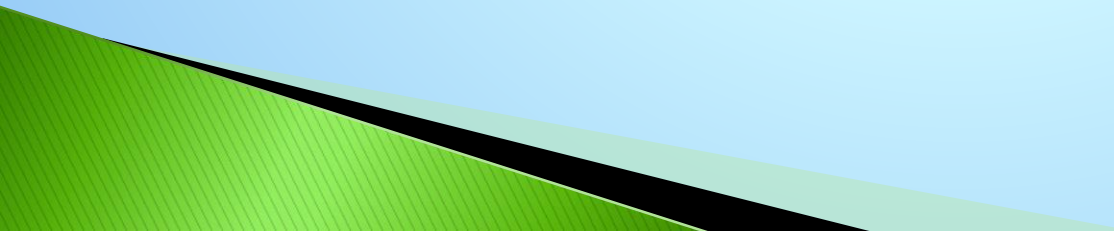


Alexander Rybalka

Plant Breeding and Genetics Institute,
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Institute of Plant Physiology and Genetics,
NAS, Kyiv, Ukraine



The research are focused on four crops:

- winter wheat

-winter triticale

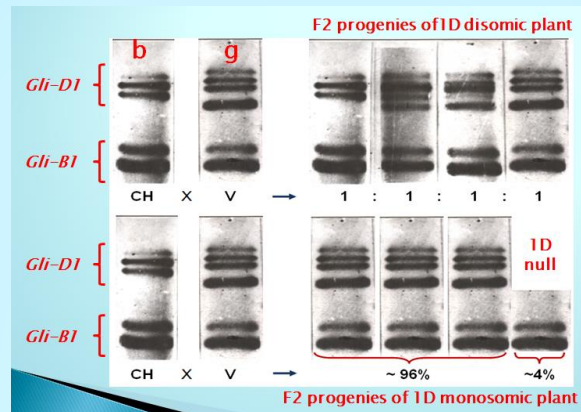
-winter hull-less barley

spring hull-less barley

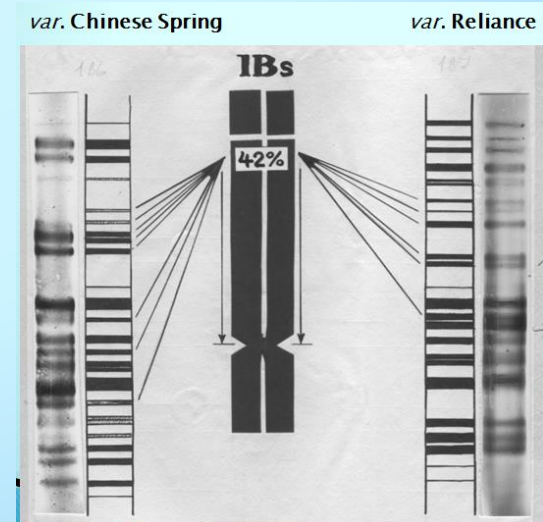
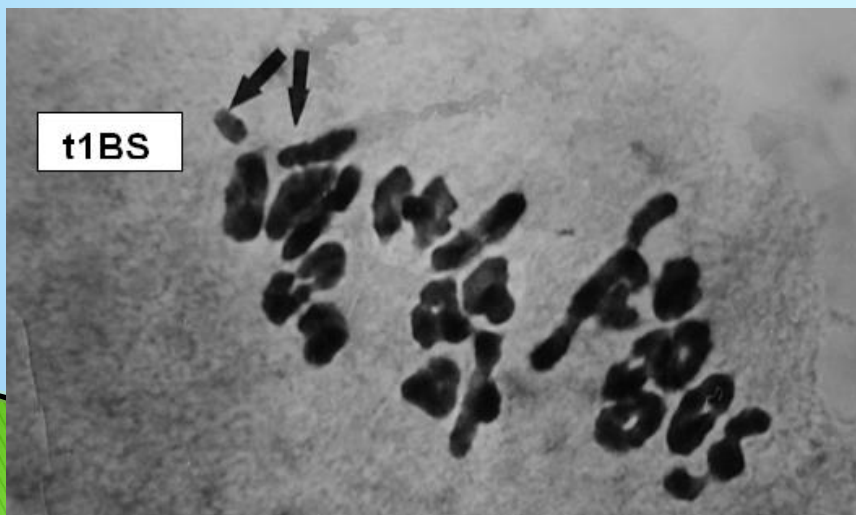
CYTOGENETIC RESEARCH of WHEAT GLUTEN PROTEINS

(the world's priority research)

