

Features of formation, filling and ripening of triticale, wheat, and rye grain

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Abstract

Context: Triticale (*<Triticosecale* Wittm.) is a crop valued for food and forage usage. However, excessive precipitation during harvesting cereals, typical for Central regions of Non-Cherozem zone, leads to pre-harvest sprouting and enzyme-mycosis exhaustion of grain, and significant degradation its technological and sowing qualities.

Objective: The aim of the research was to compare the dynamics of formation as well as the physical, biochemical, and technological properties of winter triticale, wheat, and rye grain

Methods: The research was carried out in the Russian Federation, Moscow region in 2017-2018. It was conducted on the following varieties: rye Saratovskaya 6, wheat Moskovskaya 39, triticale Aleksandr, Valentin, Viktor, and Timiryazevskaya 150. Sampling was carried out in the phases of grain formation, at the beginning and in the middle of milk stage, in wax and firm ripeness, as well as after over-maturation in a field. We measured the mass of 1000 grains, grain-unit, and grain vitreousness of air-dried grain. Whole grain flour was evaluated for protein and gluten content. Autolytic activity was assessed by the falling number using the micro-modified Hagberg-Perten method. All laboratory tests were performed twice.

Results: The mass of 1000 grains gradually increased, and then decreased a little bit due to enzyme-mycosis exhaustion. Enzyme-mycosis exhaustion was most pronounced in wheat Moskovskaya 39 and triticale Viktor. The grain-unit roughly followed the dynamics of the change in the mass of 1000 grains. The highest values of protein and gluten content were obtained at the milk stage, then their relative content decreased to dough ripeness due to the complete accumulation of starch. Afterward, the protein content increased again: in wheat and triticale Timiryazevskaya 150 to firm ripeness, in rye and other varieties of triticale, to waxy. This indicates that the processes of latent germination of grain were going on. It is interesting that the lowest activity of enzymes was also observed in dough ripeness in all the studied varieties. With further maturation, autolytic activity increased and was maximum in the firm ripeness.

Conclusions: Thus, the studied indicators of grain quality reach maximum values in the wax stage, and the delay in harvesting will lead to a strong deterioration in the technological properties of the grain.

Key words: grain forming, grain development stages, physical properties of grain, protein, gluten, falling number, enzyme-mycosis exhaustion.

Introduction

Triticale (*Triticosecale* Wittm.) is an amphidiploid obtained by combining the genomes of wheat (*Triticum* sp.) and rye (*Secale* sp.). This crop is valuable for food and feed use. However, when it is cultivated in excessively humid areas, difficulties linked to the deterioration of grain quality may appear. Problems such as in-ear pre-harvest sprouting and enzyme-mycosis exhaustion of seeds are typical.

For the central regions of the non-Chernozem zone, excessive humidity is common during the period of harvesting grain crops. Undesirable meteorological conditions also occur during the period of formation and filling of the seeds. All this leads to a delay in harvesting grain crops, and, as a result, to the deterioration of the sowing and technological qualities of the grain. One of the tasks of triticale breeding in the central regions of the non-Chernozem zone is the creation of varieties resistant to pre-harvest sprouting and to enzyme-mycosis exhaustion.

The sprouting of seeds begins when water comes into contact with the seed, which leads to the activation of hydrolytic enzymes and to the split of reserve substances. This phenomenon drastically reduces both the technological qualities of the grain and the sowing properties of the seeds. The resistance to sprouting is determined, primarily, by the duration of seed dormancy. Seed dormancy is considered as a low physiological, when there is a decrease in germination and a narrowing of the range of conditions under which the seeds germinate. It was revealed that the dormancy of seeds of winter triticale is short and unclearly expressed, and the seeds already at the age of 26 days from pollination can germinate under favorable conditions (Rubets, Pylnev & Kondrashina, 2012).

According to Temirbekova (1998), the enzyme-mycosis exhaustion of the seed is a complex phenomenon resulting from the effects of abiotic and biotic factors. First, under the influence of moisture, enzymes break down high-molecular storage compounds – starch, proteins. Cleavage products come to the surface of the seed, where pathogenic microorganisms settle and develop. All this leads to the formation of weak grains, reducing the grain-unit. However, different varieties are subject to this phenomenon to different degrees. The evaluation of the degree of weight loss of 1000 grains in winter triticale samples allows us to determine the optimal time for harvesting, ensuring minimal biomass loss.

