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Identification of Wheat Morphotype and Variety Based on X-Ray Images of Kernels

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52236

1. Introduction

High quality seeds of wheat are necessary for fast biological and technological progress. As the proverb says, "what you sow that you will reap". High quality of seeds is ensured by its variety and the extent of damage to the kernels. First of all, the seeds should be of a variety characterised by high productivity and suitable for the climate zone where it is cultivated. Secondly, the seeds should have a high level of germination capacity and be free of damage of biotic and abiotic character. To generate a variety and to obtain seed zoning requires a minimum of about ten years. The potential of variety, on average, decreases by 20% a year, and a few years later it is replaced. Transition to hybrids reduces the time of obtaining new seeds to five years, but the agricultural economy is dependent on the activities of breeding centres. Harvested grain cannot be used as seed. This state of affairs with relation to seeds stimulates the search for new approaches to an improvement of their quality.

In this chapter we propose to discuss one approach to the obtainment of seeds with improved properties. The approach proposed provides a more detailed description of the study of morphological types in the process of ontogenesis for the identification and selection of seeds on the basis of established indicators. The time required to obtain high quality seeds, ready for mass sowing, can thus be reduced from several years to hours, with multiple-fold reduction of the cost of their obtainment.

In the organism, in one form or another, "all is reflected in everything". In practical breeding selection, economically useful properties of plants are most often associated with their morphological features. Ideally, one would like to see these features in the kernels, and based on these features to select them as seeds. Suggestions about such a connection were made long

ago, but there were no arguments to substantiate such a connection, nor methods for mathematical description of the morphology of kernels, allowing identifying characteristic traits or indicators allocating particular batches of kernels to the morphological type, variety, or cultivar. Improvement of measurement techniques allows us to address anew previously known problems which have not yet matured to the point of solution, and to set new ones which previously could not even be thought about.

On the basis of the study of morphology in ontogenesis and characteristics of the wheat genome, algorithms of the kernel forming were obtained (Batygin & Demyanchuk, 1995; Demyanchuk, 1997) which allows to establish a relation between its morphology and the growth conditions of the plant, kernel position in the ear, etc. As the work conducted indicated, all of those internal and external factors determine, in ontogenesis, a specific morphological portrait of the kernel, classifying it to a specific variety under the conditions of its cultivation. Based on such a "portrait" one can identify the variety, as well as some of its properties, for example, the duration of its vegetation period.

In the next part of this chapter the required definitions necessary for the selection of morphological characters, and the obtained effects are described. To date, there exist mature technical possibilities of rapid identification of the form of kernels and of high-speed separation or grain flow on the basis of selected feature or property. The selection of kernels whose shape conforms to the specific form of a variety will ensure permanent maintenance of that variety.

The application of X-rays in this technology is due to the fact that X-rays can clearly identify the silhouette of kernels and - most importantly - identify the characteristics of their inner structure, which can be of decisive importance. For example, the presence of insects in kernels preserving their natural shapes or infestation with the sunn pest. The X-ray method can reveal hidden germination, or symptoms of germ necrosis or, alternatively, indications of increased potential productivity of seeds.

2. Description of the study object

Wheat - one of the most widely cultivated plants. It was grown over many millennia BCE. Archaeological evidence suggests that in many parts of Asia, Europe, and also in Egypt, wheat was grown for 5-7 thousand years BC. Wheat grain was found in Egyptian pyramids, the pile-buildings of Switzerland, and at many sites of ancient man. The exceptional ability of wheat to synthesise gluten, with high baking quality of flour, guarantees its monopolistic position among other crops. Wheat (genus *Triticum* L.) belongs to the order *Poales,* family *Poaceae*. In cultivation it is represented by a great many varieties adapted to growing conditions, constantly updated with new cultivars, more productive, more adaptable to local conditions, more than meeting the requirements of modern manufacturing. No other cereal species has so many varieties or cultivars as wheat. As a rule, the countries that grow wheat, apart from commonly occurring types, have their own local varieties and cultivars. Centu-

ries of experience of growing wheat in different soil and climatic conditions contributed to the formation of a large diversity of species and varieties.

Belonging to a variety is primarily determined by the main parameters of the vegetative organs – the stem and the ear, by the size, shape and colour of the grains, as well as by their chemical composition.

The real or proper wheat cultivars are characterised by elastic and flexible straw that does not get fragmented during threshing, the ear is set firmly on the straw, and the kernels are naked and during threshing get easily separated from the fitting scales.

The second group, known as spelt, is characterised by opposite features, namely, spelt cultivars have very brittle straw, easily broken during threshing, the ear is also easily separated from the straw, while the kernels are strongly attached to the scales and get separated from them only with great difficulty.

Morphology is one of the most important characteristics of plants. Despite centuries of observation of morphological features, the process of their formation in ontogeny cannot be considered well understood. If some of them can be considered as signs of species, genus or even variety, others remain highly volatile, retaining, however, in their variety, things in common which, however, are not an easy object of scientific description.

Researchers involved in the study of wheat, depending on their position, apply such a classification as is the closest to them. The most common classifications include the botanical, genetic, economic, and morpho-physiological. Let us focus on the last one. It is the one that is always used in classical breeding which has given and still gives humanity more and more productive and valuable varieties. The breeder selects plants that have some or other morphological features. Their combinations, based on his experience, knowledge and intuition, are related to the productivity, stability or some technological properties of kernel or plant as a whole, such as for example the length of straw and ear type, the size, colour and number of spikelets and flowers in the spikelet, etc., characteristics of kernels. The kernel is an integral representative of the plant. In fact, it is the whole plant in miniature.

Leading morpho- and physiologists, who left the most significant mark on the study of wheat, indicated that the kernel should carry signs of belonging to a variety. Because "everything is reflected in everything", the uniqueness of a variety should be reflected in the uniqueness of some of the morphological proportions of its kernels. The significance of this idea is implicitly recognised in the classification of wheat varieties used for admission to the collection of the Vavilov Institute of Plants (VIP) in St. Petersburg, Russia. According to the existing methods of morphology, wheat kernels are assessed by three linear dimensions (a × b × c) and their multiplication with a coefficient equal to 0.52. It is clear that the approximation of the complex-shaped kernel by means of a simple parallelepiped is an extreme simplification. In such a description both the shape of the object and its biological characteristics are completely lost. However, that was a step forward and corresponded with the level of knowledge on kernels and methods of description of their forms accumulated by that time. At the same time it was accepted that "the study of morphological characters and their rela-

tionships with the physiological functions and biochemical parameters - one of the most effective ways to improve the selection of high-protein wheat cultivars" (Henkel, 1969).

Morphophysiological systematization (Henkel, 1969) relates the type of wheat with the duration of the growing season and the weight of one thousand kernels. The characteristic features of morphotype include also the building of apexes at the moment of transition from stage of growth to the stage of development. This set of features, combination of which characterise varieties, presents a difficulty in the identification of varieties, and brings us back to the kernel. It appears to be a tempting challenge is to find such a set of morphological traits of wheat kernel that would characterise both the variety and the quality of seeds. There is a known non-formalised relationship of the form of the embryo with plant productivity. It's widely known, for example, that kernels from the upper, central and lower parts of the ear are somewhat different in shape, and that the difference in moisture content of these kernels characterises the drought resistance of the variety. In kernels most variable parameter is length. With increasing thickness of kernels (Larikova, 2007; Larikova & Kondratyev, 2002) their length and width increase as well. Compared to slender kernels, in well-filled kernels the embryo and endosperm are bigger as a rule. Depending on the degree of kernel filling, the differences in the mass of endosperm are larger than the differences in the mass of embryos.

Yielding properties of kernels as seeds are largely dependent on their place in the ear. High yield properties are typical to kernels formed in the outer flowers of the central ear. These kernels, compared to other kernels in the ear, have the greatest width. It is believed that this indication is the preferred morphological indicator of kernels for selection as the most productive seeds.

In wheat there is an interrelated system of right hand-left hand features, both for an individual plant and for the cultivar, variety or species.

In the morphology of wheat it was noted that the filling of the particular elements of the embryo (i.e. filling of leaves, roots, and other formed elements of the embryo) may be a variety-specific indicator (Henkel, 1969). Identification of these indicators with the help of a microscope was not widely developed in practice, because this involves the destruction of the kernel and therefore makes it impossible to compare its structure with the properties of the plant which would grow out of it.

In this chapter it is proposed to apply a mathematical description of the "assembly technology" of the embryo and kernel throughout the successive strictly directed cell divisions (Demyanchuk, 1997). This description is related with the morphotype of the plant, with its characteristic length of the growing season and all morphometric characteristics. This permitted mathematical description of the characteristic features of the shape of the kernel of a given morphotype and variety, and the presentation of an algorithm for the identification of kernels in complex mixture of grain in bulk. The application of X-ray techniques for non-destructive analysis of the internal structure of an object opens new perspectives in obtaining morphometric characteristics of kernels. It permits estimation of the shape of kernels as a whole, in various projections, as well as of the morphometry of the embryo. The exact lo-

cation of various defects of the internal structure, both of biotic (for example, number of in-sects) and abiotic nature (endosperm cracks), can be determined. Only the X-ray method can reveal the peculiarities of the geometry and fine structure of the embryo and then associate them with the properties of the plant obtained from the seed, beginning with its external ap-pearance and ending with the structure of its yield (number of elements, their mass, and other technological characteristics, including sensory features). Thus, selection of kernels can be conducted on the basis of their X-ray projections, characteristic for variety and for high yielding capacity. In this way it will be possible to maintain a variety over indefinite periods of time and protect it against "degeneration", while achieving increased productivity and at the same time freeing the seed material obtained from kernels that are disease-stricken, damaged, infested by insects (Varshalowich, 1958), fungal diseases, and from contamination with foreign material.

3. Peculiarity of kernel formation

The specifics of wheat kernel formation in ontogenesis consists in the combination of the growth processes of the parent organism and the kernel. Compared to the normal quadru-ple complication of the organisation of the parental structures, including those that ensure nutrition for the new structures of the embryo, at this stage radical changes take place in the organism. After the dual fertilisation, in the seven-cell eight-nucleus germ sac of the *Poligo-num* type three different processes develop:

1. Process of direct growth and development of the embryo, also characterised by quadru-ple complication compared to the structures of the preceding stage;

2. Process of the formation of endosperm, whose formation ensures such a configuration of intercellular bonds that accelerates the exchange processes many times. The intensity of those processes is not less than 21-fold greater than that of the exchange processes in the embryo;

3. Process of the formation of cells of the gametophyte (antipodes), whose intensity de-creases, producing a total of slightly over one hundred cells. As a rule, the cells of the gematophyte (antipodes) form approximately (as standard) a structure of 108 cells that undergo lysis (degradation) in the course of development.

The developing caryopsis is covered with growing structures of the parental organism. The increasing complexity and rate of acceptance of nutrition supply from the parental structure of covers is considerably less than the consumption requirements of the internal processes taking place within the caryopsis. The nutrition requirements during the formation of endo-sperm are nearly 100-fold greater than the level of requirement for the development of a ker-nel in the ear, and eventually the endosperm loses contact with the mother plant.

For analytical estimates of acceptable changes of the structures under study, we will now consider an algorithmic description of wheat kernel development.

4. Modelling of kernel development

The huge number of factors that need to be combined into a coherent picture of the growth and development reduce this problem to the area of either a description of results already available or the credibility of that result. With all the grand results of genetics, we are left only with a probability of an incomplete number of possibilities. Almost all of the designs of a biological experiment can be modelled by the study of the "black box". In practically any experiment we are left with the possibility of facing the unpredictable. This dramatically reduces the possibility of wider application of existing knowledge about the nature of things and events.

Complex systems operate under conditions of large numbers of random factors. The sources of random factors are the external environment, as well as errors and reactions that occur within the organism. In plants, growth and differentiation are so arranged as to allow talking about ontogenesis as a purposeful process. Irrespective of the diversity of conditions and ways of realisation of ontogenesis, only one and the same final result of development is constantly observed (determination).

The inductive-deductive construction allows collecting new ideas about the processes and the behaviour of an object.

Let us introduce a number of assumptions about the morphogenesis of kernel as a developing biological system. These assumptions can then be removed if necessary.

1. The zygote in conjunction with its surroundings constitute a system which includes the functions of defining and securing the structure during development;

2. The elements that determine the genetic program of implementation are in the cell;

3. The location and condition of the surrounding cells are defined by the terms of running the program of hereditary realisation, i.e. by the conditions of the program of the genome;

4. The environment meets the conditions of existence of the organism and contains everything needed to perform the processes of growth and development;

5. The organism realises itself in accordance with the provision of metabolites and following a genetic program as a response of its condition to the surrounding environment (comfort, safety);

6. The cell contains elements that are already capable of forming and eliminating connections;

7. If not enough of these elements initially, the cell system receives all that is necessary during the cycle of division as a result of exchange with neighbouring cells from the accessible environment;

8. Energy elements necessary for the process of cell division accumulate in the cell through the process of exchange with the environment via the developed connecting channels of the cell;

9. If during a cycle the necessary structural changes in the cell did not take place, and/or sufficient energy for cell division has not been accumulated, the division does not occur.

Schmalhausen, (1968), and Schrödinger, (1944) argued that the entire genetic information is transferred from the zygote to all the cells of the developing organism through the mechanism of cell division. Methods of encoding and transmission of information affect the organisation of connections between elements. Therefore, as the basis of the construction we used the concepts of systems theory and the assumption that information necessary for the implementation of the genetic programs is contained in the ancestral cell chromosomes. Our system (cell, cellular system) is defined as an ordered set, a connection pool which, through interaction, leads the system to a particular purpose. Hence an important interaction among the three fundamental components of the concept of the system: elements, relationships and operations. The algorithm usually represents the method for computing functions, and in our case it determines the sequence of actions to be performed by the organism in the process of morphogenesis.

The solutions for such an algorithm represent the chromosome sets of cells, groups of cells, organs of plants, forming a connected hierarchical structure. In formulating the problem, the study of morphogenesis in ontogeny, the very "purpose" of computing the algorithm, is secondary. The main task is to follow the scheme of calculation (self-construction) of a developing system. That scheme, in our case, defines the parameters of morphogenesis of the system. The defined, repeatable sequence of steps and fulfilment of the conditions lead to cell division.

Operations on the elements and relationships of the system should take into account:

1. Direction of the development process;

2. Obligatory character of hierarchy of structures, typical for each stage;

3. What elements trigger the development process;

4. Multiplicity of choice of pathways between the stages;

5. Oscillatory (cyclic) nature of the processes in the system being a form of its existence.

The algorithmic construction describing the development of the system allowed to achieve agreement between the object and the formal concepts of systems theory. The large number of factors of different nature that determine the development and growth of the organism does not allow the selection of a dominant biophysical and biochemical interpretation of the processes. At the initial stage, the algorithm we propose for the description of development is of a formal character. In the case of the construction being effective, there will be a most credible systemic, biophysical and biochemical, interpretation of events. If our starting hypotheses are able to substantiate the main morphological properties of the organism, not previously combined into a whole in ontogenesis, we will assume that our algorithmic construction is suitable for the prediction of the morphological features of the organism.

