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Currently we continue our research with four crops: winter wheat, winter triticale, winter hull-less and spring hull-less barley (slide 2).

My research experience has been started since 1972 in cytology and cytogenetics (slide 3). My PhD was aimed on cytogenetic study of monomeric gluten proteins of wheat – gliadins. At the beginning of our research of gliadins it was known just about the location of gliadin-coding loci in variety Chinese Spring (Wrigley, Shepherd, 1973). Their findings were obtained on the base of study of Chinese Spring (ChS) nullisomic-tetrasomic strains. At that time there *was no information available about what is the genetic basis of the gliadin proteins intervarietal diversity* that is in fact really huge. Gliadin electrophoretic patterns (one-D or particularly 2-D PAGE) are specific for each individual wheat variety. It is accepted that gliadin is one of the most heterogeneous genetic systems in wheat.

We started our research with monosomic analysis of gliadins. Monosomic analysis allows analyzing the genetic segregation among progenies of selfed normal disomic as well as 21 (each wheat chromosome) monosomic plants. Anomalous from disomic (3:1 or 1:2:1 or 1:1:1:1) segregation in the critical chromosome monosomic plant progenies allows determining of the chromosome that carries the target genetic factor.

While studying genetic segregation in progenies of disomic plants we came to the conclusion that *gliadins are inherited as six independent groups each presented with clusters of tightly linked electrophoretic bands*. Such a cluster we have identified as a discrete genetic unit (called as "block") of gliadin inheritance (slides 4,5).

On the base of our research we came to the conclusion that:

1) Each of six *Gli*-coding loci on chromosomes of homoeologous group 1 and 6 of common wheat are multiallelic;

2) *Multiallelism of the Gli-coding loci is the genetic background of gliadin proteins genetic diversity in wheat*. Latter the "block" was renamed as "allele".

The summary of our research for the wheat etalon variety Bezostaya 1 is presented on the slide 6.

The next step of our research was to get mapping of the *Gli*-loci on the corresponding chromosome arms. The first such research has been done with the use of telosomic analysis since at that time (the end of 1970th) there were no molecular markers available. On the slides 7,8,9 presented the results of our research with telosomic mapping of the locus *Gli-B1* on the short arm of chromosome 1B at a distance of 42% of crossing-over from chromosome

centromere (published in 1978). *These were pioneer research*. The similar investigations were performed in Cambridge, UK, and published just two years latter. Today we know the location for all of the *Gli-Glu* loci, but we proud that we were first who performed of the monosomic analysis of gliadins and who has mapped the locus *Gli-B1* (slide 10).

The important objectives of our research were wheat-rye translocations 1RS.1BL and 1RS.1AL broadly spread among wheat cultivars (slide 11). The translocations carry the rye locus *Sec-1* coding for rye storage protein secalins are known as highly detrimental for wheat bread-making quality. Several cytogeneticists made attempts to replace in translocations the rye locus *Sec-1* with wheat locus *Gli-B1* thereby to eliminate the defect for wheat quality.

In 1994 I had a chance to work with Prof. Adam Lukaszewski at UCR (Univ. of California, Riverside, USA). He was one of those who have successfully made chromosome engineering replacement of *Sec-1* for *Gli-B1* in the short arm of rye translocation 1RS.1BL. The engineered spring 1RSm.1BL translocatin is a triple recombinant possessing in 1RS rye chromatin two inserts of wheat chromosome arm 1BS that cause the risk of 1RSm disassembling in case of intervarietal crosses. By the use in crosses of intermediate telosomic 1BL we have modified of the engineered 1RSm.1BL translocation to winter growth habit simultaneously recombined 1BL with a strong for bread-making allele *Glu-B1al* (77+8) instead of the original *Glu-B117+18*. The modified 1RSm.1BL(*al*) translocation now involved into the breeding program of PBGI (slide 12).

In 1983 I had a chance to work with Prof. Donald Kasarda (USDA ARS, WRRC in California, Albany, USA) were some important research of wheat proteins have been performed. Two-dimensional A-PAGE separation of gliadin in wheat cultivars with 1RS.1BL translocation showed the complexity of rye secalins to be synthesized in wheat genome (slide 13). In Prof. Kasarda's lab we have isolated several of individual secalins from var. Kavkaz by the use of preparative ion-exange chromatography (slide 14). The individual secalins were purified and subjected for automatic N-terminal amino acid sequencing. On the base of N-terminal sequence the secalins were divided into two groups differing in S/L single point amino acid substitution (slide 15).