Thus, both sprouting and enzyme-mycosis exhaustion are caused by the same meteorological conditions. Both phenomena are preceded by similar biochemical processes. Perhaps, the enzyme-mycosis exhaustion of seeds occurs when the embryo is still in the dormancy stage and is not able to germinate.

Various researchers have noted the tendency of triticale, wheat and rye to seed exhaustion (Kopylov, 2001). Tyunin & Vrazhnova (1994) noted that spring wheat grains that were more susceptible to exhaustion, had a shorter seed dormancy period. These varieties were characterized by lower seed vitreousness ($r = -0.40$), 1000 grains weight ($r = -0.37$), and gluten quality ($r = -0.51$). Under the conditions of the Pre-Ural region, Eliseev & Batueva (2014) observed exhaustion of rye grain by 7.4 g (32%), wheat – by 10.3 g (25%), triticale – by 6.0 g (13%). The maximum weight of 1000 grains of different varieties of wheat and barley was reached between the dough and early wax ripeness stages, and then it decreased (Eliseev & Yarkova, 2012). In addition to the decrease of the mass of 1000 grains, Sandrykin, Kondratenko, Egushova & Pinchuk (2011) noted a deterioration in the structure of the endosperm as vitreousness of the seeds decreased.

Harvesting wheat in the phase of firm ripeness, as well as two weeks later, led not only to the deterioration of the physical properties of the grain, but also the protein and gluten content in the grain decreased (Sandrykin et al., 2011).

The aim of this research is to study the dynamics of the formation of the physical, biochemical and technological qualities of rye, wheat and triticale grains. The objectives of the study are the following:

1. Assess the dynamics of the formation of the physical properties of the grain: 1000 grains mass, grain-unit, seed vitreousness;
2. Determine the content of protein and gluten and the change in autolytic activity in the process of grain development;
3. Compare the course of the formation of the quality of triticale seeds and parent species;
4. Precise the optimal timing for harvesting triticale to maintain maximum quality.

Materials and Methods

The research was carried out in 2017-2018 in Russia, Moscow, in the Moscow Agricultural Academy named after K.A. Timiryazev. Winter rye Saratovskaya 6, winter wheat Moskovskaya 39, winter triticale Alexander, Valentin, Victor and Timiryazevskaya 150 served as material for research. Samples were taken at intervals of 7-10 days, and then the corresponding phase was determined from the seed humidity. In 2017, 4 samples were taken, in 2018 – 7 samples. The phases of plant development at the time of sampling were determined (Tables 1, 2).

Table 1. The phases of seed ripening at the time of sampling in 2017

Nº samples	Saratovskaya 6	Moskovskaya 39	Alexander	Victor	Valentin	Timiryazevskaya
1	Beginning of milk ripeness					
2	Milk ripeness					
3	Wax ripeness			Dough ripeness		
4	Firm ripeness			Wax ripeness		

Table 2. The phases of seed ripening at the time of sampling in 2018

Nº samples	Saratovskaya 6	Moskovskaya 39	Alexander	Victor	Valentin	Timiryazevskaya 150			
1	Seed formation								
2	Milk ripeness		Beginning of milk ripeness						
3	Dough ripeness		Milk ripeness						
4	Wax ripeness			Dough ripeness					
5	Firm ripeness			Wax ripeness					
6	Firm ripeness								
7	Over maturing in the field								

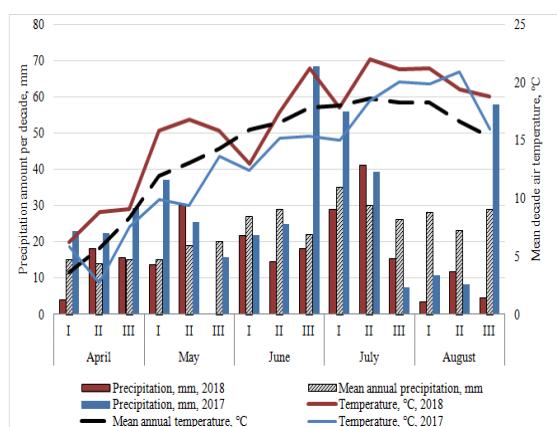


Fig. 1. Meteorological conditions of 2017–2018 in comparison with mean annual.