Therefore, let us consider the behaviour of the carriers of genetic information, genes in the chromosomes. For wheat, the basic chromosome number is a multiple of 7 (2n = 14, 28, 42). The first step to construct our algorithm is to determine the features of two functions, each of which is also a multiple of 7, and namely:

1. Function defining the conditions of the organism building cycle;

2. Function directly describing organism building in the cycle.

As far back as in the mathematics of the ancient Egyptians it was believed that a mathematical operation is determined by the two entities over which and with which it is carried out. Pythagoras, defining the concept of number, compared it to a sphere and endowed it with four dimensions (three spatial dimensions and density). From the formal point of view, the cell is similar to that concept of number, but it undergoes modification in the cycle. Our number – cell – establishes a connection with its environment, exchanges and controls connection channels whose number equals that of the chromosomes. An "operation" in the cycle, by means of the connecting channels conforming to the program of the preceding stage of development, forms cells, each of which has the same number of channels. Here ends the process of configuration of "numbers" combined into a specific form. The operation defines the process on these numbers, each of which communicates with another with the same number of communication channels, that number being also equal to the basic chromosome number. The duration of such a process is defined by the conditions of cycle completion and by the method of creation of the "cycle body". The time required for the computation is defined by the temporal complexity of the algorithm and the computing power of the computer. The temporal complexity of computation of the target function of development is defined by the duration of the vegetation period (e.g. in the case of wheat – the time of its life).

Same examples of interpretation. In the algorithmic model, the direction of metabolite exchange between the maternal and the developing organism is determined by the orientation of chromosomes in the metaphase plate. Biophysical processes (opening up of the helixes) initiate an electromagnetic pulse. The resultant force at the time of the burst pulse determines the orientation of the metabolism, and simultaneously occurring biochemical processes "fix" the structure in its current form. The "directions" (communication channels) of the processes in the cell cycle, cell division and location, are defined as the "operations" of the cell cycle. We will define the algorithm of constructing on the basis of cell division as the purpose function of development.

To clarify the composition and sequence of operations, let us consider the cycle of cell division. The cell cycle is usually divided into four periods: pre-synthetic (G1), period of DNA synthesis (S), post-synthetic (G2) and mitosis (M). Actually, mitosis accounts for 1/7-1/10 of the cell cycle (Table 1):

The phases of the cycle will be juxtaposed with operations, the implementation of which leads to doubling of the chromosomes, the direction of metabolic processes and the necessary conditions of the cell cycle (Demyanchuk, 1997). Cell division is more convenient to consider in the phase of arrangement of chromosomes in the metaphase plate. Technically speaking this phase formed a stable non-equilibrium system of implementation of a sequence of hereditary factors among which the most important are the following (Fig. 1): 1) orientation of metabolic fluxes through "channels" in the cellular environment of a developing structure, 2) biophysical processes in chromosomal band; and 3) biochemical processes that fix the shape.

Item	Cell division cycles	Interpretation
1.	In phase Gap 1 (G1) there takes place transcription (first step leading to gene expression) and translation (transfer of genes from one chromosome to another) in both cells which are a result of the preceding division. Plastids and mitochondria are multiplied. At this stage the cells of a multicellular organism perform all functions necessary for the organism.	Synchronization of metabolic processes with the surrounding cells. Setting up exchange in the plane of the perimeter of the future metaphase plate. In selected areas the "waves of incoming and outgoing" metabolic fluxes are formed.
2.	S-phase - a period when the DNA in the nucleus doubles. DNA replication begins at many but exactly defined locations. By the end of S-phase, each molecule of DNA is doubled in full. Along with the DNA the amount of histones and non-histone proteins of chromatin should double at the same time. In S-phase also centrosome doubles, the place of microtubule formation. In interphase microtubules grow from the centrosome toward the whole periphery to the cell. In late G1 phase of the centrioles move apart by a few microns, and in the S-phase next to each centriole a second centriole is built, and centrosome doubles.	Exchange. Doubling of structures responsible for development: synthesis of DNA, doubling of the chromosomes (comparative operation "×2", i.e. doubling of elements at points indicated by communication channels, so that the new elements are in agreement with the cellular environment). "The wave of incoming metabolic flux" from the surrounding cells provides a process in the cell, and the "wave of the outgoing flux" specifies the location of the cell structure which should be formed in this cycle of development.
3.	The next phase, G2 - preparation for division. At this time, the formation of the two centrosomes ends, and the system of interphase microtubules begins to break down, releasing tubulin from microtubules. The chromosomes at this time are beginning to further condense, but that is not visible under the microscope.	Formation of the "motor system of chromosomes."
4.	Actually mitosis (M phase) is also divided into several stages. The stages of mitosis - prophase, prometaphase, metaphase, anaphase and telophase.	
4.1.	In the prophase there is an additional packing (condensation) of chromosomes to the extent that they become similar to first tangled filaments, visible in the light microscope. Depolymerisation takes place in the cytoplasm present in the microtubule cell. At this point the cell, as a rule, loses its special form and becomes rounded. Around the centrosome, there appears a so-called star - a system of radial microtubules, which are gradually extended. In the process of mitosis, microtubules start renewing 20 times faster than in the interphase, and the small number of long, stable microtubules get replaced with a lot of short and unstable ones. When the microtubules extending from two poles (cell centres) meet each other, they come into contact and get connected to each other by means of certain proteins that stabilise them and	The process in the surrounding cell space. Accumulation and transformation of energy in the form required for cell division.

| Item	Cell division cycles	Interpretation
	prevent them from depolymerisation. These microtubules form the spindle of division. Microtubules from the star growing in other directions, either become ultimately destroyed or establish connections near the poles.	
4.2.	In the prometaphase the membrane of the nucleus gets defragmented into vesicles and the nucleus disappears as a structure. The contents of the nucleus are combined with the cytoplasm. A condition similar to the prokaryotic is established. During the division the nucleus disappears. In the prometaphase chromosomes condense, and finally take the form of pair formations. Each pair becomes connected at the point of crossing. In the prometaphase chromosomes, led by microtubules, get arranged in the equatorial plane perpendicular to the spindle. Microtubules act as springs. These forces are balanced when the microtubules emanating from opposite poles are the same length.	Compaction of chromosomes. Orientation of the chromosomes in accordance with the cycle of exchange flows of the cellular environment. Let us imagine that the directions relative to each other in the metaphase plate are oriented roughly with a shift at the angle of $(2\pi / 7)$. See the circle in central part of Fig. 1.
4.3.	In the metaphase, all the processes in the cell freeze. Chromosomes formed in the metaphase plates take part only in vibrational motion.	Location of bivalents in the equatorial plane. Process cycle (rhythm?), when the exchange stopps.
4.4.	The next stage - Anaphase – is started by a sudden and simultaneous separation of centromeres of the two chromatids from of each other. This is in response to a rapid tenfold increase in the concentration of calcium ions in the cell. They are released from the membrane vesicles surrounding the cell centre. Led by the attraction of microtubules, the chromosomes begin to diverge to the poles of the cell, each of the two sister chromatids to its pole.	The divergence of homologous chromosomes to the poles. Independent divergence of chromosomes included in various bivalents.
4.5.	In the next stage, telophase, a new nucleus envelope begins to form around the chromosomes gathered around each centrosome. A double membrane is recreated from the vesicles, nuclear lamina proteins are dephosphorylated and then form a proper lamina, nuclear pores are assembled again from component parts. And thus, we have considered the stages of mitosis consisting in the doubling of the nucleus. It begins with hidden from the eyes doubling of chromosomes in the interphase, and continues through its self-destruction as a structure during mitosis. When the nucleus has doubled, it is necessary to divide the cytoplasm - to carry out cytokinesis.	The formation of two haploid nuclei in the cell, which may differ genotypically. To perform the "division operation," it is believed that one of the nuclei forms structures in the cell, and the other defines the conditions and the number of cycles of division of the cell system.

Table 1. The phases of the cell cycles.
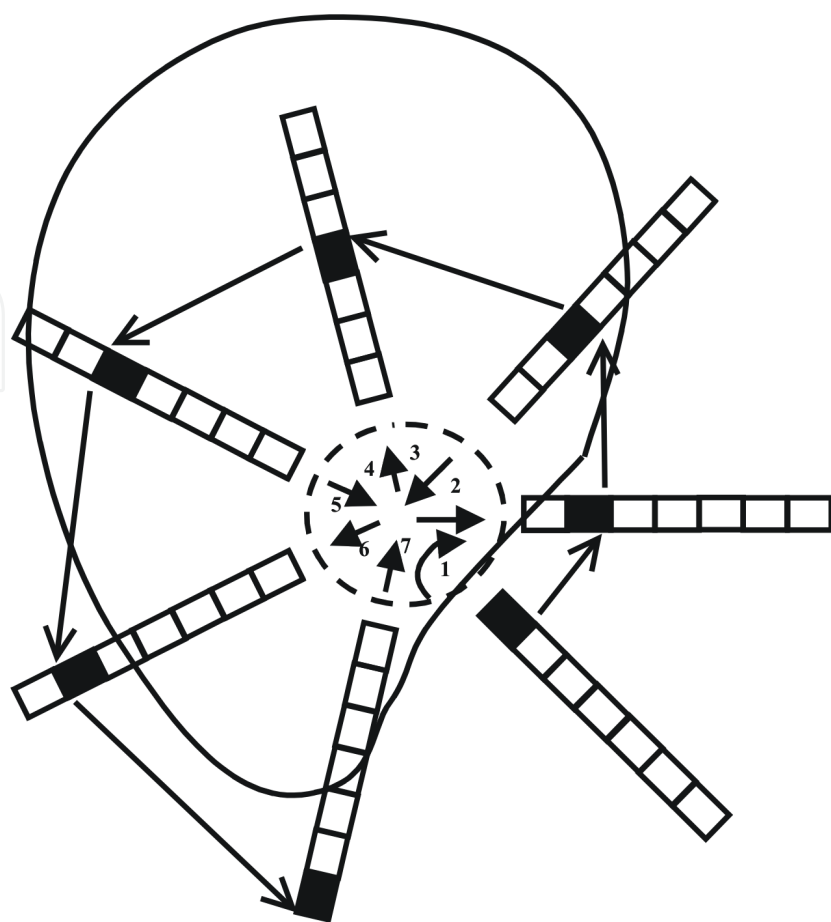
Figure 1. Scheme of the formation of embryo cells of cereals with the base chromosome numbers multiple of "7" at the initial stage of development, with the critical number of 588 cells. The arrows in a circle indicate the direction of flows, the shaded rectangles - the location of the cells, and the shape of the envelope lines around the perimeter of the shaded rectangles - the shape of the embryo.

Electromagnetic pulses of the breaking valence bonds of chromosomes determine the selectivity of resonance in the metabolism of functionally related groups of cells that have no direct contact.

During preparation for zygote division, in the surrounding space there appears a scheme of the general number and arrangement of cells which should be formed in the first phase of development. For wheat it is - ($2 \times 3 \times 2 \times 7 \times 7 = 588$). Thus, the growing cell structure then forms a shape when the "operation of cycle completion" in space will set the future structure of the embryo formation phase. Only then each of its dividing cells will be able to take the position specified in this process. To ensure such positioning of the cells, in each step of the exchange there should be a "link" of each cell with each.

The total number of cycles of the creation of structures according to the algorithm coincided with the number of stages in the development of wheat. The list of structures constituting the plant, the critical number of cells in the initial forms, the achievement of which is necessary for the completion of a stage of development and transition to the next stage of growth in the experiment, also fully coincide with the calculated ones. The angles of displacement in

the formation of metameres on the stalk (leaves, buds) and the placement of other organs of the plant also "obey" the rule of displacement "points" of their formation introduced in this manner (Fig. 2.). In Fig. 3. X-ray image of barley seed embryo is presented.
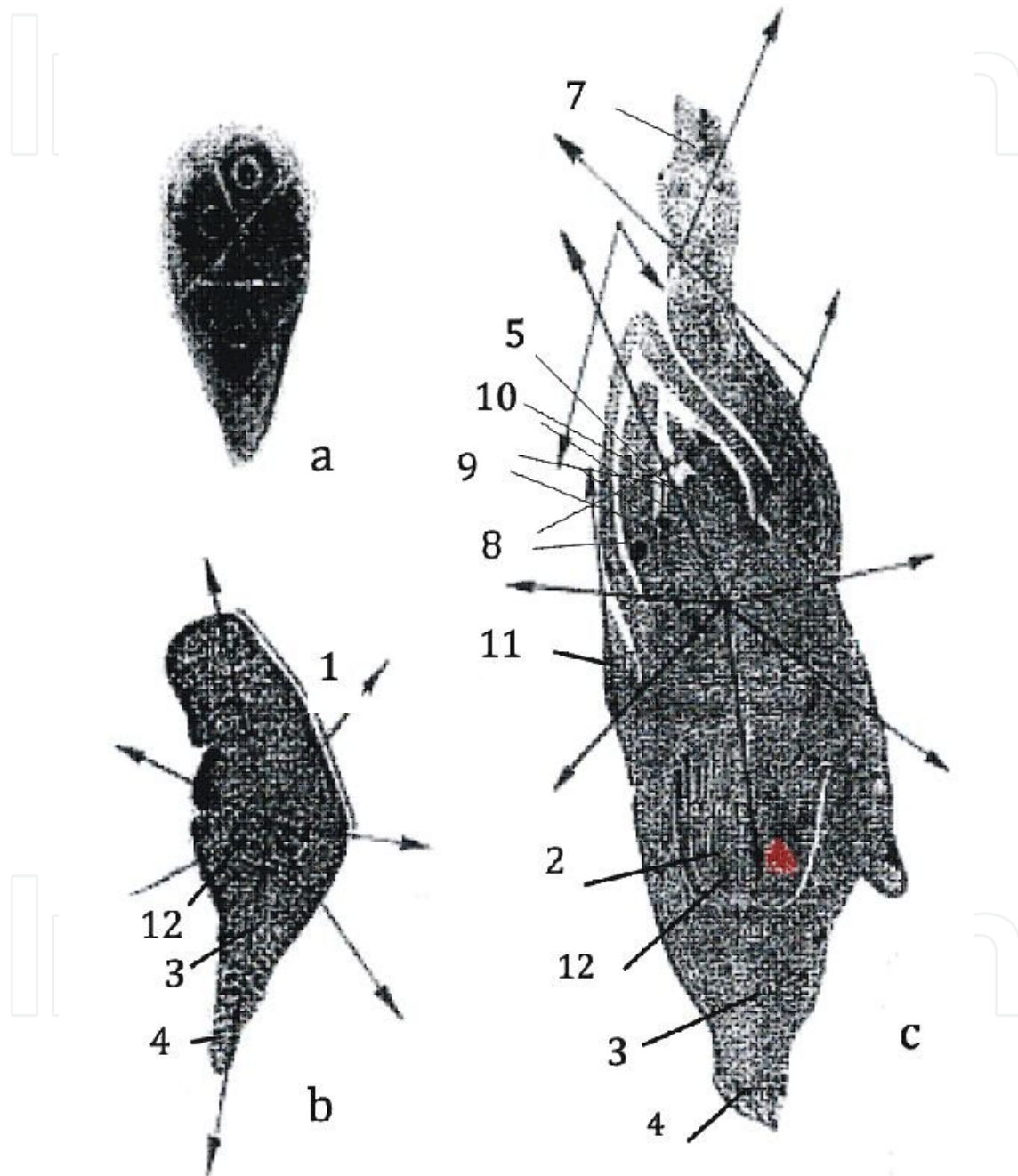
Figure 2. Development of wheat germ (Batygina, 1974; Batygina, 1987): a - four-cell embryo in the dorsal-ventral section; b and c - subsequent stages of embryonic development. 1 - plate, 2 - embryonic root, 3 – coleorhizae, 4 - suspensor, 5 - point of growth, 6 - coleoptiles, 7 - ligulas, 8, 9, 10 - first leaves, 11 - epiblast, 12 - root cap. Directions of the formation of structures and of the "organism as a whole," according to the algorithm of wheat morphogenesis (Demyanchuk, 1997), are indicated by arrows in the figures.