The reason *why the secalins cause a defect of wheat quality for a long time was unexplained*. To understand the negative effect of secalins on wheat bread-making a very simple experiment has been performed. We compared the 2-D electrophoresis protein patterns of wheat cultivar with 1RS.1BL translocation. The flour sample was extracted in two variants first with 70% ethanol and second with DM water alone. We have found that *secalins differing from wheat gliadins are very well dissolvable in both alcohol and water*. Unlike wheat gliadin difficult to

dissolve in water *secalins are readily dissolvable in water alone*. *Rye secalin do not form the insoluble gluten protein aggregates with wheat proteins and this is a reason why they cause the wheat quality defect* (slide 16).

The wheat A-gliadins were also individually isolated, purified and subjected to N-terminal amino acid sequencing. The single point amino acid substitutions seems to be the main reason causes genetic diversity of A-gliadin proteins related to celiac disease (slide 17).

The N-terminal molecular structure of two allelic variants *Gli-D1f* and *Gli-D1g* of two different wheat cultivars have been studied. The two individual gliadin ω-1 and ω-2 coding for the same locus *Gli-D1* were completely different in the N-terminal sequence chain. At the same time they were similar to some individual gliadins of the *Gli-D1g* allele in the manner as showed on the slide 18. The N-terminal amino acid sequences of gliadins coding by the locus *Gli-D1* were different from that coding by the locus *Gli-B1*. The sequencing data suggest a complexity of *Gli*-coding loci genetic divergence.

The study of tryptic peptides of reduced and carboxymethylated (iodoacetic acid or iodoacetamide) α-gliadins allowed us to develop a simple test for detection of peptides contained with SH-groups of cysteine amino acid very important for BM quality (slide 19).

The other protein system in wheat showing considerable genetic polymorphism is the seed amylases complex. We have studied the intervarietal diversity of α- and β-amylases in the Ukrainian wheat varieties, amylases inheritance as well as chromosome location of α- Amy (homeologous group 6 and 7) and β-Amy loci (homoeologous group 4).

The large and very important project in our research is the interspecific crosses as the powerful instrument to enhance the useful for wheat breeding genetic variability. Our interspecific crosses focused on the D genome chromosome manipulations as a key genome of the common wheat. As the donors of the D genome possessing valuable for breeding characters we use mainly two landraces such as local indigenous Ae. *cylindrica* (genomic formula CCDD, 2n=4x=28, in direct crosses with common wheat) and Ae. *tauschii* (genomic formula DD, 2n=2x=14, we use in form of hexaploid synthetics with genomic formula AABBDD, 2n=6x=42) (slides 21,22 (slides 21,22).

By the use of interspecific crosses we transferred from landraces to common wheat an array of the valuable for breeding characters such as new exotic alleles of the *Glu-D1* locus (slides 23,24), Unfortunately, rare of them appeared to be positive for bread-making. One of the exotic *Glu-D1* alleles derived from Ae. *cylindrica* has been found as positive for both bread-making and grain hardness (takes as much as 9% increase of the total hardness variability) (slide 25).

We have transferred from gout grass Ae. *tauschii* to common wheat chromosome 5DS the exotic allele of the gene *Ha* (hardness) coding for wheat starch granules protein friabilin. This allele determines the over-expression of friabilin with electrophoretic mobility slightly faster than normal friabilin in common wheat. The new exotic allele of the gene *Ha* determines the extra-soft common wheat endosperm texture (slide 26). On the base of the new *Ha* allele first in Ukraine extra-soft biscuit variety Oksana was developed and listed in 2007 (author A. Rybalka) (slide 27).

In the interspecific crosses pre-breeding program the tolerant to drought advanced wheat breeding lines with grain yield potential up to 12 t/ha were developed and applied to wheat state trials.

The white wheat becoming to be more popular in the world due to: a/ superior to red wheat white flour extraction; b/ its health promotion due to increased bran share in the white wheat-based baking products (slide 28). In Ukraine today 100% of bread wheat cultivars are red grained. We were first who started the white wheat pre-breeding program in Ukraine. We develop the white common wheat breeding material on the base of both soft and hard endosperm texture.

In the white wheat pre-breeding program we use the molecular markers for detection of the *R* gene alleles determining of the red caryopsis color (slide 29) as well as *Ppo* gene alleles responsible for the enzyme seed polyphenoloxydase activity, tightly linked with dough darkening during the baking products processing (slide 30). The white wheat pre-breeding program resulted in development of the white extra-soft biscuit wheat variety Byljava listed in 2014 (author A. Rybalka) (slide 31).