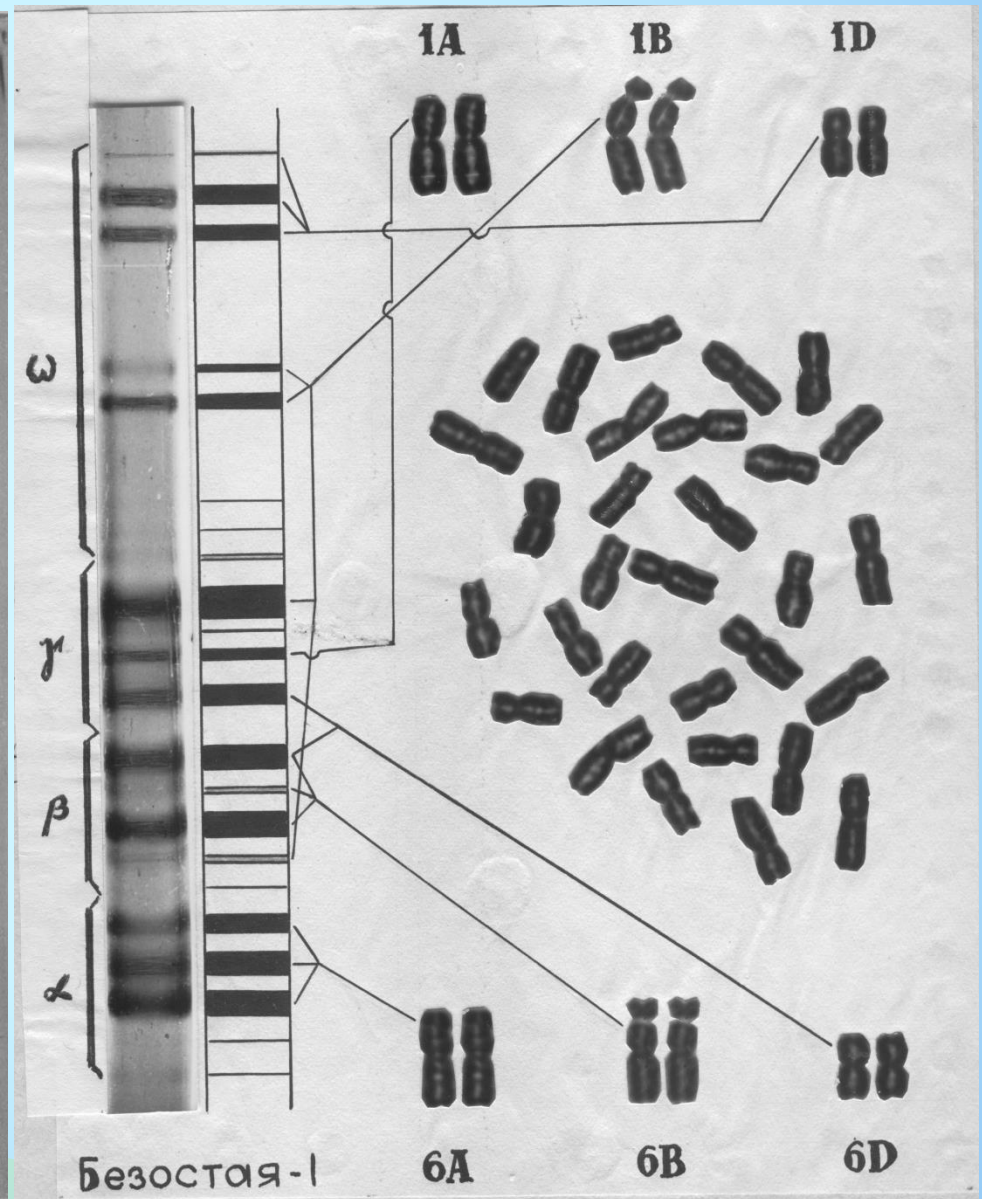
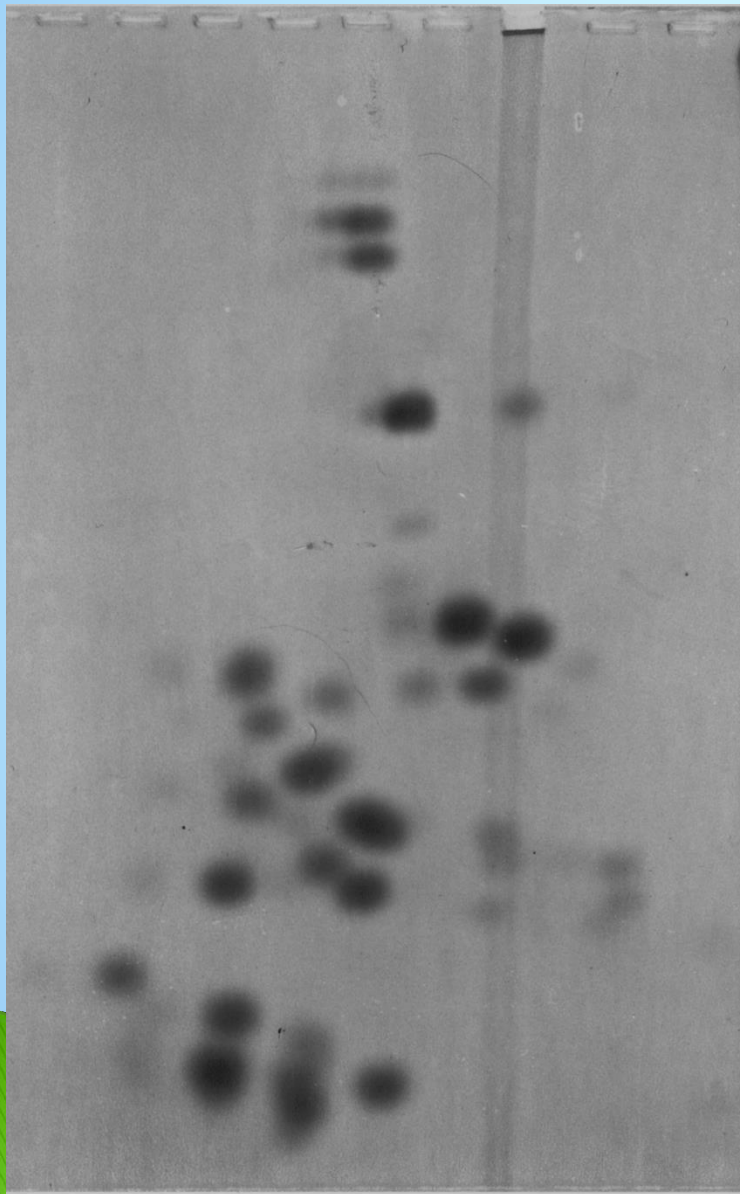
- Monosomic analysis of wheat gluten protein gliadins