The earliest samples were threshed by hand, the rest by using a spike thresher. In an air-dry grain, the mass of 1000 grains was determined by an accelerated method, and the nature was determined using a microtube of 10 ml. The vitreousness of the grain was evaluated on a diaphanoscope. Their meal was evaluated for protein and gluten content. Autolytic activity was determined by the falling number using the micromodified Hagberg-Perten method. All laboratory tests were performed twice. In 2017, only the content of protein and gluten was determined and the falling number was measured. In 2018, these studies were supplemented with a study of the physical properties of the grain, followed by an assessment of the degree of enzyme-mycosis exhaustion of the seeds. The absolute protein content was calculated based on the percentage of protein and the mass of 1000 grains.

The triticale plants phenological phase was determined from the moisture content of the grain. The classification for wheat proposed by Kuleshov was taken as a basis. The seed filling period includes the phases of milk (when moisture content of seeds is between 70 and 50%) and dough ripeness (when moisture content of seeds is between 50 and 42%). During this period, there is an active accumulation of dry matter in the grain, and the humidity depends little on meteorological conditions. The ripening period is characterized by phases of wax (grain moisture from 42 to 22%) and firm ripeness (grain moisture below 22%). At this time, the flow of plastic substances into the wheat seed ceases, and its humidity depends on weather conditions. The phase of full ripeness begins after a dormancy period and is characterized by conditioned sprouting of seeds.

The meteorological conditions of the years 2017 and 2018 varied significantly. The growing period of 2017 was cold and rainy until mid-July, which led to the formation of seeds with a relatively deep dormancy. The year 2018 was more typical for the Moscow region. During the period of seed formation, the increased air temperature was combined with a uniform distribution of precipitations, which could lead to the formation of seeds with an increased amylolytic activity (Fig. 1).

Statistical analysis of the data was performed by single-factor and two-factor analysis of variance.

Results and discussion

Dynamics of the formation of physical and biochemical properties of grain in 2018

In the wheat variety Moskovskaya 39 the mass of 1 grain in mg (or mass of 1000 grains in g), vitreousness and absolute protein content reached a maximum in the phase of wax ripeness (Fig. 2e,

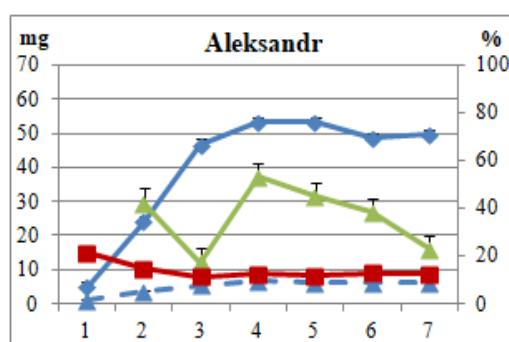
sample 4). The relative protein content (%) decreased during grain formation due to starch accumulation. After over maturing in the field, the weight of the grains decreased by 4.8 g, or 9.6%, and the protein content – by 1.8 mg per grain, or by 26.8% compared to the maximum. This is a consequence of a significant exhaustion of seeds. Vitreousness also decreased sharply due to the deterioration of the structure of the endosperm. Our results confirm the well-known fact that the best phase for harvesting wheat crops is the phase of wax ripeness.

Similarly to wheat, the sizes and quality of grain in the triticale Alexander, Victor and Timiryazevskaya 150 varieties were formed. In the Alexander variety, the weight loss of the seeds was 3.6 g, or 6.7%, and the loss of protein was 0.47 mg per grain, or 7% of the maximum content (Fig. 2a). A slight increase in the weight of the seeds during the over maturing in the field is not statistically significant.

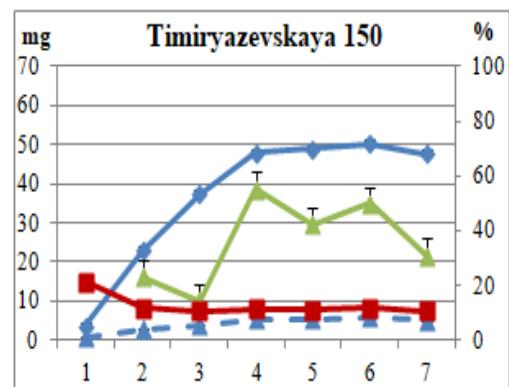
In Victor, unlike in previous varieties, the highest rates were observed in the phase of firm ripeness (Fig. 2c, sample 5). After over maturing the weight loss was 4.4 g, or 7.6%, and the protein loss was 1.27 mg per grain, or 16% of the maximum content. The vitreousness decreased as well.