Our pre-breeding waxy wheat program aimed on the development of the advanced breeding material of waxy wheat for distilling end-use varieties as well as for development of feed cultivars. For efficient selection of waxy and partial waxy wheat genotypes we use the molecular markers for detection of the *Wx-A1*, *Wx-B1* and *Wx-D1* genes suppressing of the GBSS synthesis in the starch granules (slide 32). The waxy wheat pre-breeding program is resulted in the first in Ukraine waxy wheat variety Sophia listed in 2014 (author A.Rybalka) (slide 33).

The alternative renewable fuel and edible ethanol are still important industrial demand in the world. The most perspective starchy crops in Ukraine for industrial end-use are as follows: winter wheat, winter triticale, corn and sorghum (slides 34,35). We compared the efficiency of starch-to-ethanol transformation of several varietal groups of those crops. Corn and sorghum were found as superior if compared to wheat and triticale in term of ethanol yield (l/1000 kg) per unit of the dry feedstock (slide 36). Nevertheless, efficiency of starch-to-ethanol

transformation expressed as ethanol yield in l/1000 kg of starch was higher in wheat and triticale (slide 37). The DDGS (distiller's dried grains with solubles) yield (kg/1000 kg of dry feedstock) was found not considerably different between four starchy crops (slide 38), but sorghum was the best in protein content (%) per DDGS unit if compared with other crops (slide 39).

We consider triticale as the best starchy crop for distilling end-use in Ukraine because of the following reasons: a/ some cultivars of triticale are not much less or equal in fermentability with corn and sorghum; b/ triticale crop in Ukraine is indefinite in the technological end-use; c/ triticale to be used for industrial distilling is free from possible negative social reaction.

In the triticale pre-breeding program we use (5B)5D chromosome substitution line (originated by A. Lukaszewski) allows to get the wheat-rye chromosome recombinations resulting in a wide genetic variability. We also develop the triticale waxy since waxy starch is a factor positively correlated with grain fermentability (slide 40). The triticale cultivars are considerably different in the ethanol yield (slide 41) as well as in performance of starch-to-ethanol transformation (slide 42) and crude protein content in DDGS (slide 43). We pay attention on triticale starch granulometry mainly focusing on the small size of starch granules rates (B and C types) expressing in the larger active starch granules surface to be attacked by enzymes (slide 44).

In the fermentation process, the grain proteins hydrolyzed to the small peptides migrating out of the gel during electrophoresis (slide 45). It means that DDGS proteins should be well accessible for the animal intestinal assimilation.

We develop a large healthy grain research program comprising several independent projects (slide 46).

The nutrition related research of the recent years characterizing the modern wheat varieties as unhealthy first due to at least several reasons listed in the slide 47. One of the nutrition related pre-breeding project is development of the black grained wheat on the base of Chinese source Dong 10. This project resulted in development of the black wheat variety Chornobrova listed in 2014 (author A.Rybalka). This variety characterized with elevated grain protein content, better protein solubility in 50% 1-propanol as well as improved mineral balance (slide 48). We are close to develop the variety of spelt wheat with black caryopsis (slide 49). We have found that wheat genotypes with soft endosperm texture are generally more suitable for the wheat grass juice production (slide 50).

The high wheat grain protein status has been for years as a priority of breeding related research. However, protein content as a breeding character is highly variable, very complicated for genetic manipulation and improvement due to its multigenic control as well as considerable dependence of climate and

agronomic influences. The strain of wheat T. *turgidum* var. *dicoccoides* from Israel has appeared to be as a donor of the QTL-factor *Gpc-B1* tightly linked with protein and some minerals Fe and Zn in wheat grain enhancement (A. Distelfeld et al., 2006). We use in crosses the substitution line 6B(6B) (obtained from Prof. Dubcowski, UCD, USA) possessed with 6B chromosome (carries *Gpc-B1*) of T. *turgidum* var. *dicoccoides*. Molecular markers tightly linked with *Gpc-B1* we use to facilitate the control of this factor in hybrid populations (slide 51).

The high GI (glycemic index) as well as negative postprandial glycemic and insulinemic responces of the modern wheat baking products seem to be a nutritional factor linked with metabolic syndrome and diabetes mellitus type 2. One of the promising ways to ameliorate the healthy status of the modern wheat cultivars is to enhance the RS (resistant starch) content with properties similar to dietary fiber. It is possible to achieve this goal by changing the amylose/amylopectin ratio to increase the amylose less susceptible to amylolysis than amylopectin. The enzyme *SBEIIa* is one of the amylopectin branching enzymes being genetically blocked may cause increase the amylose content. We use in crosses with bread wheat varieties the durum wheat line (originated by Univ. Tuscia, Italy) with genetically silenced *SBEIIa* contained with up to 70% of amylose. For *SBEIIa* and *bar* genes detection we use molecular markers facilitating the hybrid populations screening (slide 52).

