the components of protein-carbohydrate complex

N A Galochkina, I A Glotova and N V Podlesnykh

Voronezh State Agrarian University named after Emperor Peter the Great, 1, Michurina St., Voronezh, 394087, Russia

E-mail: galochkina na@mail ru

Abstract The aim of the study is histochemical identification of the components of protein-carbohydrate complex of wheat grains with selenium sources during germination. We formulated a hypothesis about the stimulating effect of 4,4-di [3 (5-methyl dipirazoly] selenid (DMDPS) on the biosynthesis of substances with a bactericidal effect, which include mucoid substances and glycoproteins in the composition of the components of the protein-carbohydrate complex. We confirm the hypothesis on histochemical identification of the components of the protein-carbohydrate complex of wheat grain during germination with sources of selenium. To identify acid mucopolysaccharides, transverse and longitudinal sections of the grain were stained with Alcian blue according to Steedman. Schiff-iodic acid revealed neutral glycoproteins and was clarified in xyllol. The sections were then placed into the balm and viewed in a Zeiss Axioscop 40 FX light microscope using a Levenhuk C 510 NG digital camera. To detect methionine, sections were stained with Schiff’s reagent; to identify cysteine (SH groups), sections were treated with a 0.05% solution of 2.2-dioxy-6.6-dinaphthyl disulfide. The obtained images were analyzed using Photoshop CS 5 tools. Positive effect of wheat germination with DMDPA on the content of both neutral glycoproteins and mucopolysaccharides, with a direct correlation relationship, and on the content of methionine and cysteine was proved.

1. Introduction

Selenium is an essential trace element, but its deficiency in the soil limits the possibilities of natural biochemical transformation of this element in food chains and leads to its deficiency in food and feed rations [1]. A promising direction of improving the functional properties of food biopolymer systems in plant raw materials, increasing its nutritional and biological value is the realization of its own metabolic potential by activating enzyme systems during seed germination [2, 3]. The products of hydrolytic transformations of food substances of seeds during germination are potential exoregulators of metabolism [2, 4]. Under fixed conditions of seed germination, such as environmental temperature, humidity, pH and ionic strength of an aqueous solution of salts in the liquid phase, the variable parameter is the duration of the germination process [2, 5]. From a technological point of view, it is important to regulate the duration of the germination of grains to obtain food with the highest nutritional value and functionality.

Known from the literature data on the effects on biochemical processes in the germination of cereals relate to sodium selenite, as a source of selenium. It is of interest to assess the feasibility of introducing the organic selenium-containing preparation DMDPS into the liquid phase when soaking wheat grain, the effectiveness of which as a regulator and stimulator of the antioxidant system of the body in high-risk conditions was shown by a number of authors [6,7].
The purpose of the work is the histochemical identification of the components of the protein-carbohydrate complex of wheat grain during germination with DMDPS.

2. Materials and methods
In the study, the seeds of soft winter wheat “Alaya Zarya” were used in the Central Black Earth Region [10]. The variety combines the signs of winter hardiness and productivity with high baking qualities of grain, and the process of induced autolysis during germination allows targeted enrichment of raw materials with biologically active components. It can be used in the development of new innovative food products.

The sources of selenium during germination were sodium selenite (FSP 42-0250-1024-01, manufactured by MCD, Moscow) and 4,4-di [3 (5-methylidipyrazolyl)] selenide (DMDPS) with a content of 0.657 g DMDPS per 100 cm³ of the preparation (manufacturer - LLC Safron, Moscow, sanitary-epidemiological conclusion № 77 99 13 003 T 000518 03 03 06). According to [10], DMDPA today is the lowest toxic compound of selenium, obtaining weak cumulativeness.

Wheat seeds were germinated in accordance with the recommendations [10] in germinators on filter paper under the conditions of optimal wetting at a temperature of 20 °C for 40 hours. The ratio of the liquid phase and grain is 4:5 [10]. As the liquid phase we used tap water (sample “Wheat+H₂O” - control); aqueous solutions of sodium selenite Na₂SeO₃ (sample “Wheat +Na₂SeO₃”) and DMDPS (sample “Wheat+DMDMS”) with a concentration of 0.005%, calculated on the basis of selenium. The repetition of experiments is threefold.