In the Timiryazevskaya 150 variety, similarly to Victor, the accumulation of reserve substances proceeded up to the phase of firm ripeness (Fig. 2b, sample 6). However, this variety turned out to be sensitive to rains, which occurred during the phase of dough ripeness (sample 4), and that sharply reduced the vitreousness of the seeds. Since the process of accumulation of protein and starch did not stop until firm ripeness, the vitreousness somewhat recovered and then strongly decreased already during the over maturing in the field due to seed exhaustion. The weight loss of the seeds was 2.5 g, or 5%, and the loss of the protein was 0.91 mg per grain, or 15% of the maximum content.

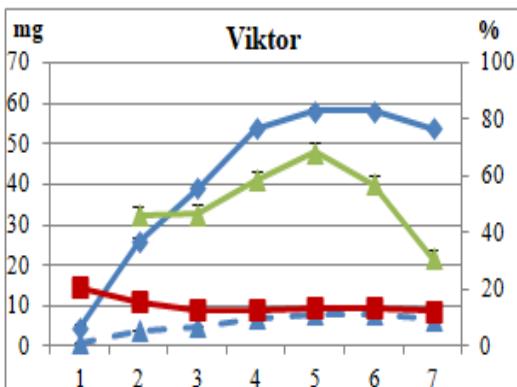
The seed formation in the Valentin variety differed from the previous varieties in that it reached maximum quality during the dough ripeness phase (Fig. 2d, sample 4). However, the accumulation of protein continues until firm ripeness: 7.33 mg per grain. The weight loss of the seeds was only of about 1.2 g, or 2%, the protein loss was 0.45 mg per grain, or 6% of the maximum content. Such data indicate that in reality the exhaustion of seeds was more significant, however, due to processes of accumulation of reserve substances that were going in parallel, it turned out to be imperceptible.