Figure 3. X-ray image of barley seed embryo. Magnification of 30x.

In fact, the method allowed specifying all the critical numbers that must be achieved in all the transitions in the development. Deviations from the common "standard" of the formation of cells of cereals with the basic chromosome number equal to seven (7), are characterised by stable formation in the species- and variety-related additional functionally related groups of cells, in multiples of 49. Depending on the location within the organism, the ways of placement of cells into connected groups differ from one another. For example, the laying of cells according to the scheme, with a constant shift of the direction of the location of the next cell along the line of arrows (7 - 4), indicated in Fig. 1, with a shift at the end of the circle in the perpendicular plane and the angle of $2\pi /7$, forms the shape of the sprout. Analysis of subgroups of cells, corresponding to the phase of establishment of metameres (leaf, shoot, and bud) showed also that in the structure of leaf the observed proportions, necessary for its construction, were maintained (Demyanchuk, 1997). Each variety has its own stable scheme of the formation of cells which constitutes its morphological specificity. The formation of the endosperm is based on interactions of haploid groups of chromosomes of the

three nuclei. Algorithmic description of the development of specific forms of cereal grains will correspond to reality if the process is presented in a species (Fig. 4).
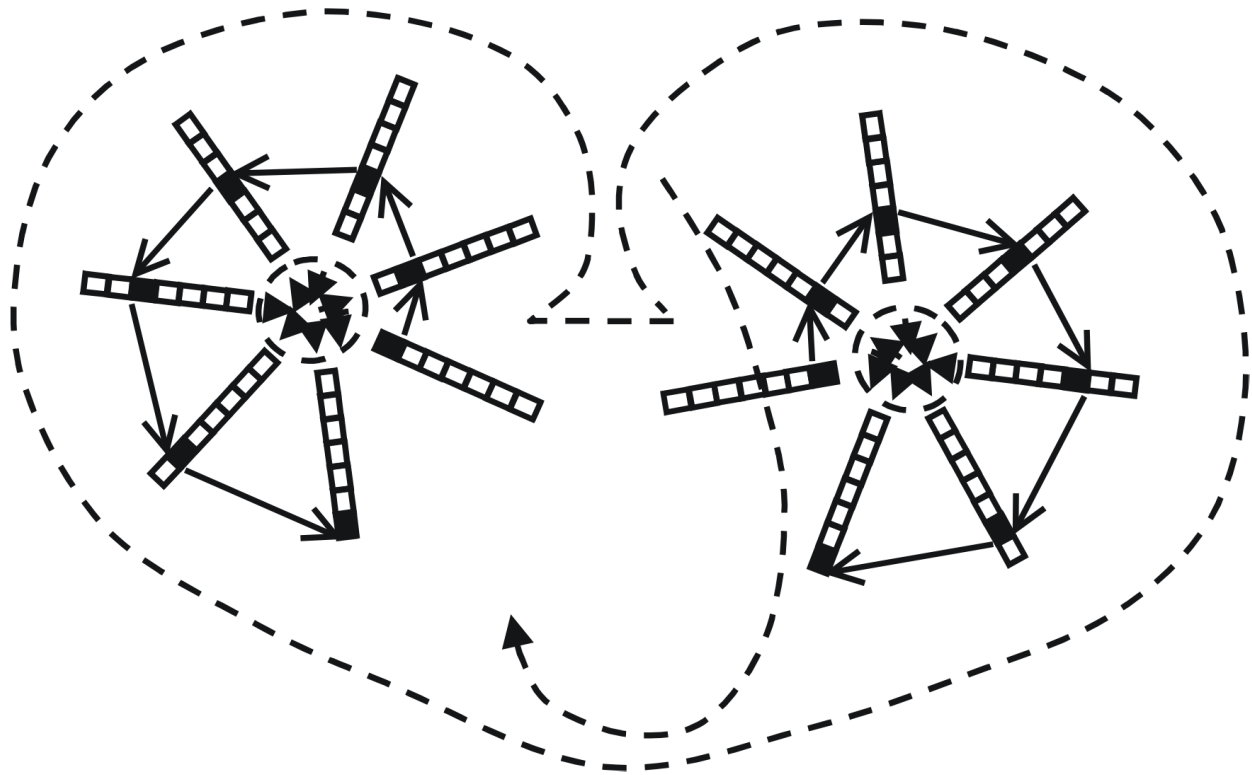
Figure 4. Diagram describing the development of the endosperm by the "closing" of the three groups of connection channels.

The form assumed by the cell system is one of possible choices from a list of assemblages of cells at specific angles to each other. The characteristic angles of deviation in the construction of the embryo are shown in Fig. 2a. The most distinct combination of cells under these angles is observed in the primary divisions of the embryo, during the period when the cells are initially placed in a certain plane of division. As can be seen from Fig. 2b, in a formed embryo, the angles formed by the elements of the embryo, as well as their orientation relative to each other, are also located at a deflection angle equal to or a multiple of $2\pi/7$, i.e. approximately 51.4 degrees.

Of key importance for the specificity of form is the condition of the formation of a critical number of cells. If the conditions for transition to the next stage of development are not met, the laying of the critical number of cells is initiated again. In such a case, the groups of cells initiated in the preceding attempt at establishing the critical conditions of transition remain in the organism and continue to grow. The external manifestation of the process will be an

increase in the "life cycle" and addition of a certain number of functionally related groups of cells, multiples of 49, of a given stage, which will change the shape of the specifics.

The development of the endosperm corresponds to a system that defines the formation of cells under the control of three groups of channels. Two of those groups indicate the distribution of cells in conformance with Archimedes spirals (Fig. 4.). Deviation from the conditions for achieving the critical number of endosperm cells will also lead to a repetition of the full cycle of formation of the previous critical number.

In the current morphophysiological classification there are 10 major types of soft and hard wheat. Some of them are presented as 2-5 subtypes. Let us now consider the morphological characteristics of kernels of the known morphophysiological types. Unfortunately, the material available at the time did not permit to process data for all known morphophysiological types, however, the results showed that the geometric characteristics of shape have distinct specific symptoms that can be used both in the practical and the theoretical aspects.

4.1. The first morphophysiological type

This type includes very early and early maturing varieties of spring wheat - North Circumpolar, East Siberian and Far East selections and is divided into subtypes - a, b, c (Henkel, 1969). The length of their growing season (from germination to maturity) equals 68 - 85 days, at least 75-100 days, respectively. Wheat cultivars of this type are characterised by the ability of seed germination at 0 °C, resistance to relatively low temperatures in spring and even to weak frosts, short first stage of development; they are adapted to develop under the conditions of a short summer and an early fall. They accelerate their development at daytime length of 18-24 hours and under predominance of light flux in the long wavelength region of the spectrum (red-orange). They do not have high heat requirements in stages X-XII of organogenesis. Maturation can take place even at +12, +14^{0}C.

Due to the rapid passage of stages II-V of their organogenesis, they form 5-6 leaves. The leaves are short (8 - 10 cm), narrow (0.7 - 0.8 cm), light green, with a slight pubescence. Leaf sheaths are usually smooth. Nodes not pubescent. Short internodes, plant height of about 70 cm. Stem is thin, relatively strong. Long day accelerates their development in stages VII-VIII of organogenesis, therefore they form short ears under such conditions (4.5 - 6.5 cm). High transparency of the air and lower temperature do not contribute to increased length of segments of the spike in stages VII-VIII of organogenesis, therefore, even at small size the ears are usually dense. The kernels are small. Weight of 1000 kernels varies from 14 to 18 g.

Varieties included in the first morphophysiological type are represented, to a considerable extent, by red-colour, non–pubescent cultivars.

Subtype "a" of the first morphological type is represented by the soft spring wheat variety 'Alenkaya' (Fig. 5-7.). The second subtype, "b", is represented by variety 'Balaganka' (Fig. 8-10.). The third subtype, "c", is represented by variety 'Amurskaya 77' (Fig. 11-13.).
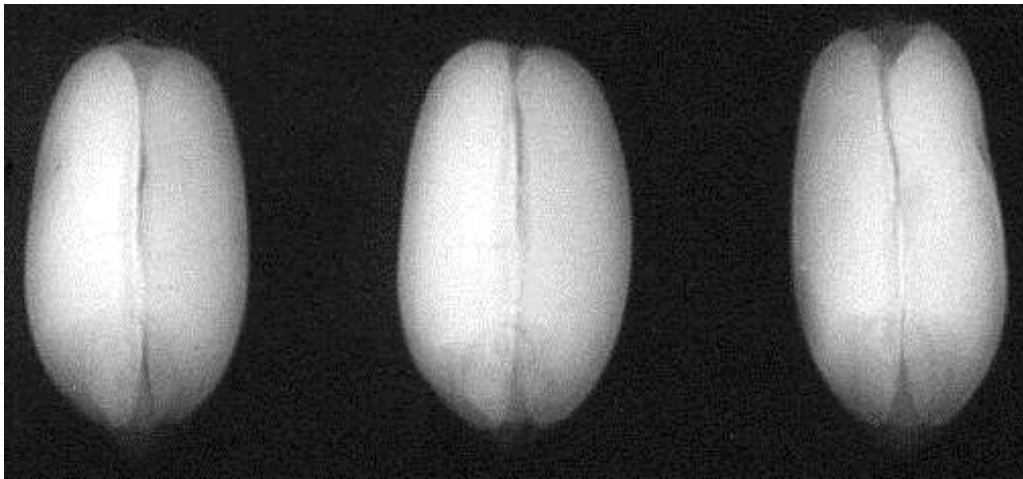
Figure 5. Var. Alenkaya. Front view: Kernels extended, i.e. significant predominance of length over width. In the embryo half of the kernel visible varying thickening at the apex of the embryo (little "chubby cheeks"). The radii of curvature of both ends of the kernels are approximately the same, large enough (blunt ends). The projections of the grooves along the length of the kernels are narrow. At the top end a little shadow in the form of an equilateral triangle.
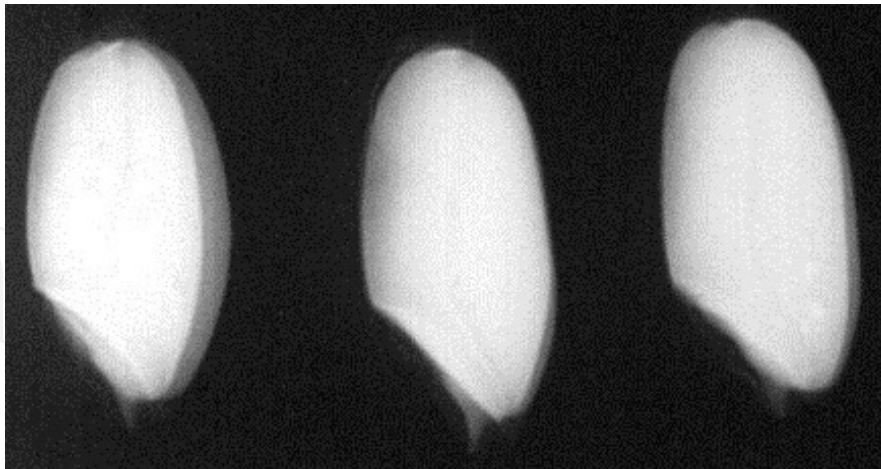

Figure 6. Var. Alenkaya. Side view: Ventral side of the projection is clearly rounded, but in the middle third is nearly straight, that is, the middle third of the kernel can stably lie on a plane. Profile section of the embryo - almost straight or slightly concave line. The back contour is slightly convex. Line at the bottom of the grooves can be seen going in the middle of kernels, with a bend repeating the bend of the back contour.
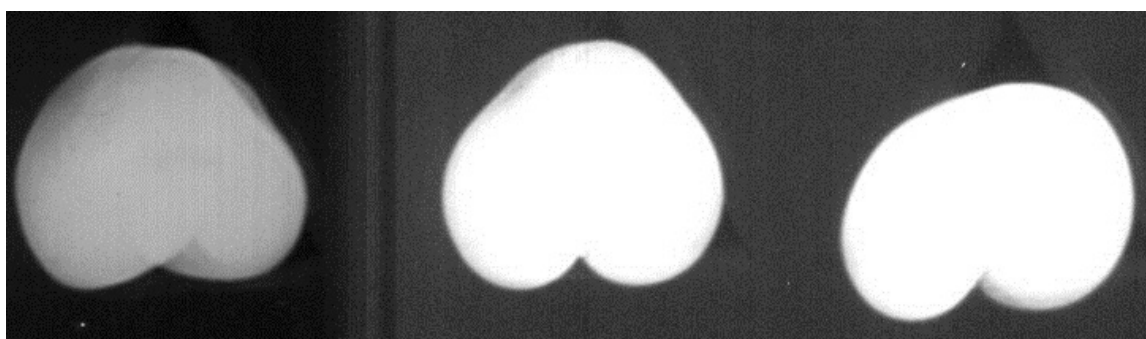
Figure 7. Var. Alenkaya. Up-down view: The up-down projection most often resembles the card colour "diamonds", but there are asymmetries usually caused by a thickening in a random location.