- Telosomic mapping of wheat *Gli*-coding locus



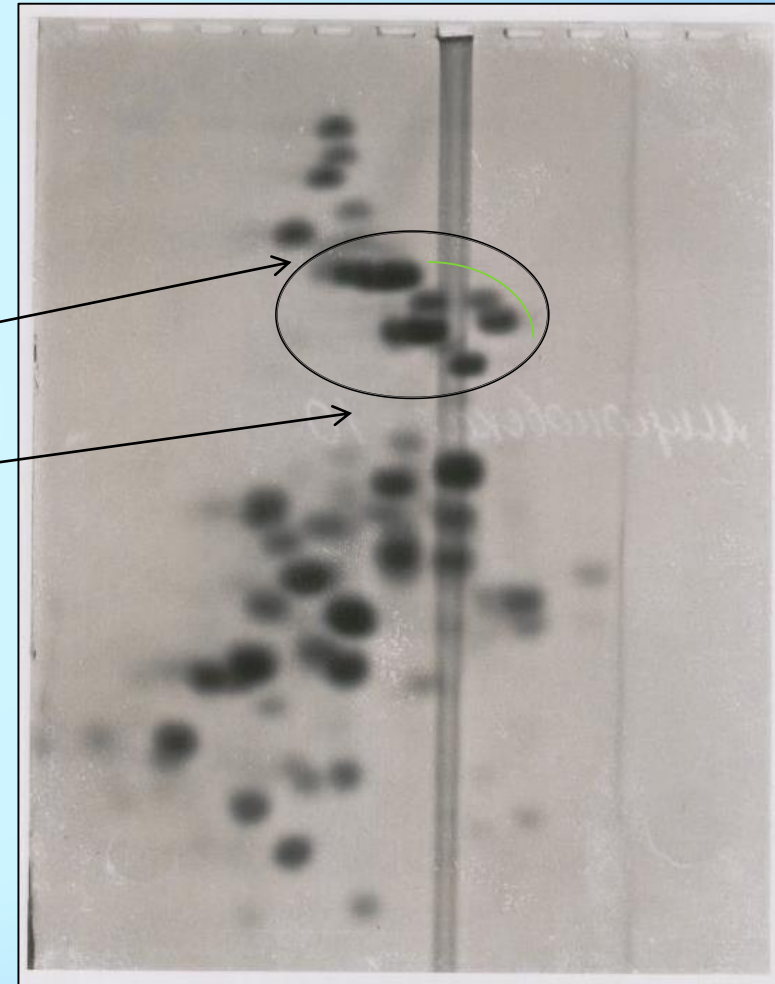
Gli-1 and *Gli-2* loci on chromosomes of homoeologous group 1 and 6 in bread wheat var. Bezostaja-1 (the world's priority research)