Grains treated with organic and inorganic selenium and water were fixed in 10% neutral formalin and processed by common histological method dehydrated in alcohols of increasing concentration and kept in chloroform, followed by pouring in paraffin. Paraffin sections 6–7 µm thick after dewaxing were stained with. Alcian blue by Steedman to detect acid mucopolysaccharides, the images are shown in Figures 1–3. Schiff-iodic acid revealed neutral glycoproteins and was clarified in xylene, the images are presented in Figures 4–6. Using this method, all the compounds containing oxy groups can be detected, which can be converted into aldehyde groups [11]. The arrows indicate the location of the corresponding components.

To detect methionine, the sections were stained with Schiff’s reagent and then washed in 0.5% sodium hydrosulfite solution with addition of 10 cm³ of 1N HCl solution for 2 minutes. After washing in water, the sections were dehydrated in alcohols of ascending concentration, clarified in xylene, contained in balsam. The aldehyde is condensed with Schiff’s reagent, during the reaction a chromophore is formed, and at the same time tissue structures containing amino groups turn red. The results are presented in Figure 7.

![Figure 1](image-url)  Identification of acid mucopolysaccharides in wheat grain germinated in water (cross section); coloring with Alcian blue by Steedman; × 400
Figure 2. Identification of acid mucopolysaccharides in wheat grain germinated in a DMDS solution (longitudinal section); coloring with Alcian blue by Steedman; × 400

Figure 3. Identification of acid mucopolysaccharides in wheat grain germinated in sodium selenite solution; coloring Alcian blue by Steedman; × 400

Figure 4. Identification of neutral mucopolysaccharides in wheat grain germinated in sodium selenite solution (longitudinal section); CHIC reaction; × 400

Figure 5. Identification of neutral mucopolysaccharides in wheat grain germinated in DMDPA solution (longitudinal section); CHIC reaction; × 400
Figure 6. Identification of neutral mucopolysaccharides in wheat grain germinated in water; (a - cross-section, b - longitudinal section); CHIC reaction; × 400

To identify cysteine (SH-groups) sections, the sections after deparaffinization were treated with a 0.05% solution of 2,2'-dioxy-6,6'-dinaphthyl disulfide (DDD) in 30% ethanol prepared in phosphate buffer with pH 8.5 for 1 hour at 50 °C. After cooling to a temperature of (20 ± 2) °C, the sections were washed with distilled water, the alcohols of increasing concentration were dehydrated in a battery, washed twice in ether for 5 min, and then brought to a distilled water through alcohols of descending concentration. For 2 min, the sections were stained with a 0.1% solution of strong blue B, prepared in 0.1 M phosphate buffer at pH 7.4. In this case, structures containing SH groups were colored blue [12]. The results are presented in Figure 8.

Figure 7. Identification of methionine in wheat germinated in solution a - sodium selenite, b - water, c - DMDPS; Schiff reagent × 400.

Figure 8. Identification of cysteine in wheat germinated in a solution of sodium 1-selenite, 2 - water, 3 - DMDPS; durable blue coloured B; × 400

Then the sections were enclosed in balm and examined in a Zeiss Axiscop 40 FX light microscope. With the help of a digital camera Levenhuk C 510 NG, in each section of the grain, 5-6 randomly selected fields of view were selected, from which digital micrographs were obtained. The obtained images were analyzed using Photoshop CS 5 tools. Using the histogram tool, the area of all tissue
structures of the grain was calculated, and then the area of the dyed structures and those containing acid mucopolysaccharides or neutral glycoproteins. The latter value was expressed as a percentage, taking the first for 100%. The results of measurements and calculations were subjected to statistical processing.

The average proportion of the structures containing acid mucopolysaccharides or neutral glycoproteins (%) in all groups was determined by the formula:

$$\bar{M} = \frac{1}{n} \sum_{j=1}^{n} x_j \times 100\% ;$$  \hspace{1cm} (1)

where $n$ is the total number of pixels attributable to all grain structures, $x$ is the number of pixels per structure containing mucopolysaccharides or glycoproteins. Then, the standard deviation $\sigma$ and the representativeness error $m$ of each fraction were calculated:

$$\sigma = \sqrt{\frac{1}{n-1} \sum_{j=1}^{n} (x_j - \bar{M})^2} ;$$  \hspace{1cm} (2)

$$\sigma = \sqrt{\sigma^2} ;$$  \hspace{1cm} (3)

$$m = \frac{\sigma}{\sqrt{n-1}} ;$$  \hspace{1cm} (4)

In assessing the significance of differences between different groups, Student’s criterion was used. The distribution of parameters in this case corresponded to the normal one. To calculate this criterion, instead of the absolute average values of the shares, their angular relations $\varphi$ were used, where

$$\varphi = 0,0349 \arcsin \sqrt{\bar{M}} \text{ (radians)} ;$$  \hspace{1cm} (5)