Figure 8. Var. Balaganka. Front view: Elongated projection. The predominant form - with longitudinal and lateral symmetry. The projection of the groove is narrow, but with weak darkening along it. At the top it turns to black in the form of an isosceles triangle with the sharp end down.
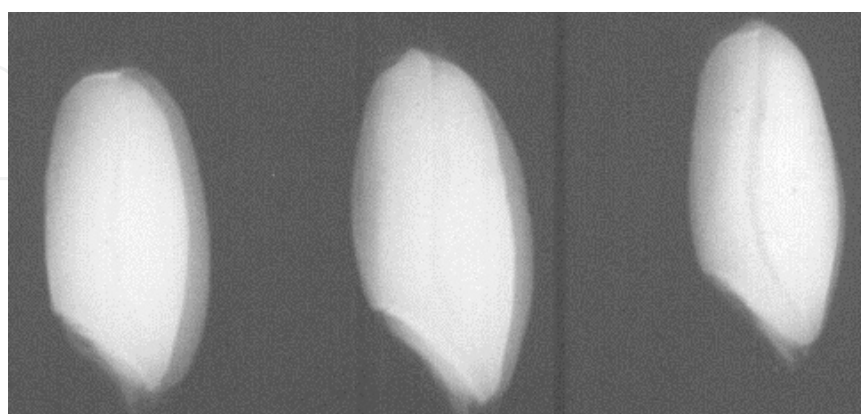
Figure 9. Var. Balaganka. Side view: Ventral and dorsal sides are convex and have an approximately constant radius over the entire length. The embryo contour is straight or slightly concave. The projection of the bottom of the groove is rather broad, indicating an expansion of the groove at its very bottom in the kernel.
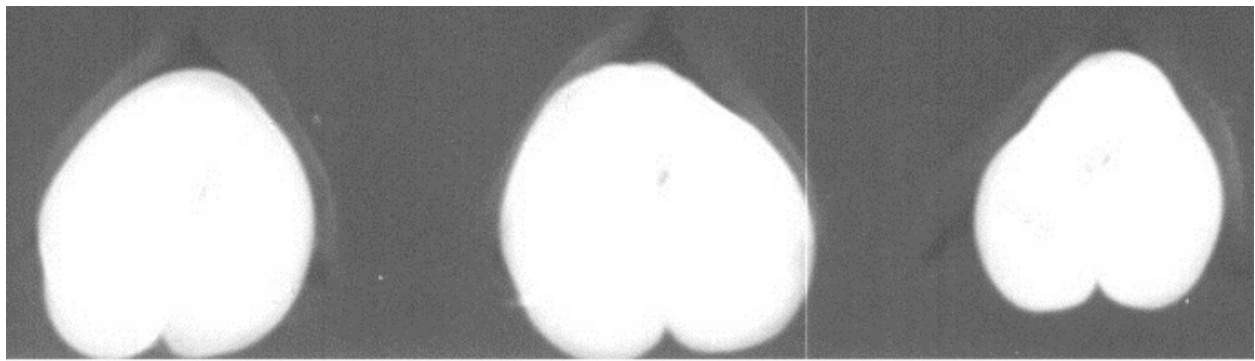
Figure 10. Var. Balaganka. Up-down view: Often asymmetrical, the shape is close to pearform.
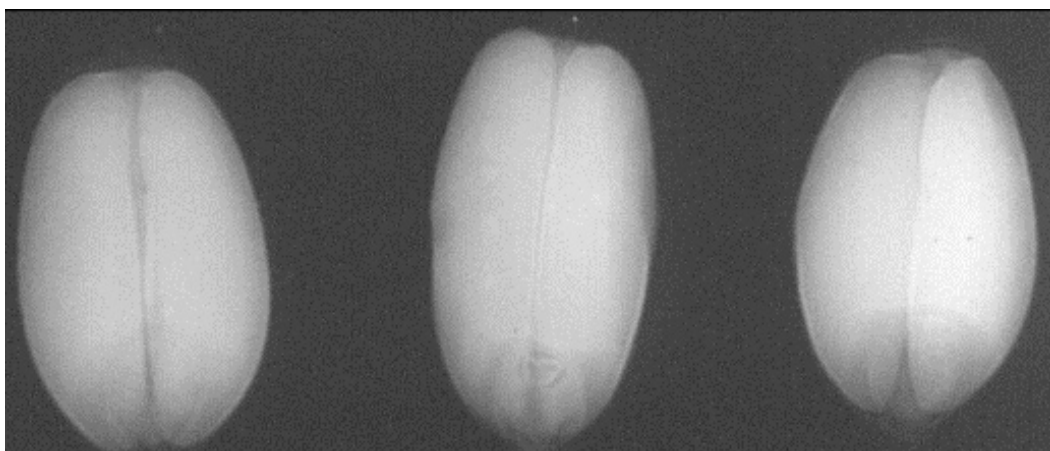
Figure 11. Var. Amurskaya 77. Front view: The projection is mainly barrel-shaped, at least - with a thickening of the end of the embryo. The groove is thin, and at both ends turns into acute-angled shadows, almost identical in shape, size and optical density. In some kernels - along the lateral edges of the shadow line - a sign of enzyme-mycosis infection of moderate severity.
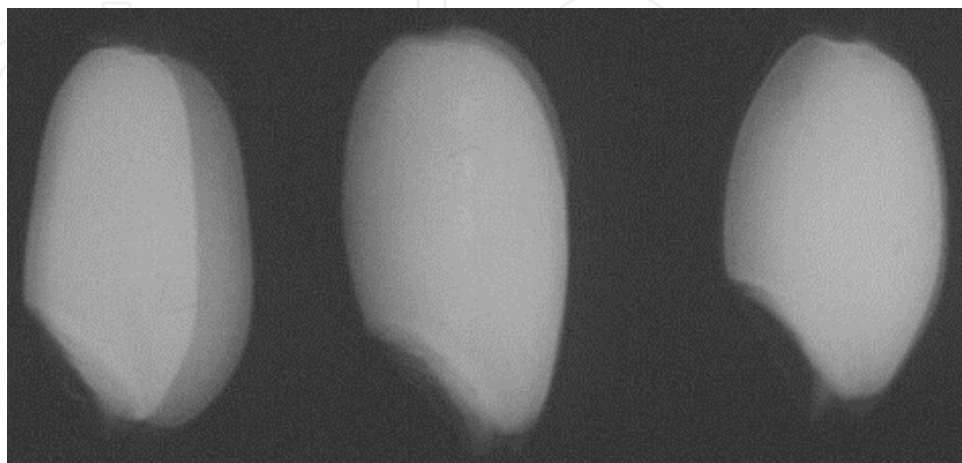
Figure 12. Var. Amurskaya 77. Side view also wide, indicating considerable height of kernel.
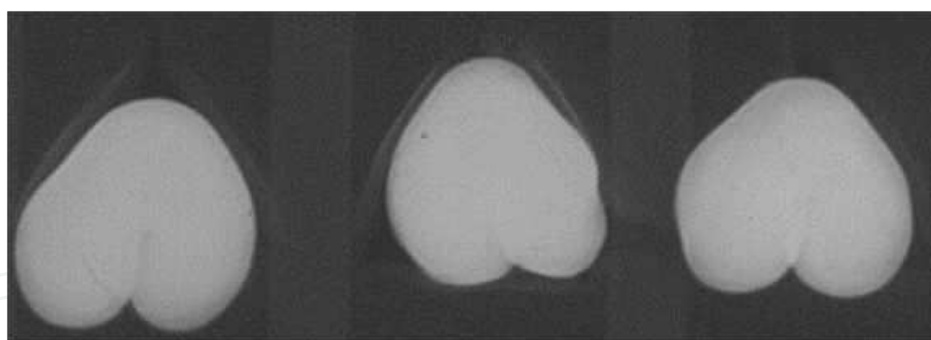
Figure 13. Var. Amurskaya 77. Up-down view. Contours are partially asymmetric, and in a half of the kernels the top is sharpened, that is, on the back there is a narrow ledge. In some kernels a deep-going groove can be observed.

4.2. The second morphophysiological type

This type includes medium-early and medium-late varieties of spring wheat (Henkel, 1969). They are local and selected varieties of temperate latitudes, developing mainly thanks to the winter and early spring precipitation in areas with moisture deficit in the second half of the summer (winter varieties resistant to drought). The length of their growing season is 80-105 days. Under the conditions of increasing length of the day, they pass very quickly through stages I-II, which results in plants with sparse foliage and early transition to stage III. Moisture deficit and low relative air humidity during stages IV-VI of organogenesis inhibit the growth of leaf blades (short and narrow) and contribute to the development of predominantly columnar parenchyma. The first phase is short (stages I-II), the second (stages III-IV) is relatively long. Therefore, the formation of the bottom of the embryonic ear manages to go through thanks to winter and early spring precipitation. The top of the ear, due to moisture deficit in the spring, is often undeveloped. As a result, there is a pronounced spindle in the structure of the ear or even a complete reduction of the upper spikelets. In cultivars of this type the passage through stages III-IV of organogenesis gets accelerated by two or three days (in conditions of 16-20-hour photoperiod), as well as the passage through stages V-VI (with prevalence of red and orange rays in the light spectrum). The cultivars of this type are relatively resistant to high temperatures and to moisture deficit in stages VII-VIII and X-XII of organogenesis. Plant height is 75 - 80cm, but depending on the availability of moisture in stages VI-VIII it varies greatly - from 30 to 110 cm. The ears are of medium size (7-9cm) and medium density.

The specific features of the physiology of development and the high drought tolerance permit the cultivation of many varieties of the second morphophysiological type both in the steppe regions of south-eastern European part of Russia and in many parts of Western Siberia.

The second morphophysiological type, subtype "a", is represented by var. 'Saratovskaya 29' (Fig. 14-16.). Subtype "b" of the second morphophysiological type is represented by variety 'Artemovka' (Fig. 17-19.).

Figure 14. Var. Saratovskaya 29. Front view: The lateral edges of the projection for the most part parallel to each other. The groove is thin and only the upper end has a distinct acute shadow. The shadows along the side edges of the projections under the shell - a consequence of enzymatic-mycosis infection.
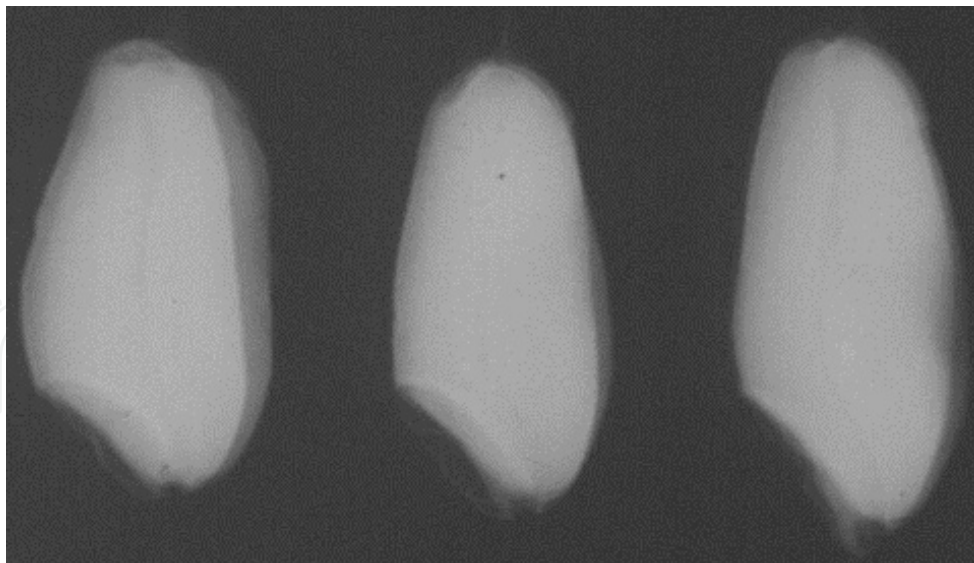

Figure 15. Var. Saratovskaya 29. Side view: Top of the kernel somewhat sharp. Along the shell - shadows, traces of enzymatic-mycosis infection. Embryo section slightly concave.
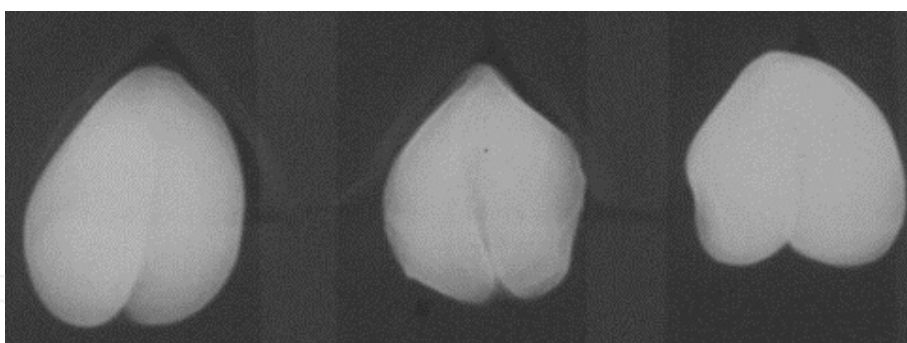
Figure 16. Var. Saratovskaya 29. Up-down view: A characteristic feature - longitudinal grooves and wide lateral recess before the proper groove on the end make the ends of folds sharp and seemingly distant.

Figure 17. Var. Artemovka. Front view: Kernel elongated with a slight bulge at the bottom. The groove is narrow, with a subtle extension of the middle part, ending with a clear wedge shadow only on the upper end of the kernel. A faint shadow along the groove.
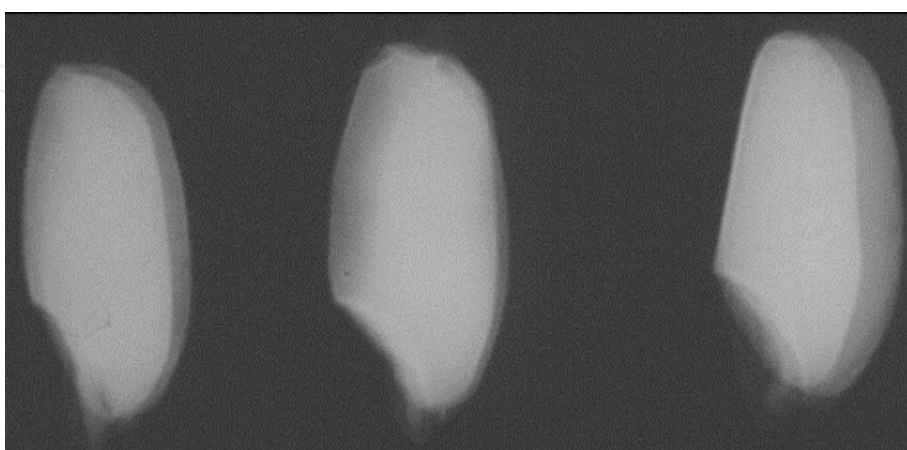
Figure 18. Var. Artemovka. Side view: Line of the ventral side of the kernel is a curve with a single radius, with a slight flattening in the middle. The line of the back of the kernel is a straight line. Concave section of the embryo.
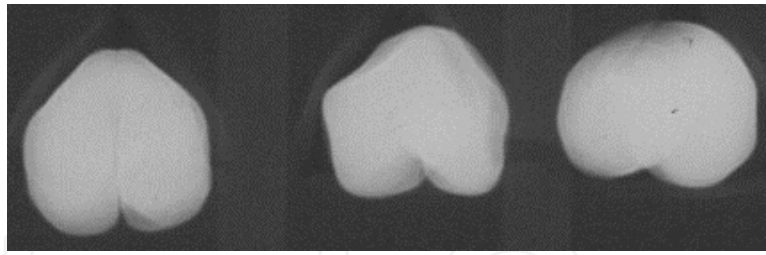
Figure 19. Var. Artemovka. Up-down view: The whole image of up-down view is like a "house with a gable roof". A shallow and narrow deepening in front of the groove, in the form of a small equilateral triangle. Thin groove is visible without any express extension at the end, that is, in the middle of the groove.

4.3. The third morphophysiological type

This type includes medium-early and medium-late maturing cultivars of soft spring wheat from the Siberian-Ural environmental group (subtype "a") as well as from the Northwest Environmental Group (subtype "b") (Henkel, 1969). They are characterised by a relatively long duration of stages I-II of organogenesis and medium duration of stages III-IV. They can develop normally with day length of 16-17 h and predominance of light flux in the red and orange range of the spectrum. Their development is inhibited at 13-14-hour day length. Duration of vegetative period is 85-100 days. Lower temperatures in spring led to a delay of stage II of organogenesis and the formation of 7-9 leaves, and to the growth of mechanical tissues of the lower and middle internodes of the stem. Good moisture availability in stages V-VI of organogenesis is conducive to synchronous formation of spikes and to the formation of a cylindrical and slightly club-shaped ear, as well as to increased growth of the leaves in length and width. Favourable moisture conditions in stages X-XII of organogenesis cause the formation of large kernels, but low temperatures in stage X of organogenesis inhibit the growth of kernels in length. In varieties of this group the kernels are relatively short and often have a low weight of 1000 grains (28 - 30g).