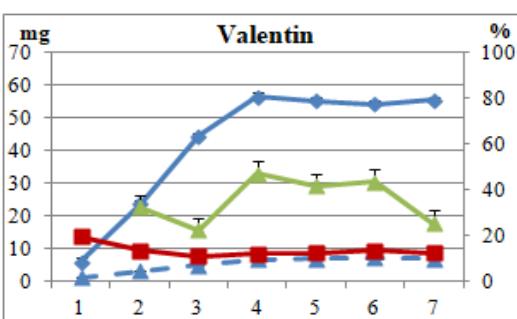
a



b



c



d

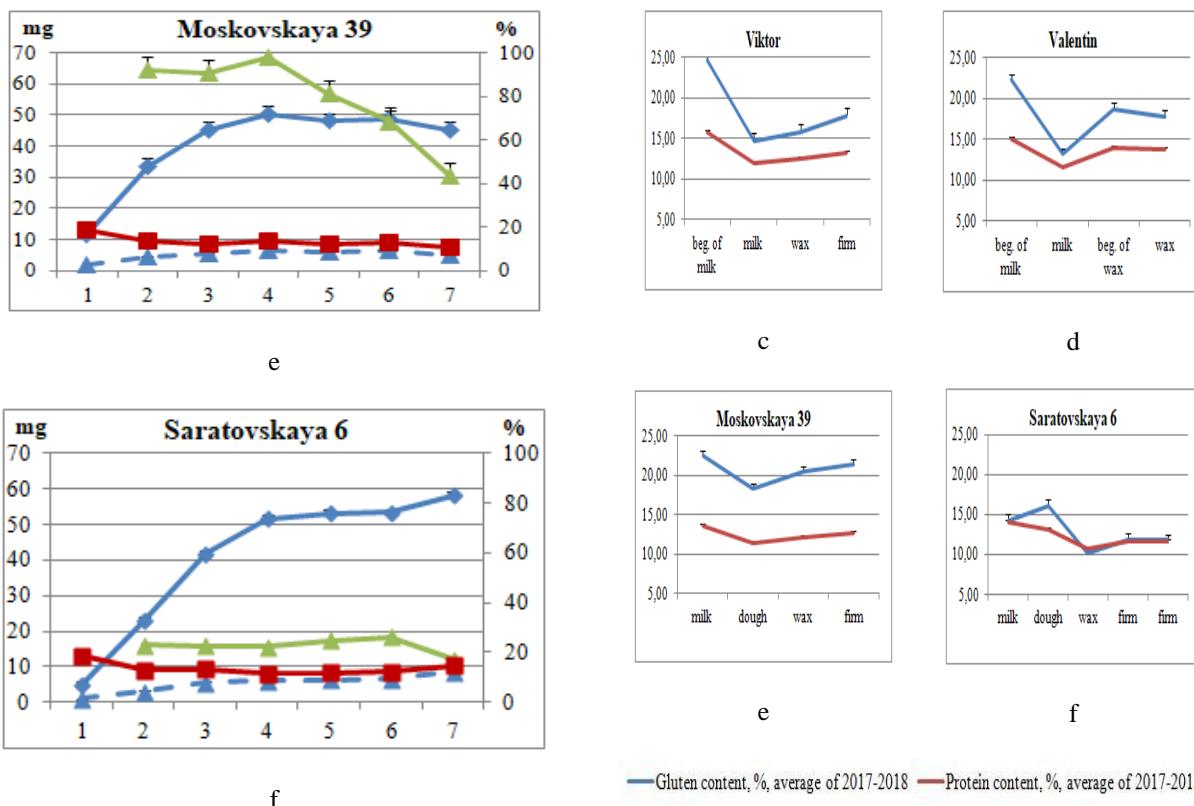


Fig. 2. Dynamics of formation of physical and biochemical properties of grain in 2018.
Phenological phases 1-7 are indicated in accordance with Table 2.

The smallest significant differences are presented in the form of error bars.

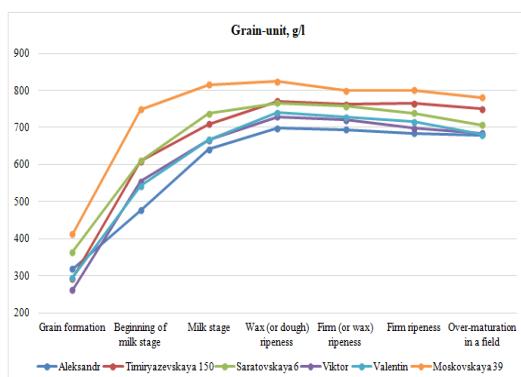


Fig. 3. Grain-unit, 2018.

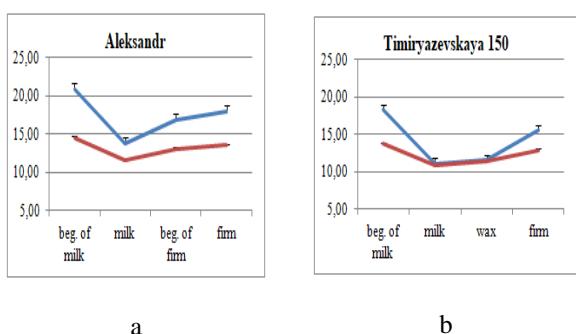


Fig. 4. Protein and gluten content in different phases of grain ripening (average for 2017-2018).

The accumulation of reserve substances in rye Saratovskaya 6 went on without interruption up to the latest sample (Fig. 2f). Its' vitreousness was the lowest of all the studied varieties and fluctuated slightly during the entire period of maturation. No seed exhaustion was found.

The studied varieties reached the greatest grain-unit by the wax ripeness phase, and then it declined after over maturing in the field. In the triticale varieties Alexander and Timiryazevskaya 150, there was no significant reduction of the grain-unit. Other varieties of triticale and rye showed a significant decrease of the grain-unit, by 6 to 8%, and the wheat Moskovskaya 39 variety – by 12.5% (Fig. 3).

Changes in protein and gluten content in 2017-2018

As in 2017 fewer samples were taken, for the objective comparison only those samples were selected from the 2018 data that coincided by the ripening phases of the grain with the 2017 samples.

The relative content of protein and gluten are strongly correlated: in the wheat variety Moskovskaya 39 $r = 0.962^{**}$, in triticale varieties $r = 0.992 \div 0.996^{**}$, in rye variety Saratovskaya 6 $r = 0.76^{**}$.