In this case, the error of the representativeness of the angular ratio of the fraction $m_{\varphi}$ depends only on the total number of the group:

$$m_{\varphi} = \frac{1}{\sqrt{m}} ; \quad \text{or} \quad m_{\varphi}^2 = \frac{1}{m} ;$$  \hspace{1cm} (6)

Then the Student’s coefficient $t$ is calculated by the formula:

$$m = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}} ;$$  \hspace{1cm} (7)

Differences were considered significant at $t \geq 1.96$, i.e., $p<0.05$, considering the number of degrees of freedom:

$$v = n_1 + n_2 - 2;$$  \hspace{1cm} (8)

To identify the degree and nature of the relationship between the content of acid mucopolysaccharides and neutral glycoproteins, the linear Pearson correlation coefficient ($r$) was calculated using the formula:

$$r = \frac{\sum x_{1j} x_{2j} - \left(\frac{\sum x_{1j} \sum x_{2j}}{N}\right)}{\sqrt{\left(\sum (x_{1j} - \bar{M}_1)^2 \times (x_{2j} - \bar{M}_2)^2\right)}} ,$$  \hspace{1cm} (9)
where $X_{j1}$ is the measurement result of the first feature; $X_{j2}$ - the measurement result of the second feature; $\bar{M}_1$ - arithmetic average of the first row of signs; $\bar{M}_2$ - arithmetic average of the second row of signs; $N$ - the number of compared pairs

3. Results and discussion

It was established that the nature of selenium preparations has a significant effect on the character of the processes of swelling and germination of grain. Selenium in an inorganic form inhibits the germination process, but does not protect against damage. Organic selenium stimulates the germination of grains and blocks pathogenic processes [10].

In this connection, a hypothesis was formulated about the stimulating effect of DMDPA on the biosynthesis of substances with a bactericidal effect. These include, in particular, mucoid substances and glycoproteins as the components of the protein-carbohydrate complex. To confirm the hypothesis, a morphometric identification of the components of the protein-carbohydrate complex of acidic mucopolysaccharides and neutral glycoproteins of wheat grain was carried out during germination with sources of selenium. Cross and longitudinal sections of the grain were stained with Alcian blue by Steedman, and Schiff-iodic acid.

Selenium metabolism is catalyzed by certain enzyme systems, and glutathione contributes significantly to it [15]. According to the results of the studies presented earlier, during wheat germination, there is a sharp increase in the content of glutathione. Further, selenium passes into the composition of organic compounds, namely, into selenomethionine and selenocysteine [10].

Thus, the conversion of a trace element presents two stages (Figure 9) - at the first one, selenium is transformed to selenide with glutathione, and at the second one, amino acid synthesis and replacement of sulfur by selenium occurs in them to form selenomethionine and selenocysteine.

\[
\text{Se} \xrightarrow{\text{stage 1}} \text{G} - \text{S} - \text{Se} - \text{S} - \text{G} \xrightarrow{\text{stage 2}} \text{G} - \text{S} - \text{Se} - \text{H} \xrightarrow{\text{H}_2\text{Se}} \text{selenohydrogen} \]

\[
\text{stage 1} \xleftarrow{\text{selencysteine}} \xrightarrow{\text{selenmethionine}} \text{stage 2}
\]

**Figure 9.** Stages of the metabolism of selenium

The reactions of the first stage are hydrolysis, reduction and oxidation, which takes place with the participation of the functional groups -OH, -NH2, -SH and COOH. The metabolism of the second stage consists of glucuronization, sulphation, acetylation, methylation, as well as conjugation with glutathione and amino acids [15-17].

**Table 1.** Average proportion of the identified components of the protein-carbohydrate complex

| Groups                  | Average share of structures, % | $\sigma$ | $m$  |
|-------------------------|--------------------------------|---------|------|
| with acid mucopolysaccharides |                               |         |      |
| Wheat +Na$_2$SeO$_3$     | 8.6                            | 2.38    | 0.68 |
| Wheat +H$_2$O           | 13.1                           | 4.26    | 1.46 |
| Wheat +DMDPS            | 21.3                           | 3.07    | 1.17 |

| with neutral glycoproteins |                               |         |      |
| Wheat +Na$_2$SeO$_3$     | 6.1                            | 0.64    | 0.46 |
| Wheat +H$_2$O           | 8.7                            | 0.83    | 0.20 |
| Wheat +DMDPS            | 16.3                           | 0.79    | 0.16 |

The results of the statistical analysis of the identified components of the protein-carbohydrate complex of wheat grain containing acid mucopolysaccharides and neutral glycoproteins are shown in Table 1. In
assessing the significance of differences between different groups, the methods of statistical analysis were used. The results of the calculations are given in Table 2. The distribution of the parameters in this case corresponded to the normal one.