In the healthy grain program the largest share takes the pre-breeding research of spring and winter hull-less barley of food end-use (slide 53). It is widely recognizable that *barley is a renewed healthy food product highly important in preventive health care against the cardiovascular, diabetes type 2 and cancer diseases*. We conduct the largest in Ukraine pre-breeding program of hull-less barley for food end-use (slide 54). One of the most important objectives in the hull-less barley program we pay particular attention on seed and seed germ morphology (slide 55). We mainly focus on the two-rowed awned and awnless barley head morphotypes (slide 55) as well as on the grain color pigmentation tightly linked with particular antioxidants important for the health care.

The hull-less healthy grain program has already been resulted in first in Ukraine high protein hull-less spring two-rowed barley cultivar Achilles listed in 2014 (author A. Rybalka) for food end-use (slide 58).

On the base of our healthy grain research program we have developed the “healthy bowel” snack composed with porridge made with naked barley grouts, black wheat bran and flax flour. The porridge harmonized in taste and health benefits (fruit fiber, antioxidants, minerals, vitamins) with the fruit smoothie mixed with grapefruit, kiwi, banana and apple (author A. Rybalka) (slides 59,60). There are two volunteers A. Rybalka and his wife Oksana have been experimented on

themselves the snack in everyday manner consumption during the two last years being in excellent physical and healthy state.

Being in chronic budget shortage we are forced to develop the self made laboratory equipment for electrophoresis as well as for wheat quality evaluation in the primary breeding nurseries of wheat breeding (slide 61). The major feature of the wheat breeding samples grown in Odessa region is permanent grain damage by the sun pest *Eurygaster integriceps*. Just take a look on the completely destroyed Mixolab profile of the extra-strong wheat variety Kujalnik damaged in the increased percentage rate by sun pest (slide 62). As a consequence we need the lab protocols for wheat samples quality evaluation with displayed (for bread-making) or blocked (for breeding quality potential) salivary enzymes of sun pest incorporated in the wheat grain. I these cases the standard quality evaluation procedures are failed to give not objective assessment.

We have developed the protocol and semi-automatic microprocessor managed device for breeding samples quality assessment, called as SDS-30. The device has already been in use for 12 years completely satisfying of the wheat breeders with proper quality evaluation. The device is introduced in the practice of the wheat breeding quality evaluation in five the largest wheat breeding centers in Ukraine (slide 63).

We have also developed the laboratory protocols and equipment for mini-gel-SDS-electrophoresis of wheat HMW-glutenins (slides 64,65) and gliadins (slide 66), normal gel format HMW- and LMW-glutenuns (slide 67). The protocols were development while working in the Angevin-Nickerson lab. I am very grateful the Angevin-Nickerson team for support of my stay and my research in the Chartainvilliers (1996-2006).

We are also actively involved into the seed purity testing in the Plant Breeding and Genetics Institute for seeds of wheat, corn inbred lines and F1 hybrids (slide 68), barley varieties (slide 69), sunflower F1 hybrids (slide 70). For the last five years we cooperate with Limagrain group in Ukraine testing the seed purity of Limagrain's corn and sunflower F1 hybrids.

We deal with some transgenic material of wheat and barley derived from USDA ARS WRRC USA (Ann Blechl). In our crosses experiments we use wheat lines with over-expression of HMW-GS *Glu-D1x5* and *Glu-D1y10* to study their dosage effects on bread-making quality (slides 71,72). In barley crosses we use the transgenes with HMW-GS of wheat *Glu-D1x5*, *Glu-D1y10* and both *Glu-D1x5+y10* integrated into barley genome from wheat. The purpose of this experimentation is to improve the mixing properties of the barley flour in composite dough obtained by blending of barley and wheat flours. For detection of

wheat gluten-coding genes in barley segregating populations we use molecular markers (slide 73).

Finally, A. Rybalka is an author of 220 publications including two book-monographs 1) Wheat quality and its amelioration (550 pages), 2011, Logos, Kyiv (in Ukrainian), 2) Barley as a functional food product (560 pages), 2016, Logos, Kyiv, 2016. I am an author (co-author) of 12 wheat and barley varieties, two corn and sorghum hybrids listed in Ukraine. I am a member of the New York Academy of Sciences since 1996.