It was found that in wheat, the relative content of protein and gluten significantly decreased in the phase of dough ripeness (Fig. 4e), while in rye, this phenomenon was observed only in the phase of wax ripeness (Fig. 4f). In contrast to the parental species, all studied triticale varieties (Fig. 4a-d) showed a decrease in the protein content already in the milk ripeness phase. Such dynamics can be explained by the fact that at this time there is an intense accumulation of starch, which leads to a decrease in the percentage of protein. If you refer to Figure 2, it is clear that the absolute protein content increases during the indicated phases, and then goes to arrives to a plateau and may decrease slightly due to enzyme-mycosis grain exhaustion. When ripe, the relative protein content increased again, which can indicate starch loss; this is the seed exhaustion.

Two-factor analysis of variance revealed a significant impact of the research year: wheat, rye and Victor triticale were characterized by a significantly higher content of protein and gluten in 2018, and the rest of the triticale varieties – in 2017.

Changes in the autolytic activity of the grain in 2017-2018

The study of the technological properties of grains harvested in different phases shows the change in the activity of hydrolytic enzymes during maturation. The determination of the falling number is based on the measurement of the viscosity of a water-flour suspension when it is placed in a boiling water bath. Low viscosity and, accordingly, a low falling number are characteristic of samples with increased activity of amylases and other hydrolytic enzymes, high-quality grains will have high viscosity and high falling number.

Two-factor analysis of variance showed that the falling number was significant in 2017 for all the studied varieties. In 2017, during the grain formation, adverse weather conditions were observed – low temperature and heavy rainfall during the grain formation period, and then – drought during the grain ripening period. Such conditions contribute to the formation of grains with a deeper dormancy (M.S Bazhenov, Pylnev & Tarakanov, 2011), which is ensured, in particular, by the activity of amylolytic, proteolytic and other enzymes.

Table 3. Falling number (sek) in different phases of grain ripening (average for 2017-2018)

Variety Year	Development phase			
	Beg. of milk stage (a)*	Milk stage (b)	Beg. of ripeness (c)	Firm ripeness (d)
Alexander				
	2017	146	337,5	134,5
Timiryazevskaya 150				
	2017	246,5	338	255,5
Viktor				
	2017	51,5	150	133,5
Valentin				
	2017	47	150	46
Saratovskaya 6				
	2017	139	315,5	373,5
	2018	108,5	118	74,5

* – samples marked with the same letters differ insignificantly by Duncan criterion (within one variety).

On average, over two years of research, the greatest falling number was characterizing the milk ripeness phase in triticale and dough ripeness in wheat and rye.

During maturation, the activity of enzymes increases slightly, and over maturing in the field leads to a significant deterioration in the technological qualities of the grain. Of the studied triticale varieties, the highest falling number was observed in the Timiryazevskaya 150 and Alexander varieties (Table 3). They are also resistant to in-ear pre-harvest sprouting. The variety Valentin, which had the lowest falling number of all, is one of the most unstable to the in-ear pre-harvest sprouting. Wheat Moskovskaya 39, which had the lowest autolytic activity of the grain, practically does not sprout in the ear.

Conclusions

1. In the process of grain ripening, the grain mass increases until the wax ripeness phase in wheat, and until firm ripeness in rye and triticale.
2. The vitreousness of the seeds and the grain-unit increase until the wax ripeness phase of wheat and most triticale varieties. The delay in harvesting leads to a sharp decrease in seed vitreousness, grain-unit and weight of the seeds. The strongest seed exhaustion was found in wheat, to a lesser extent – in triticale. In rye, no significant changes in the grain

structure have been identified when over maturing in the field.

3. The accumulation of protein continues until the wax ripeness in wheat and in triticale variety Alexander. When over maturing in the field, the protein content of wheat and triticale is significantly reduced.
4. Wheat harvesting should be done in the phase of wax ripeness and triticale harvesting should be done in the phase of firm ripeness, when the maximum amount of organic matter has been accumulated. Over maturing in the field is not recommended.

Author contributions

Yuliya N. Kotenko: research carrying out, analysis of results, article writing, final review.

Marc D. Kanaan: research carrying out, analysis of results, article translating.

Valentina S. Rubets: research planning, analysis of results, article redaction.

Vladimir V. Pylnev: analysis of results, article redaction.

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