Table 2. Significance of differences between different groups of comparison

| Groups of comparison                          | t       | p    |
|-----------------------------------------------|---------|------|
| containing acid mucopolysaccharides           |         |      |
| (Wheat +Na₂SeO₃) – (Wheat +H₂O)               | 2.78    | <0.01|
| (Wheat +Na₂SeO₃) – (Wheat +DMDPS)             | 5.46    | <0.001|
| (Wheat +DMDPS) – (Wheat +H₂O)                 | 4.39    | <0.001|
| containing neutral glycoproteids              |         |      |
| Wheat +Na₂SeO₃ – Wheat +H₂O                   | 5.27    | <0.001|
| Wheat +Na₂SeO₃ – Wheat +DMDPS                 | 9.26    | <0.001|
| Wheat +DMDPS – Wheat +H₂O                     | 9.51    | <0.001|

The average proportion of structures with neutral glycoproteins and acidic mucopolysaccharides in the samples is shown in Figure 10.

Figure 10. Average proportion of the identified components of the protein-carbohydrate complex containing acid mucopolysaccharides and neutral glycoproteins in wheat samples.

α-Amino acids are detected using Schiff ninhydrin-reagent (fuchsin acid) The method is based on the interaction of ninhydrin with amino groups (-NH₂); the aldehyde which has been formed by Schiff’s reagent Amino acid reactions in amino groups (-NH₂), carboxyl (-COOH), sulfhydryl (-SH) and disulfide (-SS) groups The results of statistical analysis of the revealed structures with amino groups (methionine) and -SH groups (cysteine) are shown in Tables 3-4.

Table 3. Average proportion of structures containing methionine and cysteine

| Groups                  | Average share of structures, % | σ    | m    |
|-------------------------|--------------------------------|------|------|
| **with amino groups (methionine)** |                                |      |      |
| Wheat +Na₂SeO₃          | 10.3                           | 2.84 | 0.83 |
| Wheat +H₂O             | 16.8                           | 2.49 | 1.27 |
| Wheat +DMDPS           | 25.4                           | 4.72 | 1.35 |
| **with -SH groups (cysteine)** |                                |      |      |
| Wheat +Na₂SeO₃         | 5.8                            | 0.49 | 0.35 |
| Wheat +H₂O            | 7.9                            | 0.63 | 0.44 |
| Wheat +DMDPS          | 14.6                           | 0.79 | 0.27 |
The data on the average share of the structures with methionine and cysteine in wheat germ samples are presented in Figure 11. Taking as a baseline the content of structures with methionine and cysteine in a wheat sample germinated with water, it should be noted that wheat germination in sodium selenite solution reduces by 10.3% content of identifiable amino groups characteristic of methionine, and by 26.6% the content of sulfhydryl groups characteristic of cysteine. Opposite results are obtained after placing DMDPS into the water during soaking and germination. In this case, the growth of structures characteristic of methionine (NH2-group) was 51.2%; the growth of structures characteristic of cysteine (SH-groups) was 84.8% compared with their baseline.

In order to identify the degree and nature of the relationship between the content of acid mucopolysaccharides and neutral glycoproteins, the linear Pearson correlation coefficient was calculated. For samples of wheat germinated in a solution of sodium selenite and DMDPS, it is 0.994 and 0.997, respectively. Such values of the Pearson correlation coefficient reflect the existence of a linear relationship between the two quantities and indicate a very high degree of positive correlation between the studied traits [18-20].

### Table 4. Significance of differences between different groups of comparison

| Groups of comparison | t    | p    |
|----------------------|------|------|
| containing methionine |      |      |
| (Wheat +Na2SeO3)–(Wheat +H2O) | 4.23 | <0.001 |
| (Wheat +Na2SeO3)–(Wheat +DMDPS) | 9.53 | <0.001 |
| (Wheat +DMDPS)–(Wheat +H2O) | 4.64 | <0.001 |
| containing cysteine |      |      |
| (Wheat +Na2SeO3)–(Wheat +H2O) | 3.74 | <0.001 |
| (Wheat +Na2SeO3)–(Wheat +DMDPS) | 9.91 | <0.001 |
| (Wheat +DMDPS)–(Wheat +H2O) | 4.98 | <0.001 |

4. Conclusion
We confirmed the hypothesis formulated by us about the stimulating effect of 4,4-di [3 (5-methylidipirazolyl] selenide (DMDPA) on biosynthesis in biochemical processes during soaking and germinating wheat grain substances with a bactericidal effect. These include mucoid substances and glycoproteins in the composition of the components protein-carbohydrate complex of wheat grain. The positive effect of wheat grain germination with DMDPA on the content of both neutral glycoproteins and mucopolysaccharides, between which there is a direct correlation link, and on the content of amino groups of methionine and cysteine has been proved.