Subtype "a" of the third morphophysiological type is represented by a variety from the forest-steppe (Siberian-Ural) group – 'Viesna' (Fig. 20-22.). Subtype "b" is represented by variety 'Gorkovskaya 20' (Fig. 23-25.).
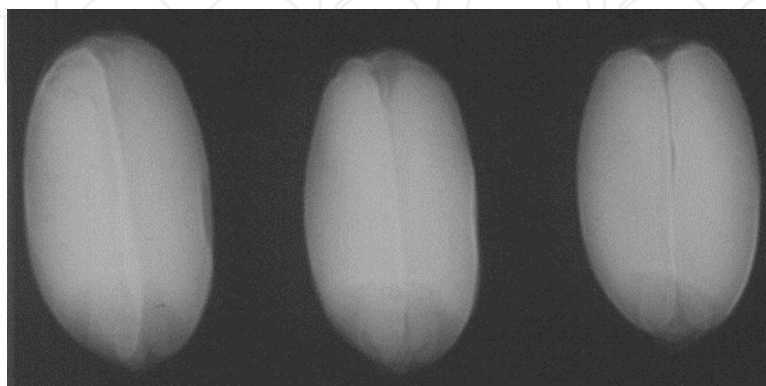

Figure 20. Var. Viesna. Front view: Basically a regular ellipse. The groove is thin, no shadows to be seen around it, wedge-shaped extension visible only at the top of kernel.
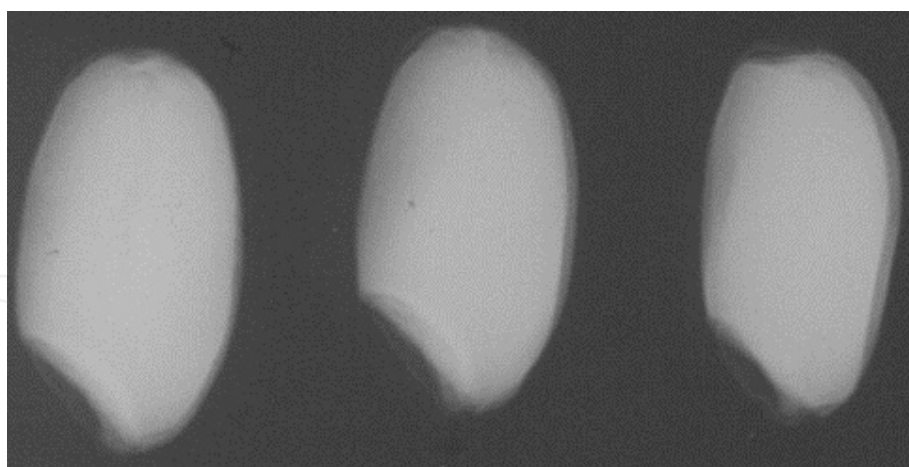
Figure 21. Var. Viesna. Side view: Basically also a regular ellipse, interrupted by the slightly concave section of the embryo. In some kernels the ellipse is slightly distorted by a small bulge in the bottom half. The groove is not visible.
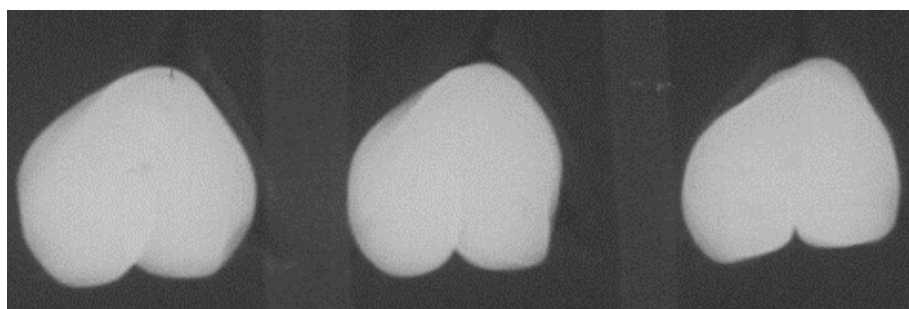

Figure 22. Var. Viesna. Up-down view: General view like a house. The lower edges of the folds are flattened. The groove is thin, with no extension on the end.

Figure 23. Var. Gorkovskaya 20. Front view. The projection is usually elliptical, with blunt ends. The groove is thin, with a slight expansion in the upper third and a triangular shadow visible at the upper end. Along the groove there is a faint and narrow shadow.
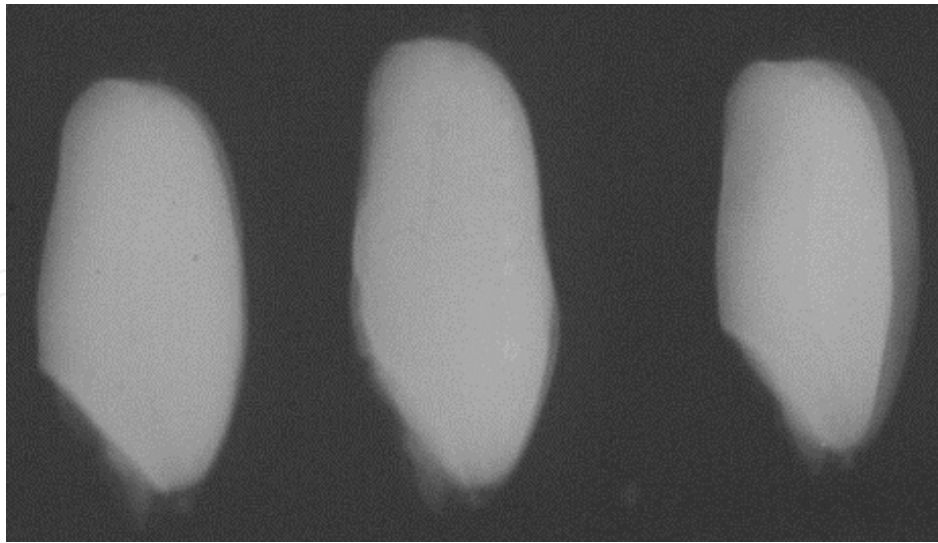
Figure 24. Var. Gorkovskaya 20. Side view: Line of the edge of the ventral part of the kernel - a curve with a single radius. Line of the edge of the back - straight. Visible line the bottom of the groove, running parallel to the back.
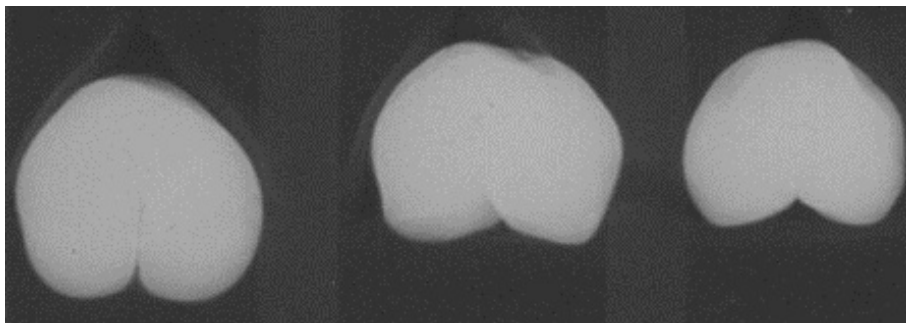

Figure 25. Var. Gorkovskaya 20. Up-down view: Projection of the "worm" type, slightly flattened, so that the width is a bit larger than the height. Before entering the groove - a narrow and low depression (a little groove along the kernel).

4.4. The fourth morphophysiological type

This type includes late-maturing varieties of spring wheat of Western European breeds. The length of growing season is 120-130 days (Henkel, 1969), with a prolonged first stage of development. Under conditions of long day and high light intensity, plants of the fourth type respond with accelerated transition through the second phase (stages III-IV of organogenesis). However, they may be slow to develop in conditions of low light intensity at considerable cloudiness and 14-15-hour day, and in the early stages – also under conditions of a shorter photoperiod. Slow development in such conditions, with good moisture availability and high rates of fertilisation in stages V-VII, causes the formation of large leaves, and of square-headed or even club-shaped forms of ear in the Western European group of varieties. Slow development at a sufficient water supply for plants in stages X-XI leads to the formation of large kernels with a high weight of 1000 kernels (38 - 45g or more).

Spring wheat varieties of the fourth type belong mostly to the forest-steppe and partially to the forest environmental groups. They are mostly prevalent in Germany, the Czech Republic, Slovakia, Denmark, Belgium, Finland and other countries, but compared to winter wheat they occupy small areas. This group includes spring wheat variety 'Peka' (Fig. 26-28.).
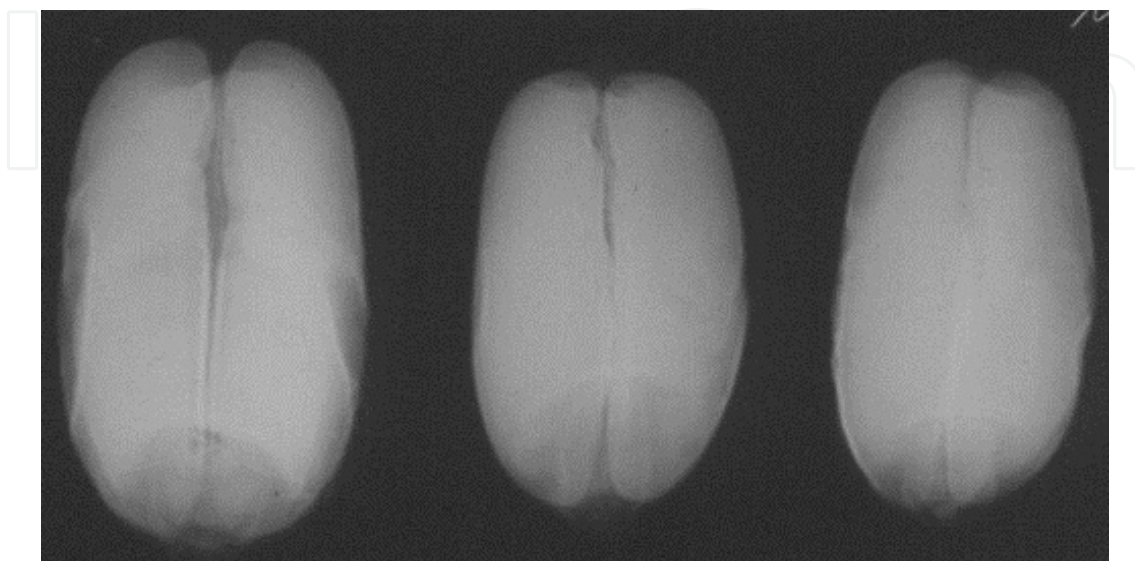
Figure 26. Var. Peck. Front view: Form close to rectangular. The edges are typical of small indentations, as if holes. Shade of the groove is well marked.
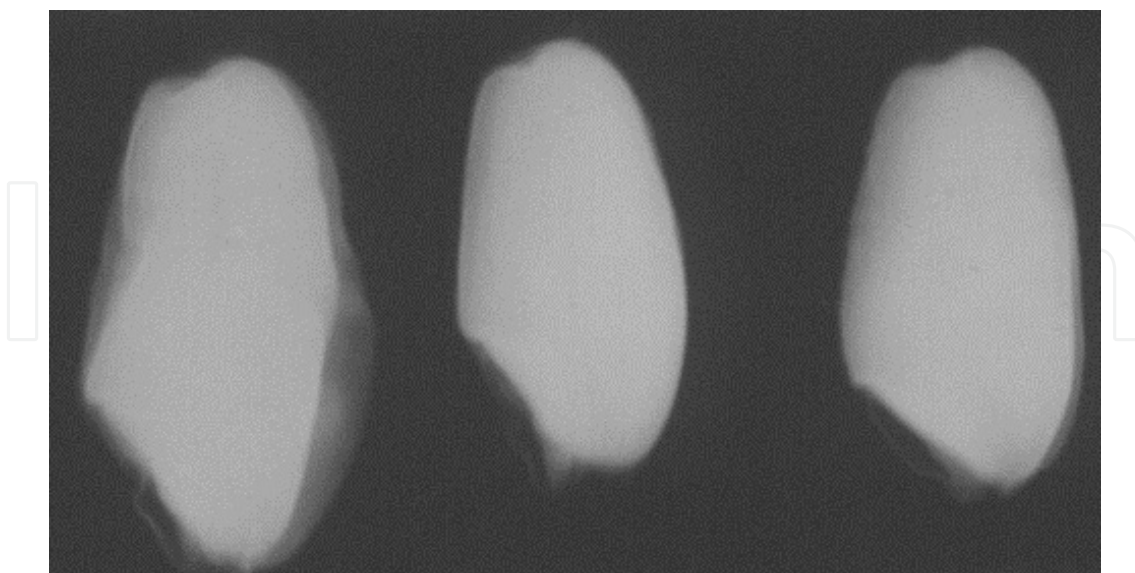
Figure 27. Var. Peck. Side view: As a rule, the contour of dorsal part of kernel is straight. Line of the ventral side is convex. The shadow of the groove can be seen, not in all kernels.
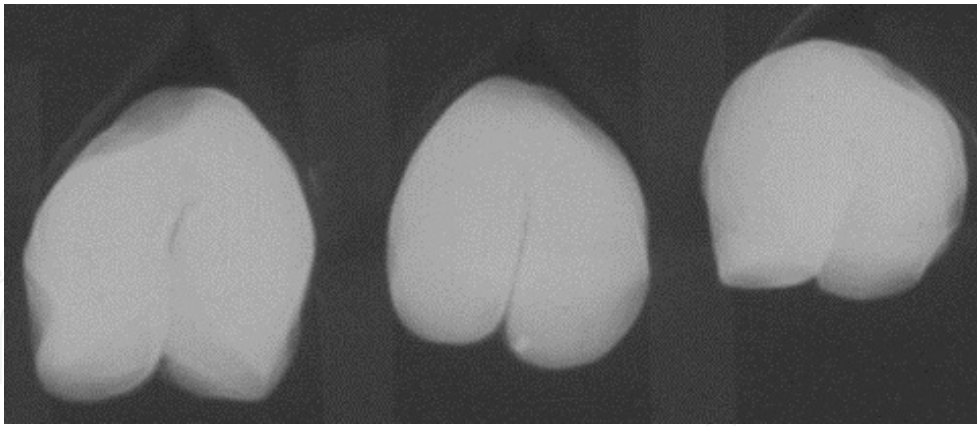
Figure 28. Var. Peck. Up-down view: A depression in front of the groove - either broad or a narrow one. Depending on this the folds are either rounded or sharp. Kernels with rounded folds are seemingly flattened (width greater than height); those with sharp folds have height greater than width, and angular.

4.5. The fifth morphophysiological type

This type comprises mid- and late-ripening varieties of the West Siberian breeding (Henkel, 1969). The specific features of climatic conditions - cold and dry April, May and first half of June, relatively high rainfall in late summer (July), low temperature in August, formed a special type of Siberian forest-steppe ecological forms of wheat.

The length of the growing season is 95-110 days. The slow development and long passage through stage II of organogenesis in the presence of favourable conditions for plant growth lead to increased tillering of plants of this morphological type. Delay in development at stages III-IV of organogenesis causes the possibility of forming an increased number of rudimentary spikelets. Delay at stage II of organogenesis permits significantly more efficient use of late summer rainfall for the formation of large ears and many-flowered spikelets. The ability of going through stages X-XII of organogenesis even at relatively low temperatures ensures the ripening of wheat in late August and in September. The long duration of stage II contributes to the formation of high foliage of plants. The leaves are large, dark green, and with medium- and strong pubescence. No grain of cultivars representing this type was obtained for analysis.

4.6. The sixth morphophysiological type

This type includes early-maturing varieties developed in Central Asia, mainly in the conditions of both spring and autumn sowing under periodic watering, less often in rain-fed crops (Henkel, 1969). They are distinguished by their resistance to soil and air drought, especially in stages II-V and X-XII of organogenesis. They include winter and spring forms, as well as transient forms (spring and winter). In Central Asia, Iran and Afghanistan, high temperatures and direct solar radiation during stages VI-VII of organogenesis often lead to complete closure of the glumes, complicating threshing, and to the development of mechanical tissues in glumes and awns.

The rapid passage through stage II of organogenesis at high temperatures and intense direct solar radiation leads to a sharp decrease in the number of leaves and stem internodes formed at this stage, and to a reduction in the process of tillering.

In stages IV-VI of organogenesis, the plants need watering to ensure normal moisture content of the generative organs. Stages XI-XII proceed normally at high temperatures.