Rye secalin (detrimental for quality) in wheat

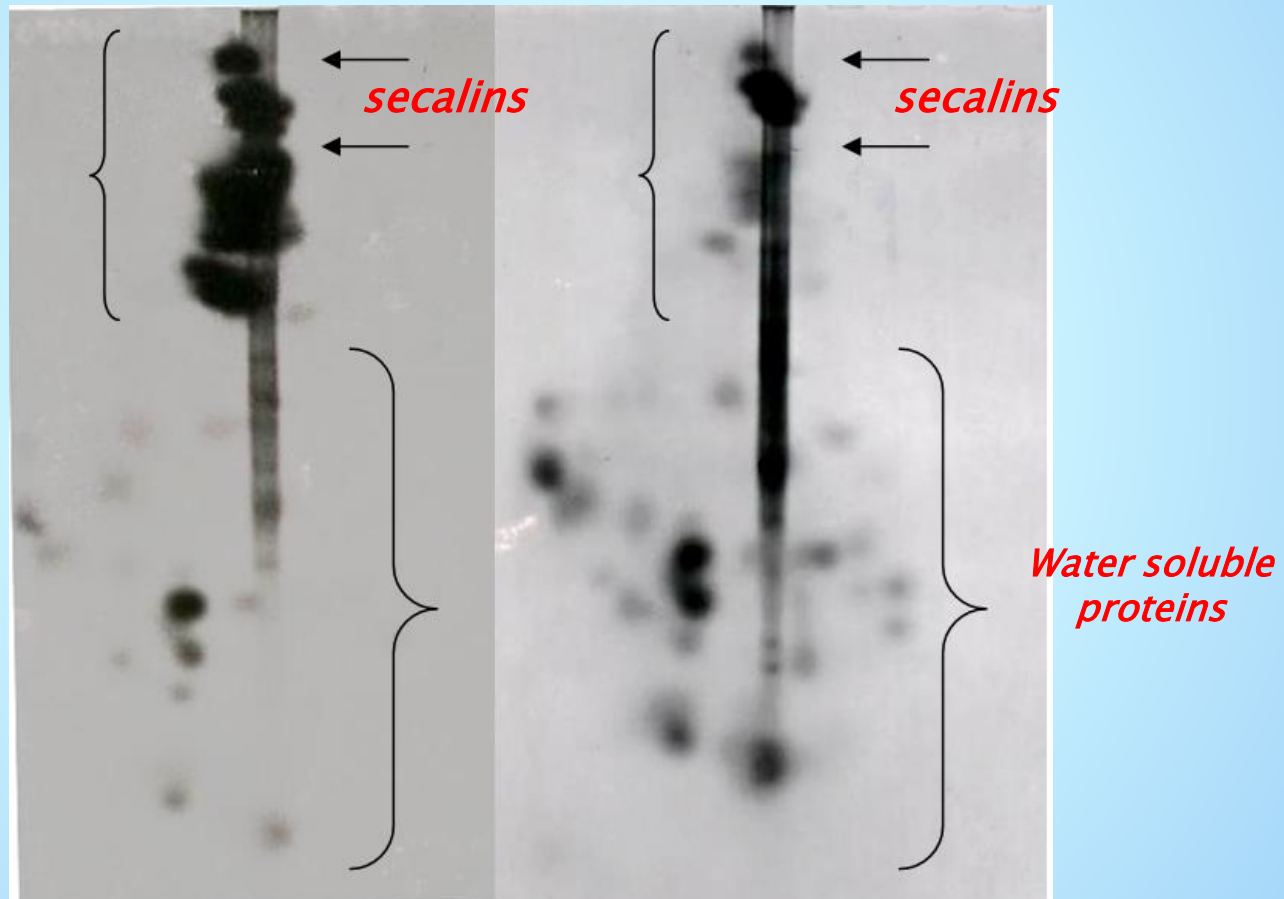
1D-A-PAGE

2D-PAGE



Secalins are soluble in water – the reason why they cause the bread-making defect (the world's priority res.)