References
[1] Lyons G H, Stangoulis J C R and Graham R D 2005 Phytotoxicity, selenium, sulphur, wheat (Triticum aestivum L) to high soil and solution selenium levels Plant and soil 270(1-2) 179-178
[2] Dobrutskaya E G, Kurbakova O V and Golubkina N A 2010 The role of selenium in the formation of germination of carrot and dill seeds *Bulletin of the Russian Academy of Agricultural Sciences* 4 41-43

[3] Stashkova N O Technological aspects of obtaining dry germinated wheat grains *Storage and processing of agricultural products* 2 37-38

[4] Lazo-Velez M A, Aviles-Gonzalez J, Serna-Saldívar S O and Temblador-Perez MC 2016 Optimization of wheat sprouting for production of selenium enriched kernels using response surface methodology and desirability function *LWT-food science and technology* 65 1080-1086

[5] Obroucheva N V and Sin'kevich I A 2010 Aquaporins and cell growth *Russian Journal of Plant Physiology* 57(2) 153-165

[6] Pekhovskaya T A, Tarasov Yu A, Katkovskaya I N, Elchaninova M A, Alftan G V, Prokopchik N I and Mosesenok A G 2009 Selenomethionin and dimethylhydrazolylselenide (selector) as protectors of oxidative stress *Health and environment* 13 408-413

[7] Vostroilova G A and Belyaev V I 2011 Toxicometric parameters of dimethyl dipyrrozolyl selenide *Veterinarian* 1 7-9

[8] Kuchmenko T A and Shuba A A 2017 Informativeness of the "electronic nose" output signals on piezosensors *Analytics and control* 21 (2) 72-84

[9] Skvortsova L N, Zaika Ya G and Karmushakova G N 2010 The use of ion exchange for the separation of various forms of selenium in the analysis of aqueous plant extracts and dietary supplements *Sorption and chromatographic processes* 10 (2) 266-272

[10] Glotova I A and Galochkina N A 2017 The influence of selenium sources on biochemical processes during the swelling and germination of wheat grain *Himija rastitel'nogo syr'ja* 4 211-216

[11] Sarkisov D S and Perov Yu L 1996 *Microscopic technique a guide* (Moscow: Medicine)

[12] Yang X Tian, Y Ha P and Gu L 1997 Determination of the seleno methionine content in grain and human blood *J Hyg Res* 26 113-116

[13] Suzuki K T, Kurasaki K and Suzuki N 2007 Selenocysteine beta-lyase and methylselenol demethylase in the metabolism of Se-methylated selenocompounds into selenide *Biochim Biophys Acta* 1053-1061

[14] Xia Y, Hill K E and Byrne D W 2005 Effectiveness of selenium supplements in a low selenium area of China *Am J Clin Nutr* 81 829–834

[15] De Souza M P, Lytle C M, Mulholland M M, Otter M Z and Terry N 2000 Selenium assimilation and volatilization from dimethylselenone by Indian mustard *Plant Physiol* 122 1281-1288

[16] Neuhierl B, Thanbichler M J, Lottspeich F and Bock A 1999 A family of S-methylmethionine-dependent thiol/selenol methyltransferases *Role in selenium tolerance and evolutionary relation* *J Biol Chem* 274 5407-5414

[17] Sunde R A 1997 *Selenium in Handbook of nutritionally essencial mineral elements* (D L O'Dell, R A Sunde eds) Marcel Dekker, Inc N Y Chap 18 493-556

[18] Skvortsova L N, Zaika J G and Karmushakov G N 2010 The use of ion exchange to separate different forms of selenium in the analysis of plant aqueous extracts and dietary supplements *Sorption and chromatographic processes* 10(2) 266-272

[19] Skirdenko V V, Tonyuk M L, Karpushina O S, Sheiko A A and Rossikhin V V 2012 Biologically active substances of wheat germ *Traditional and alternative medicine* 10 18-19

[20] Glanz S 1998 *Biomedical statistics* (Moscow: Practice)