No grain of cultivars representing this type was obtained for analysis.

4.7. The seventh morphophysiological type

This type incorporates spring wheat cultivars with spring and autumn sowing times (?) and intermediate (semi-winter) forms of wheat (Henkel, 1969). These are local and breeding varieties of Georgia, Azerbaijan, Tajikistan, the Mediterranean countries, Ethiopia, etc. They are characterised by a relatively short first phase of development (stages I-II of organogenesis) and medium duration of the second (light) phase (stages III-IV of organogenesis), which plants can normally go through at 14-15-hour day length. In the case of vernalisation of sowing material they require from 15 to 30 days of low temperatures; in the vegetative state the first phase of development of many of them will be completed within 10 to 15 days. Early warm spring and rare cases of the return of cold weather permit the transition to stages III-V of organogenesis under short-day conditions. Delay in development caused by short day in the period of differentiation of the embryonic ear promotes the formation of multi-flower spikelets, and subsequent delay in stage V under conditions of still a relatively short day and high moisture content due to precipitation of spring and the first half of the summer ensures synchronous development of multi-flower spikelets.

Favourable conditions for photosynthesis, large number of leaves (8 - 10 leaves) and high moisture content in stages X-XII of organogenesis ensure the growth and ripening of grains which, in this group of wheat cultivars, reach extreme sizes and weight. No grain of cultivars representing this type was obtained for analysis.

4.8. The eighth morphophysiological type

This type includes wheat varieties formed under the conditions of the Crimea, southern Ukraine, Moldova, Georgia, Armenia, Azerbaijan, South Yugoslavia, Bulgaria, India and other areas with a relatively mild winter (Henkel, 1969). In the vegetative state (in the phase of emergence and tillering), they can pass the first phase of development (stages I-II of organogenesis) at temperatures of +7, +12° C. Therefore, they can quickly move on to stages III- IV of organogenesis in early spring at 12-13-hour day length. As in the beginning of the formation of rudimentary spike there is a certain delay in stages IV-V of organogenesis, so they can form multi-flower spikelets, with simultaneous development of flowers. In conditions of late spring and rapid rise of heat, in many varieties the flowers in upper spikelets may be underdeveloped. In such years the form of the ear is close to the spindle, in the middle part of the ear 3-5 fertile flowers develop, in the top part - 1-2 flowers. In this connection, depending on spring conditions, spike length and number of kernels in ear vary dramatically. The ability of wheat varieties of this type to develop at relatively high temperatures in the first phase and to accelerate their passage through that phase under the effect of low tem-

peratures, as well as their ability to begin the second phase in short-day conditions and dramatically accelerate the development at lengthening photoperiod, resulted in a high flexibility of those varieties. No grain of cultivars representing this type are available.

4.9. The ninth morphophysiological type

This type includes wheat varieties with seed material vernalisation for periods from 40 to 85 days (Henckel, 1969). Beginning in spring and later, the development of plants of this type is similar to the development of varieties of the second morphophysiological type. Representative of this type is variety Mironovskaya 808 (Fig. 29-31.).

Figure 29. Var. Mironovskaya 808. Front view: Projection elongated, often close to an ellipse, sometimes with a slight thickening towards the embryo end of the kernel. Expansion of groove in the middle due to the particular case of damage by *thysanos*. At the upper part of kernel the groove ends with a well-defined wedge-shaped shadow. Along both sides of the groove rather broad shadows.
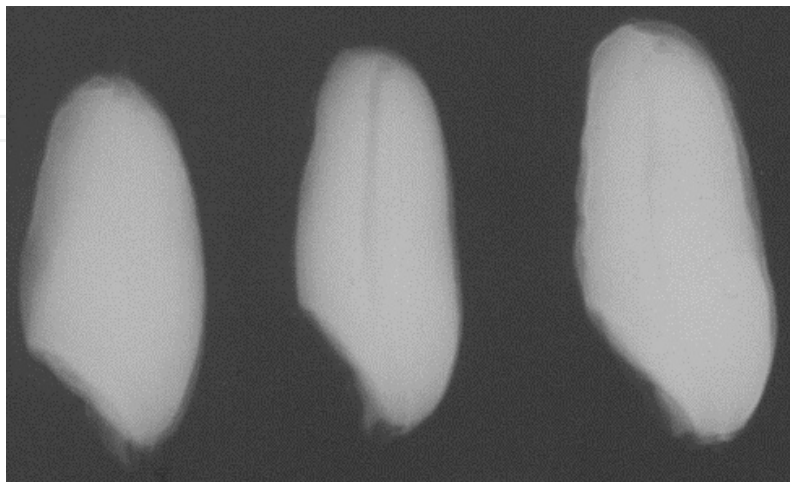

Figure 30. Var. Mironowskaya 808. Side view: The profile of the elipse is narrower with a clear thickening of the bottom. Thye line of the dorsal view is straight line, and at the ventral side – convex, some – with a direct plot in the middle. Section of the embryo – a straight line. Almost always seen the shadow line of the bottom grooves.
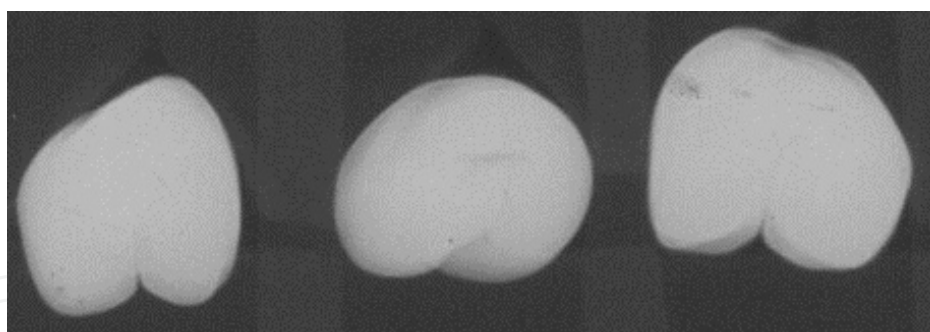
Figure 31. Var. Mironowskaya 808. Up-down view: Asymmetric :round loaf". Distinct broad but not deep ditch in front of the groove itself.

4.10. The tenth morphophysiological type

This group includes winter wheat varieties cultivated in Western Europe and the Baltic states, in the Leningrad region and adjacent areas of the Russian Federation (Henkel, 1969). Since the spring, with the transition to stages III-IV of organogenesis, the development of wheat varieties of this type is similar to the development of varieties of the fourth morphophysiological type. They also form square-headed and club-like ear, late developing and well leaved.

5. Identification of seed appurtenance to variety

The objective of the study was to explore the possibilities of application of X-rays to determine with the morphological features characteristic of wheat varieties. It is proposed to use chosen characteristics for the selection of seeds of regional varieties, which will bring them to the highest sowing condition, prolonging the active "life" of the varieties, as well as to consider those characteristics as a highly efficient tool in plant breeding.

For the study of the varietal morphogenetic specificity we chose the wheat Mironovskaya 808 (in 2004 it was replaced in the registered by the variety 'Volgaskaya 16'), derived from the "spring wheat in winter wheat" cultivars under the guidance of an outstanding breeder of cereals, Academician V.N. Remeslo. The variety is extremely interesting in that under field conditions it allowed, in the central zone and southern regions, to harvest up to 56 quintals per hectare, that is at least twice the average value of the current level of productivity. Mironovskaya 808 is classified as soft winter wheat, morphotype IX, subtype "c", with a long vegetative period of 290-305 days. It was derived through repeated mass selection of morphologically homogeneous plants from initial material obtained through directional modification of the spring wheat 'Artemovka' into a winter form. Group selection of 11 morphologically homogeneous and highly productive plants of the third progeny was the beginning of the variety Mironovskaya 808 (Remeslo, 1977). The leading varieties, in terms of yield and grain quality, are the winter wheats, Bezostaya 1, and Mironowskaya 808, and the spring wheat Saratowskaya 29 (Grundas & Wrigley, 2004 a).

The variety Mironovskaya 808 is characterised by a broad morphological diversity. The differentiation of its forms bears the character of a separate genotype. These differences are comparable to the differences between individual varieties of wheat. This finding echoes the results of a recent study in the VIP. Among 230 varieties of spring wheat from Asia and Africa, twelve (K-202015 in Egypt; K-43720, K-43730, and K-55728 in Iraq; K-14317, K-14333, K-38598, K-38674, K-38675, and K-60213 in Iran; K-55 733 in Syria, and K-44513, Ethiopia) also proved to be morphologically heterogeneous (Mitrofanova, Wael Al-Youssef, 2008). It was shown that wheat may contain in its composition intravarietal groups of plants whose differences, in terms of their qualitative and quantitative characteristics, are comparable to the intervarietal ones.

Within the scope of the problem, attention was focused on factors affecting the length of the growing season as an approach to the control of subsequent change of form. The morphological varietal specificity of cv. Mironovskaya 808 was studied on combinations of loci Vrn1 - Vrn3, each of which has its registration number in the collection of wheat in the VIP, St. Petersburg, Russia (K-60657, and K-60662). Mironovskaya 808 belongs among the strong varieties of wheat, as a transient variety (Stelmach, 1987).

The variation of ripening time for various Vrn- and Ppd- genotypes in terms of their heading time averaged at a multiple of ±4 days. The distribution by rank of early ripening (acc. to Vrn) was as follows:

1. Vrn1 Vrn2 Vrn3;
2. Vrn1 Vrn3;
3. Vrn1 Vrn2;
4. Vrn1;
5. Vrn2 Vrn3;
6. Vrn3;
7. Vrn2.

The difference in speed of heading between the extreme variants amounts to an average of 19 days. The speed of transition from the second to the third stage of organogenesis was the maximum for Vrn 1 and the minimum for Vrn 2, and in the case of Vrn 3 it was at a medium level for the spring genotypes. This leads to the conclusion that the final stages of organogenesis in plants of various genotypes take place in various periods of vegetation. The duration of those periods and the climatic conditions during those periods determine the number of forming ovules of the reproductive organs.

The differences in the duration of the vegetation period for various genotypes are reflected in the varied form of kernels. This study had the objective of estimation of specific morphological indicators on the basis of variation in the duration of the vegetation period. X-ray images of kernels permit accurate recording of their contours in a form in suitable for identification. Using this method we can register the kernel in three planes, each of which carries its own specific information about its form, suitable for identification.

For morphological classification of cereal grains it is proposed to distinguish two levels of X-ray magnification: the first – image magnification by a factor of 2-10, which allows to study the morphology of kernels as a whole, and the second – magnification of images to x20-x40, to identify variety-specific features of particular elements of the embryo, or signs of kernels damage by mycosis.

The formalisation of the differences for computer identification must be based on the use of a method of recognition of "model of elementary figures" and their combinations, characteristic for wheat kernels.

To detect the presence of fungi in the caryopsis it is necessary to apply image magnification of x27-x30. Fig. 32. presents clearly visible fungal filaments (hyphae).
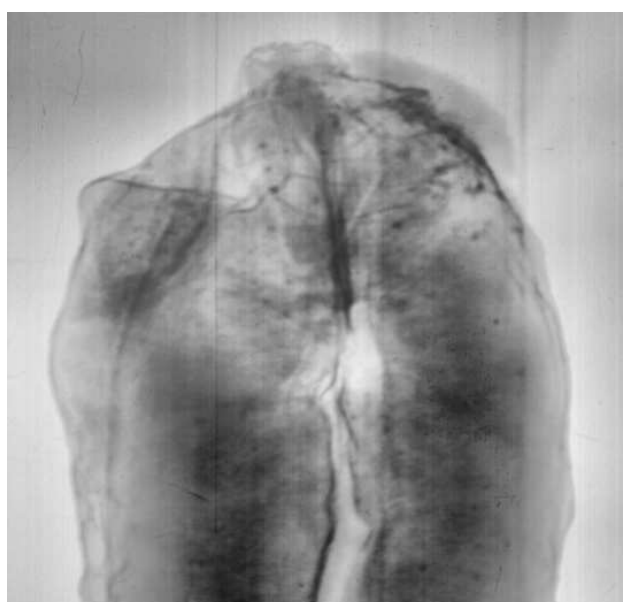

Figure 32. The presence of fungi in the caryopsis of wheat.

6. Features of intravarietal morphological variability

Despite the importance of morphology in breeding work, in estimates of dispersion of the parameters of morphological characteristics of varieties in generations no quantitative studies of the dependence of the shape of kernels on the properties of the parent and daughter plants were previously systematically conducted. The method of X-ray analysis allows us not only to assess mathematically the shapes as a whole, which in itself is important, but also to study the relationships of internal structures of kernels.

Figs. 33-38 present the main and typical forms, identified as characteristic for the variety Mironovskaya 808.
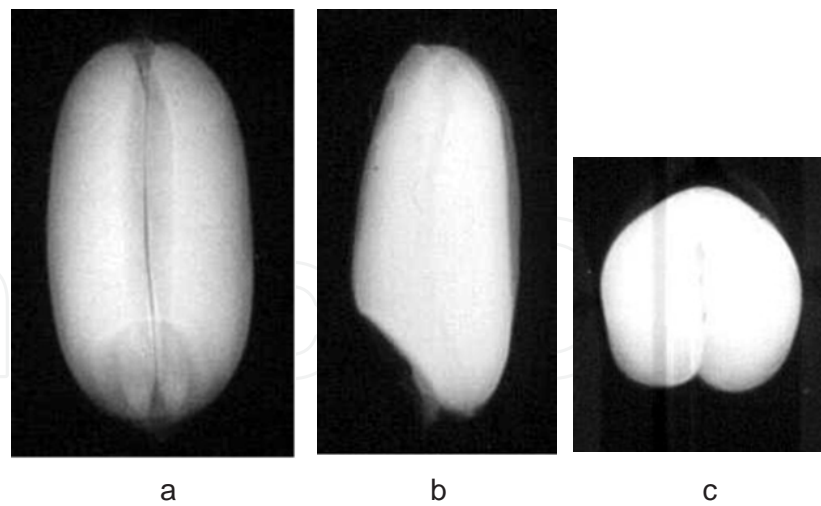
Figure 33. Morphotype 9 (K-60657). Front view (a): The side walls are almost parallel, slightly convex. The ends are approximately the same curvature. Side view (b): The surface from the groove side is flat. Up-down view (c): Close to the shape of a circle ("high round loaf").
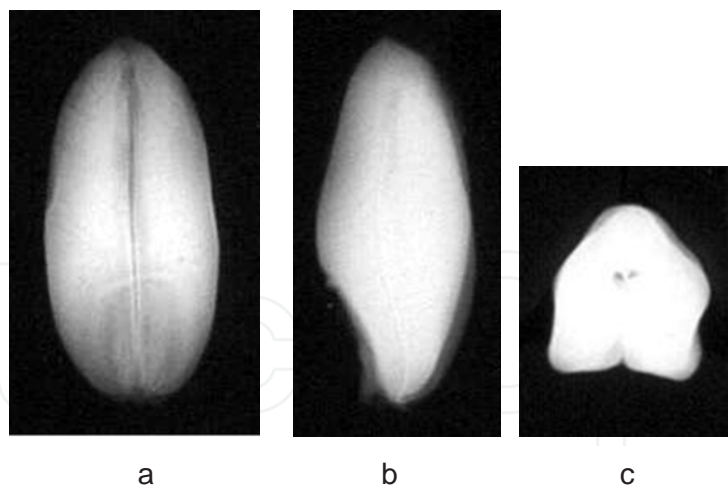

Figure 34. Morphotype 10 (K-60658). Front view (a): Narrowing towards the top of the kernel. Side view (b): The surface of the groove is slightly convex. Up-down view (c): Outline of this shape is rectangular ("house"-type).
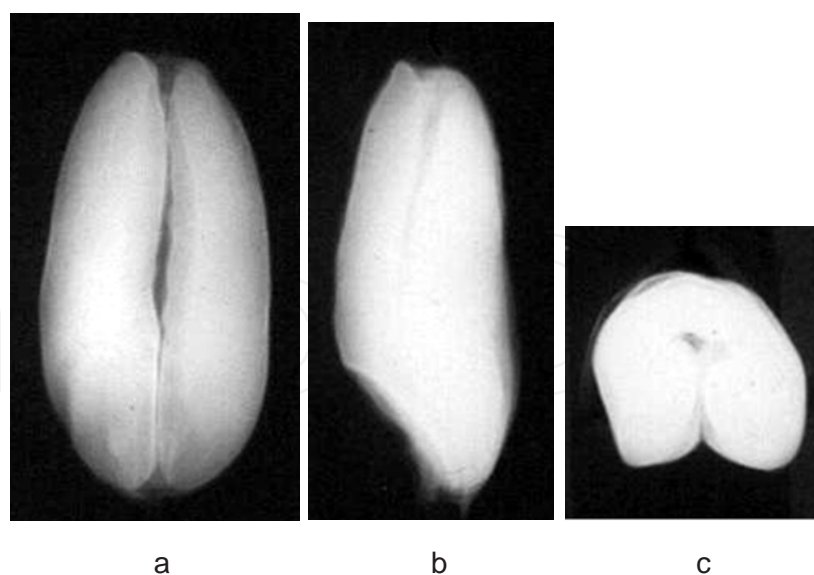
a   b   c

Figure 35. Morphotype 10 (K-60658). Front view (a): Narrowing towards the top of the kernel. Side view (b): The surface of the groove is slightly convex. Up-down view (c): Outline of this shape is rectangular ("house"-type).