Kavkaz – wheat variety with centric wheat-rye 1RS.1BL translocation

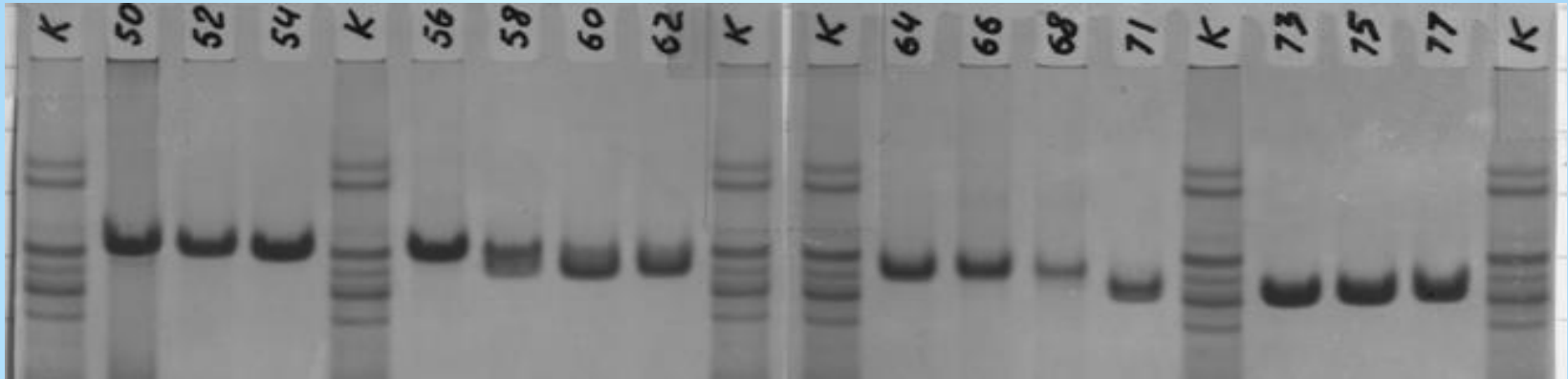


Ethanol extraction

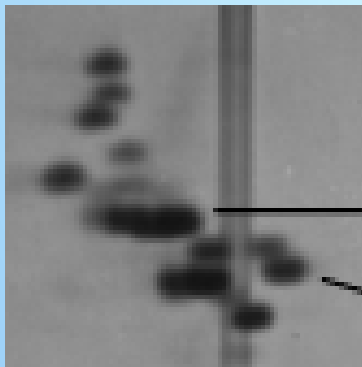
Water extraction

Rye secalin N-terminal sequencing

Individual secalin preparative isolation / purification

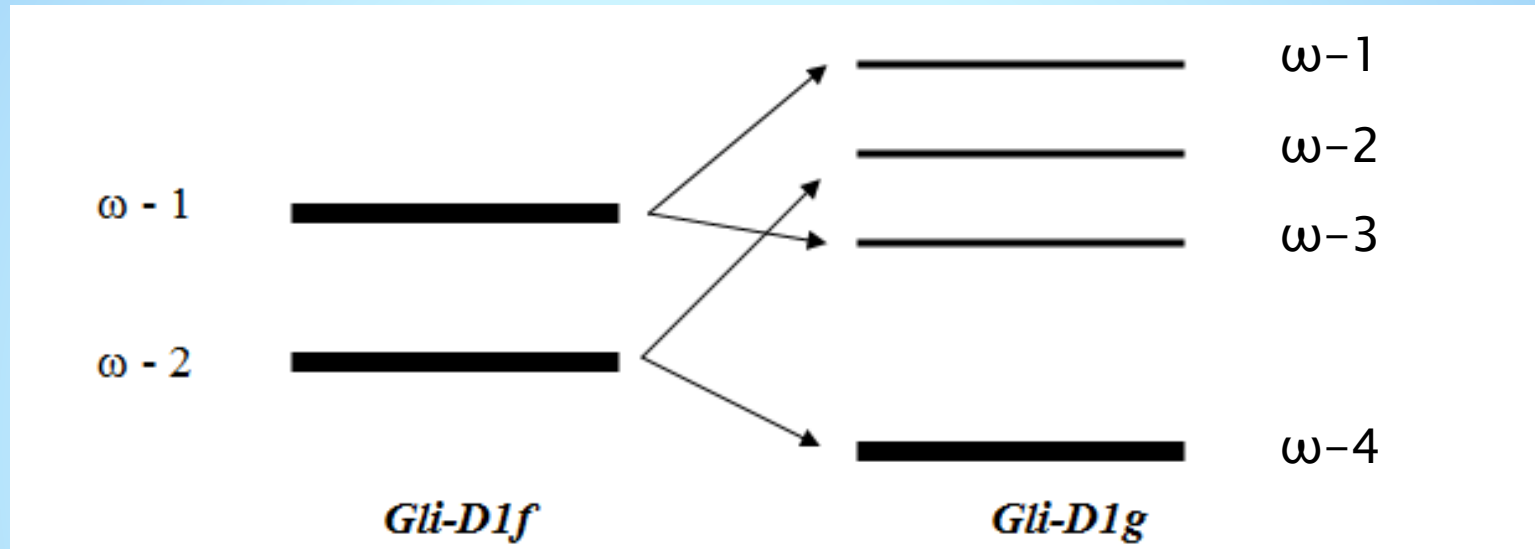


Individual secalin N-terminal sequencing



1	5	10	15
NH ₂ -R-Q-L-N-P-S-E-Q-E-L-Q-S-P-Q-Q-P-V.			
1	5	10	15
NH ₂ -R-Q-L-N-P- L -E-Q-E-L-Q-S-P-Q-Q-P-V.			

N-terminal molecular structure of two alleles *Gli-D1f* and *Gli-D1g* for gliadin-coding locus *Gli-D1* in comparison with locus *Gli-B1*



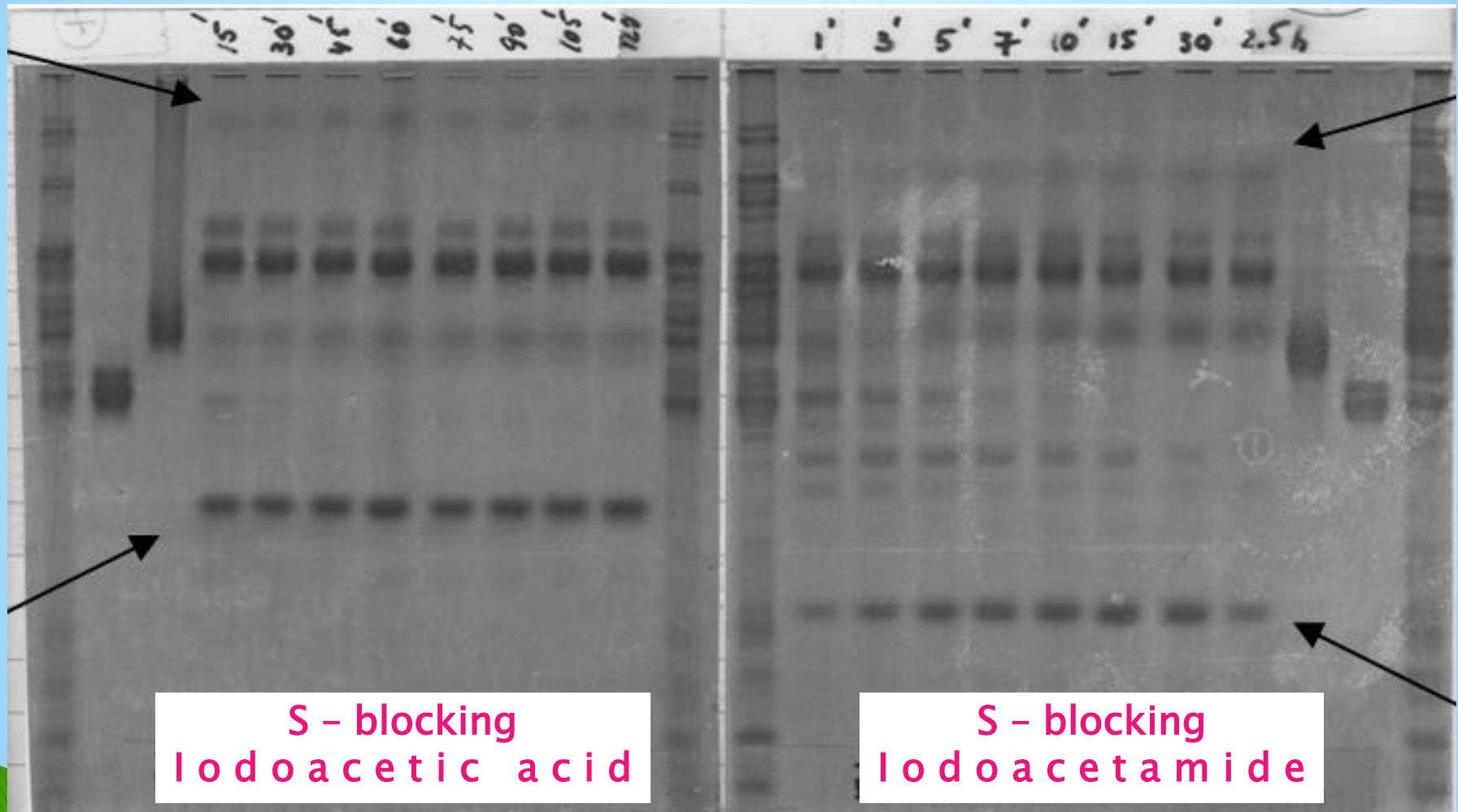
Locus *Gli-D1*

	1	5	10	15
$\omega-1$	NH ₂ -K-E-L-Q-S-P-Q-Q-S-F-S-H-Q-Q-Q-P-F			