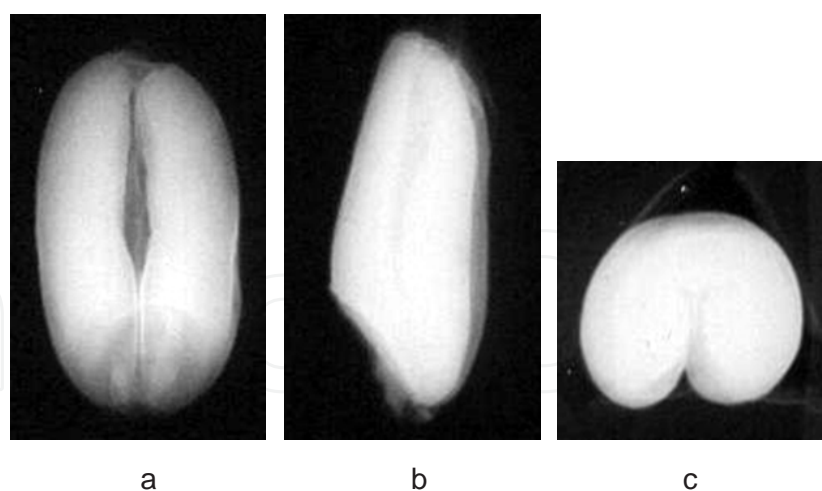a   b   c

Figure 36. Morphotype 12 (K-60660). Front view (a): The side lines are parallel, the ratio of length to width is lower than that of morphotype 9 (kernel shorter). Side view (b): The surface of the groove side is slightly concave, from the

back side the top is sharpened. Up-down view (c): The ratio of height to width of the projection is lower than in the previous morphotype ("flattened round loaf").
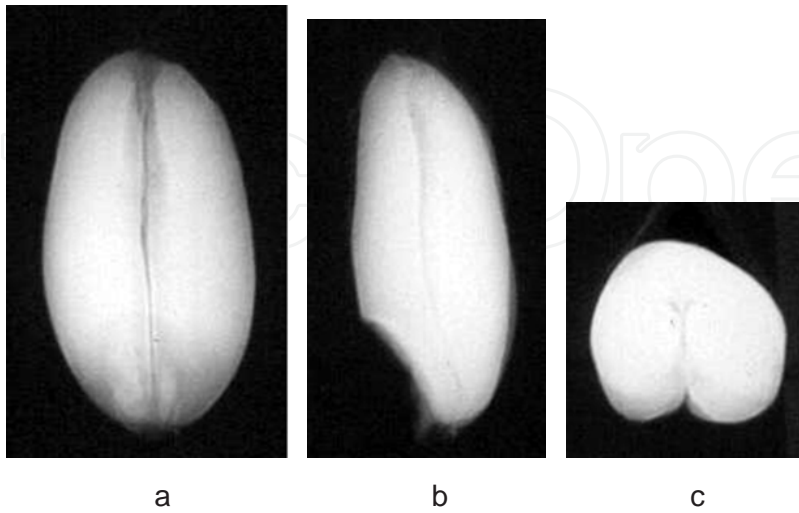

Figure 37. Morphotype 13, (K-60661). Front view (a): Rounded ends with approximately the same radius, but maximum diameter of the kernel dropped below the middle of the kernel. Side view (b): The surface of the groove side is convex, tapering at the upper end of the centre, on the extension of the projection small grooves, bending towards the back. Up-down view (c): Type "low loaf with a slightly pointed top".
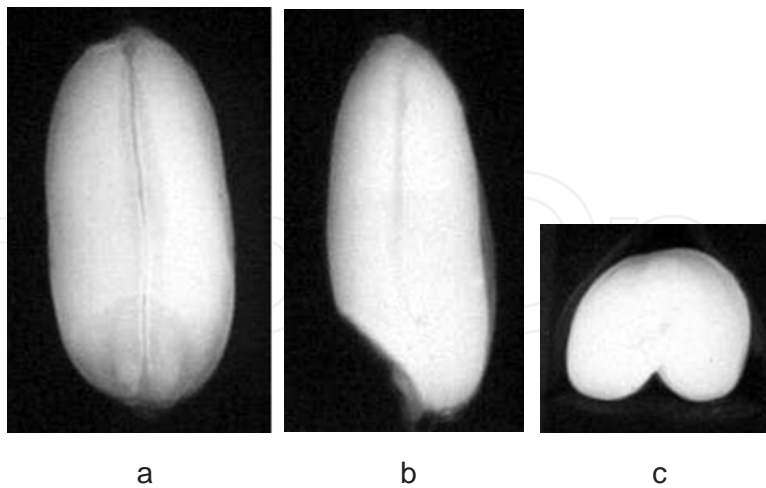

Figure 38. Morphotype 14 (K-60662). Front view (a): Lateral line projection is slightly concave, in contrast to morphotype 9. Side view (b): The surface of the groove side is flat, slightly concave. The upper end is tapered symmetrically. Up-down view (c): Top contour is rounded with height to width ratio greater than that of morphotype 12.

It was found that in the morphology of kernels and varieties, and in intravarietal variations, there exists a dispersion that is not taken into account in the varietal identification. This variance/dispersion is due to the position of kernels in the ear and to inevitable loss of varietal properties in reproduction of seeds for production sowings. The proposed combination of analytical and X-ray techniques can be regarded as a method of fine separation to ensure varietal purity and to meet industrial requirements for seeds of high productivity.

Analysis of the profiles of typical representatives of kernels allows the following results to be presented for discussion:

1. The forms of kernels from an intravarietal group, with conditionally the same Vrn features and also the same ID number, will be different.

2. Various Vrn groups contain kernels with a similar form, which confirms the need for their purity selection already at the stage of accepting a variety for inclusion in the collection.

3. X-ray images of kernels from a group with an individual number in the collection can be the basis for a description suitable for recognition.

In this study the identification was conducted on the basis of geometric similarity of representatives of morphotypes. Primarily, a differentiation was established with relation to the size of kernels – with the formation of a longer spike the kernels formed are considerably larger. In the selection of seeds, apart from checking for defects, seeds with the highest length/width ratio are chosen. The decision on the selection is based on prior calibration performed on a test batch of kernels.

To determine the form of geometric similarity the following algorithm was applied:

1. Projections of every kernel were set apart and described by surface area corresponding to the area of the projections;

2. The projections of areas in the kernel samples areas were situated in the same orientation of each projection and were scale calibrated to match one of the characteristic linear dimensions;

3. We evaluated the percentage differences on the line of maximum discrepancy of figures on the common surfaces (see Fig. 39-41.).

It was found that the differences in the selected morphological representatives of a variety, at least in one of the projections, amounted to no less than 10%. Assessment of morphological differences was performed using the following formula

$$(100 \times (L_{K.X} - L_{K'.X'}) / L_{K.X}),$$

where: L - the line of maximum divergence of calibrated figures, describing the compared kernels; K and K' - indicate the type of projection and can assume the values of F (Front view), S (Side view) and U (Up-down view); X and X' - indicate the sequence number allocated to identify the morphotype.
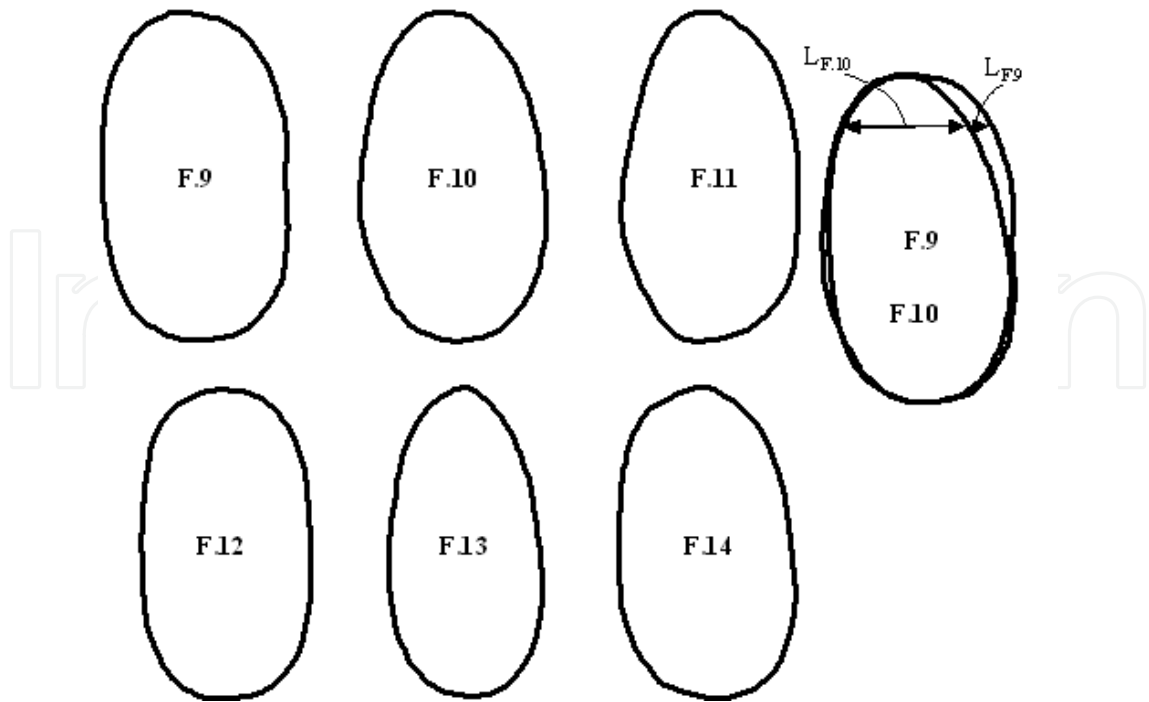
Figure 39. Forms of geometric similarity of wheat var. Mironovskaya 808. Front view (F): To morphotype 9 – F.9, to morphotype 10 - F.10, respectively, etc.
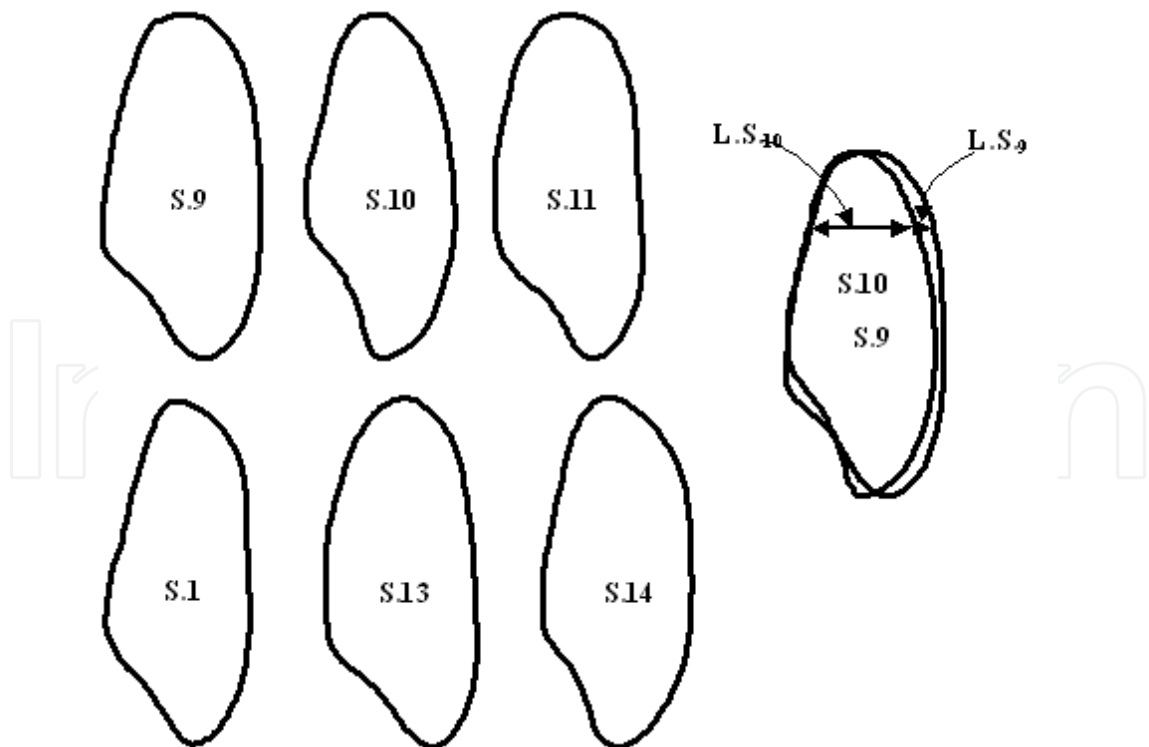

Figure 40. Forms of geometric similarity of wheat var. Mironovskaya 808. Up-down view (U): For morphotype 9 - U.9, to morphotype 10 – U.10, respectively, etc.

Figure 41. Forms of geometric similarity of wheat var. Mironovskaya 808. Up-down view (U): For morphotype 9 - U.9, to morphotype 10 – U.10, respectively, etc.