$\omega-2$	NH ₂ -A-R-E-L-N-P-S-N-K-E-L-Q-S-P-Q-Q-S			

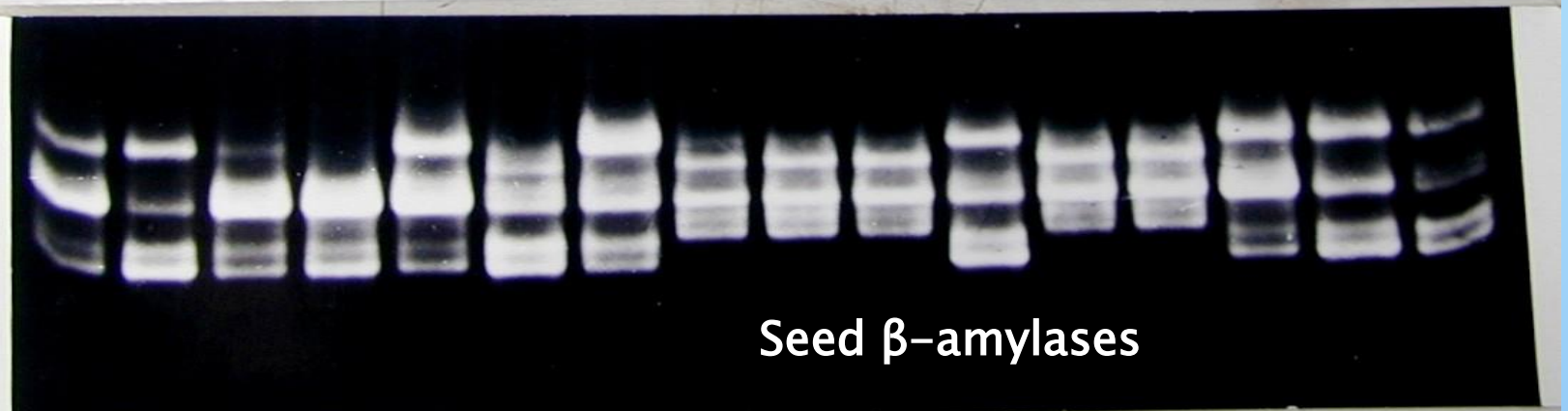
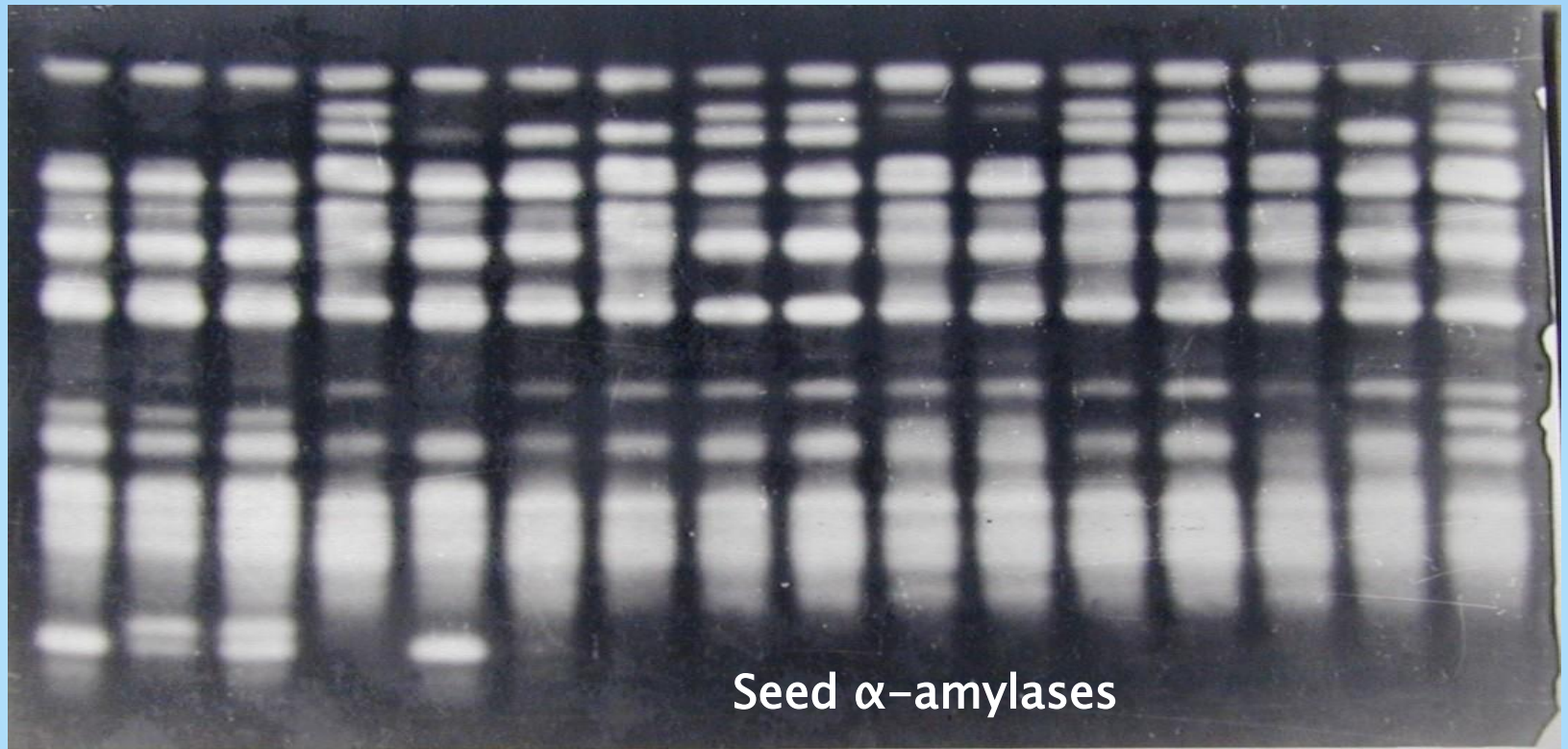
Locus *Gli-B1*