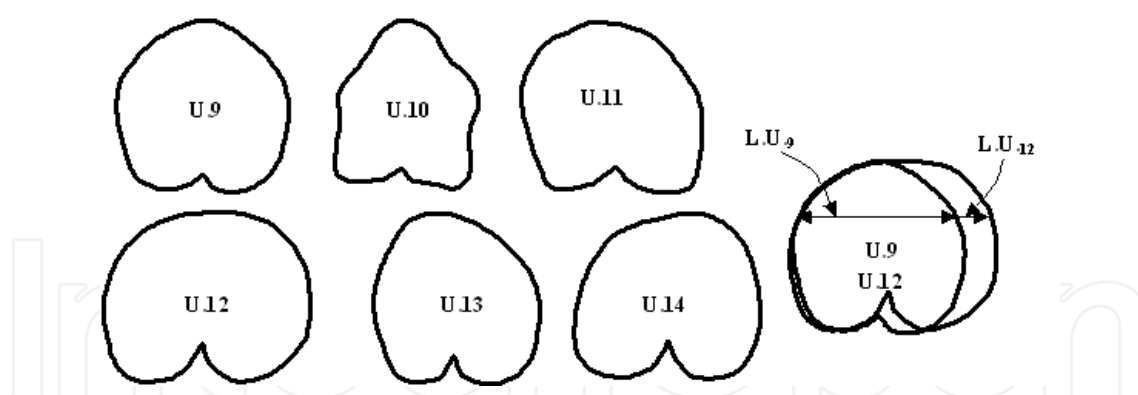# 7. X-ray inspection of grain

The quality of grain is determined through characterisation of more than 20 of its physical, biochemical and technological properties. Among the approximately three dozen methods for the evaluation of grain quality, the position of the X-ray method (XRM) is more than modest. This method is included in the standard as a method of assessing the rate of infestation and populations of grain insects, including quarantine species (Varshalovich, 1958). XRM allows, without destroying the object, to identify its internal structure and make it observable and accessible for qualitative and quantitative evaluation (Grundas, Velikanov, 1998; Grundas, et al., 1999).

Using XRM we can detect many defects in the internal structure of grains, significantly affecting their quality, such as:

1. Fractures or cracks caused by both natural and technological factors;

2. Damage caused by the sunn pest (*Eurygaster maura)*;

3. Occurrence of enzymatic-fungal damage/weakening;

4. Damage to the embryo, of various nature and extent;

5. Infestation with insects, including the earliest stages of larval development;

6. Occurrence of internal sprouting, which had begun in the field or in a pile and was stopped by drying;

7. Infestation and damage by fungi.

All of these defects reduce the quality of grains as seed and as a raw material for processing, and in some cases make the kernels unsuitable for planting or for processing (the presence of insects, embryo broken off).

XRM permits determination of the physical parameters of individual kernels as well as of kernels in bulk or heap (size of kernels, grain nature, the presence of impurities, etc.). In re-

cent period also other desirable capabilities of the XRM are being discovered in its application for analysis of grain quality. Over the past 20 years a lot of attention has been devoted to the XRM by research teams in Russia, Poland, and other East European countries (Velikanov, et al, 1994; Velikanov, et al., 2008; Demyanchuk, et al., 2011; Grundas, et al., 2011).

The results of research carried out by using XRM showed significant differences in grain endosperm cracks between common wheat varieties. Natural wetting of dry grain (below 15% of moisture content) during rainfall when wheat is standing in the field is one of the reasons of its cracking. The susceptibility of wheat grain to mechanical damage is determined by genetic factors (e.g., grain hardness), environmental effects (climatic conditions during pre-harvest period), and by the conditions of grain storage (especially excessive humidity). The combination of these properties determines the quality of grain material for industrial purposes (Grundas & Wrigley, 2004 b).

Given below are some examples of X-ray images of kernels of basic cereals with internal defects of different nature, with comments:

The kernel can acquire a fracture or a crack in the field during its ripening, due to diurnal changes in temperature and humidity, as well as in the processes of harvesting, drying and transportation of grains (Fig. 42.). Strongly expressed fractures decrease both the sowing quality of seed and the technological properties of grain (oxidation of reserve substances, reduction of allowable storage time, impossibility of obtaining good quality flour).
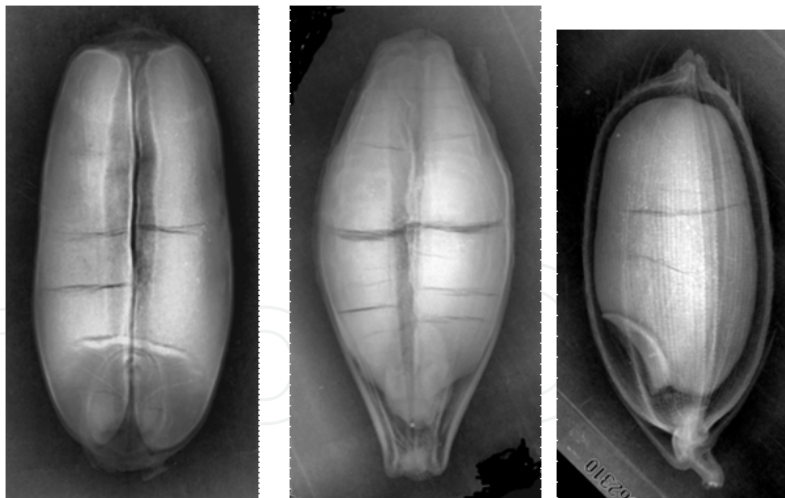

Figure 42. Endosperm cracks in wheat, barley and rice, respectively.

Complete or partial loss of the embryo (for comparison, in the far left picture - wheat kernel with a normal embryo) in kernels of any cereal (in the photo - wheat and rye) leads to non-germination of seeds, and for grain - to damage to seed coat, opening access to air and malicious agents, if that is not done on purpose, within the scope of process conditions directly before milling (Fig. 43.).
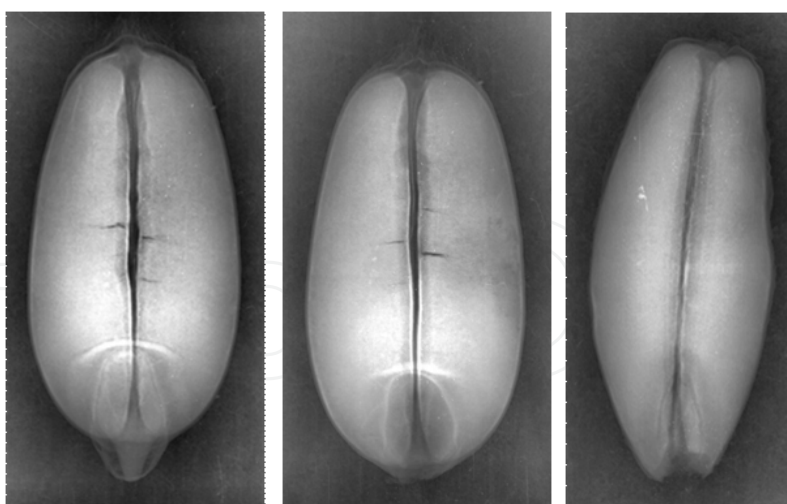
Figure 43. Complete or partial loss of the germ in wheat and rye seeds, respectively.

At high humidity and air temperature, grain can germinate in the field while still standing or in a heap, or in storage (Fig. 44). The initial stages of germination, identified on X-ray images, cannot be detected visually, but the biochemical and morphogenetic changes in kernels reduce their sowing and technological properties (Grundas, 2004; Bechtel, et al 1990). In hulled varieties even advanced internal germination can be visually observed (far right image of two kernels of oats, taken at lower magnification - in the kernel on the left the length of the germ, hidden by the seed coat, almost equal to the length of the endosperm).

Figure 44. Hidden germination of wheat, barley and oats seeds, respectively.

Cavities, eaten out by insect larvae inside the kernels, are easily detected on the images in the form of characteristic darkened areas (Fig. 45.), regardless of their size (two images of rye kernels on the left). Repeated taking of an image in a few seconds makes it possible to

identify live insects in the kernel (two images of wheat kernel on the right). The presence of insects in the grain is not allowed (Varshalovich, 1958; Nawrocka, et al, 2010; Nawrocka, et al, 2012). Selective visual analysis is unreliable. Only X-ray selection can ensure the absence of infected kernels.
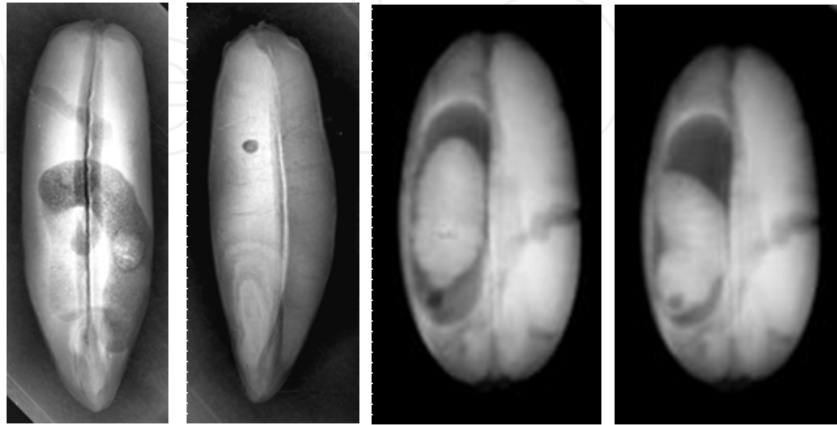

Figure 45. Infestation and populations by granary weevil (*Sitophilus granarius*) in kernels of rye, rice, and wheat, respectively.

Typical moire-effect darkened patterns on kernel images clearly indicate loosening of the endosperm tissue, resulting from the introduction of active hydrolytic enzymes of the sunn pest (Fig. 46.). The presence, in a batch of grain, of 2-3% of kernels with this kind of damage means a change of wheat grain classification from strong to weak one. Visual analysis leads to an underestimation or overestimation. X-ray analysis makes it possible to better quantify the damage.

Figure 46. Wheat and rye kernels damage by sunn pest (*Eurygaster integriceps*)

Loss of tissue density in the lateral parts of kernels and along the grooves as a result of the activity of their own enzymes at high humidity in the field, and thus also of enzymes of fungi that have evolved on the surface of kernels, with the resultant hydrolysates (Fig. 47.). Visual identification is difficult, especially in hulled kernels. X-ray analysis is accurate, it is possible to quantify the defect. Kernels with symptoms of enzyme-mycosis damage have lower sowing and technological parameters.
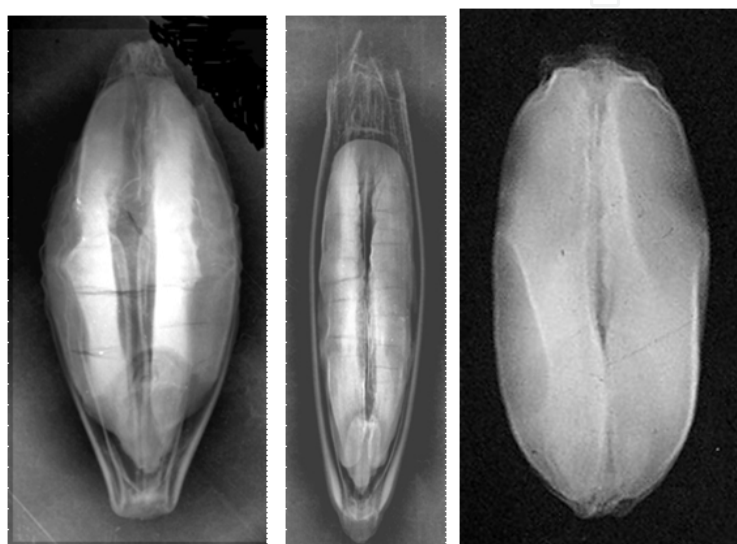

Figure 47. Mycosis-enzyme depletion of barley, oats and wheat kernels.

In spite of the considerable progress in the development of XRM, its application for the diagnosis of the quality of seeds and kernels remains extremely limited. So what is the situation with the whole issue of quality of seeds and kernels?

Great volumes of grain are evaluated for their sowing and technological properties on the basis of extremely small samples. Based on long years of research, the size of these samples (2 kg) is sufficient for a reliable assessment of the material. The risks are great, especially on the grounds of parameters with minimal thresholds of acceptability, such as the presence of insects or the percentage of kernels damaged by the sunn pest. Quantitative evaluation of kernels on the basis of those indicators may be over- or underestimated, and decisions are made on the fate of the entire batch of grain. Getting the diagnosis of the state of a batch of grain in the present conditions has no effect on changing this state in the case of its failure. Such a diagnosis can be the basis for lowering the price, the decision can be taken to treat it

with insecticides, the buyer may decide to utilise the grain, using improving additives, but the quality of the original batch has no prospects for improvement. The situation can change radically only through separation based on a critical parameter, that is the presence of insects. However, without the X-ray method such a separation is impossible, because with the current standard diagnosis the source material is destroyed.

The large number of features reliably detected by XRM already today sets this method apart from other methods. In spite of the undeniable advantages, X-ray techniques are limited by two factors. First, identification of the variety and quality assessment of grain often requires assays of proteins and other biochemical parameters of grain. Second, the techniques should allow total control (Demyanchuk, et al., 2007). Today all the prerequisites for the solution of these problems are available.

Thus, as far back as in 1953 Watson and Crick (Watson & Crick, 1953), by comparing the data from X-ray analysis with a cardboard model based on them, determined the structure of the double helix of DNA, for which they were awarded the Nobel Prize. The biochemical diagnostics required for the assessment of quality of kernels and seed can be made using the methods of X-ray structural analysis. This results from the possibility of studying the atomic structure of matter using X-ray diffraction. From the diffraction pattern one can determine the electron density distribution of matter, and based on that the kind of atoms and their arrangement. X-ray structural analysis can determine the structure of crystals, liquids, protein molecules, etc. In order to obtain images of large molecules with atomic resolution rays with shorter wavelengths are applied, i.e., hard X-rays rather than soft.

The solution of the second problem requires rapid analysis of large volumes of grain material. Usually, to ensure the sharpness of images in X-ray filming, the object is translucent only in the period of exposure. For this purpose, the control grid of X-ray tube X-ray machine is fed from the switch current pulses associated with the mechanism of filming apparatus. In this way, by using X-ray tube with cold emission, times of exposure and 10^{-7}s at a frequency of 100 frames per second can be achieved. Currently achievable filming rates are from a few thousand to 100 thousand frames per second, at exposure times of up to 15ns. Adaptation of these techniques of diagnostics to the needs of the selection/separation of kernels will almost completely eliminate poor-quality material. The transition to the industrial application of X-ray separation of grain, from a few kilograms per hour up to several tons per hour, will initiate a review of the whole range of concepts (Demyanchuk, et al., 2007). To address the issues of arbitration it is not enough to say that we see indications of quality, variety or defects. The limit values of parameters should be determined quantitatively and validated by specifically developed regulations. This means that the current problems of X-ray application require a radical revision of existing standards of quality of cereal crops. This will allow the move to full control of parameters of seed variety and kernel quality. This will require the mandatory inclusion of elements of rapid selection of seeds into systems of X-ray diagnostics.

Thus, the X-ray techniques have the potential of becoming a universal method of diagnosing and bringing the quality of original batches of grain to a high status.

8. Conclusions

1. The study showed that using X-ray images with direct X-ray magnification of 2-10 times and computer image analysis the variety-specific morphological parameters of cereal grains can be successfully determined.

2. The algorithm by which the plant is running self-building genome in ontogeny on the principles of complex systems is presented. Its high convergence with the experimental data gives the basis for the application of this approach to the consideration of the morphological characteristics of kernels as a tool for the identification of their morphological type and variety.

3. To identify the kernel, besides three dimensions, it is proposed to determine, by X-ray, the contour of kernels (seeds) in at least two projections. It is advisable to plan a continuation of the research with a collection of cereal cultures to create a database for the identification of kernels and for decision making on the basis of X-ray selection.

4. It is proposed to use the mass-scale X-ray separation of kernels for their selection on the basis of varietal and production characteristics, as reflected in their morphology, and for the elimination of contaminants, including kernels with internal defects, with a view to a permanent renewal and maintenance of resources of industrial high-quality varietal seed.

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