	1	5	10
	NH ₂ -S-R-L-L-S-P-R-G-K-E-L-H-T-		

Detection of cysteine SH-group in reduced and carboxymethylated wheat α -gliadin tryptic peptides (USDA-ARS, WRRRC, 1982)



Genetics of α - and β -amylases in wheat



Interspecific crosses pre-breeding program focused on the **D-genome** manipulations as a **key genome** of bread wheat

Ae.cylindrica (CCDD) $2n=4x=28$



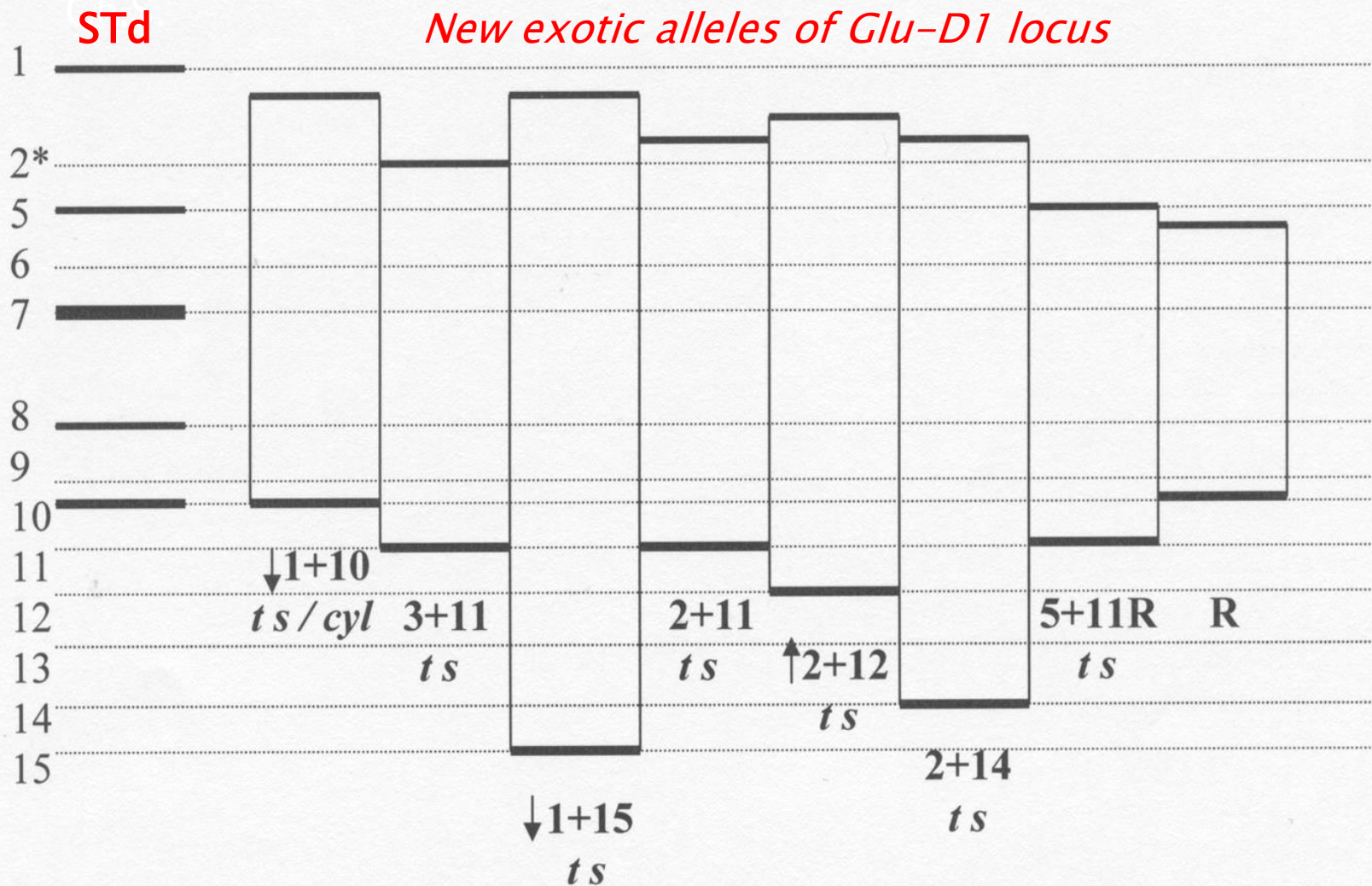
Indigenous species

Ae.tauschii (DD) $2n=2x=14$



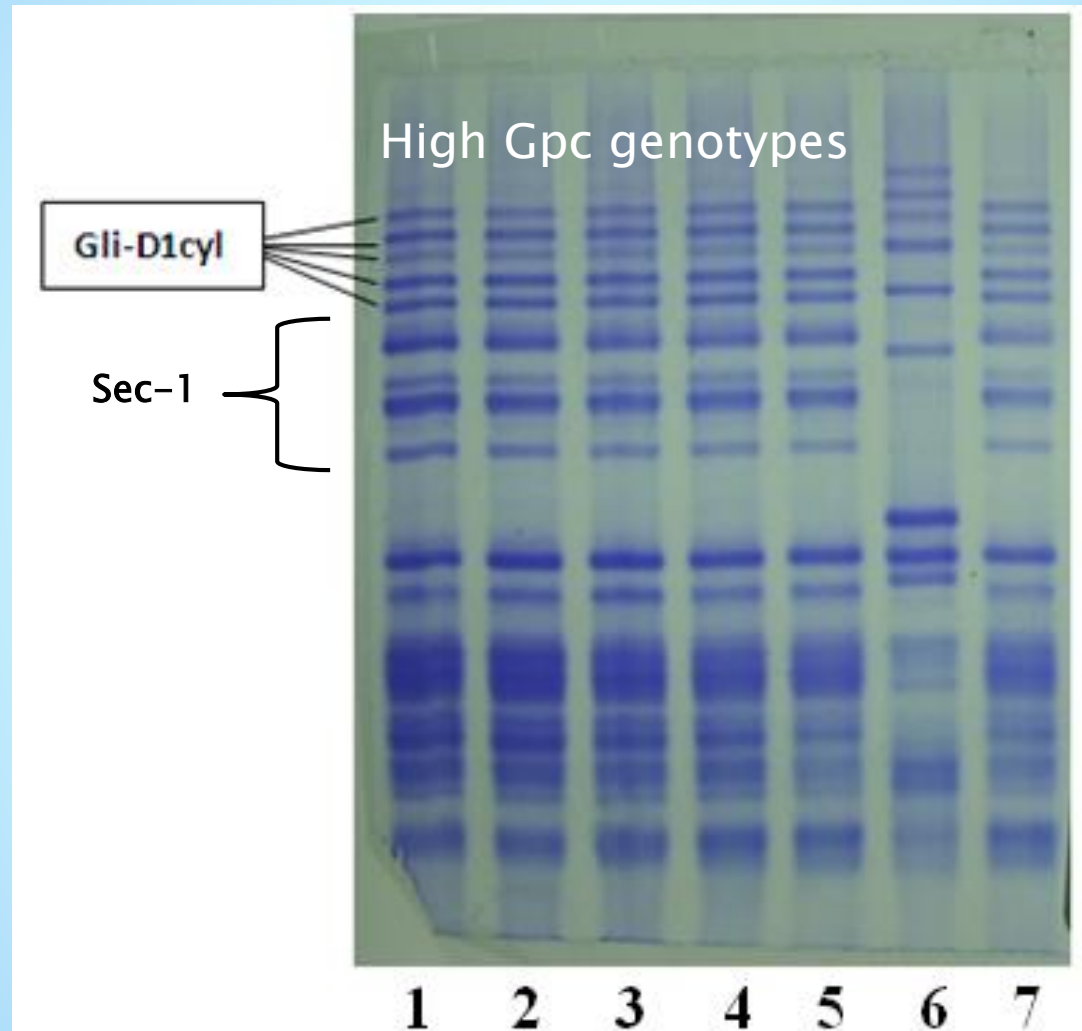
Synthetics *AABBDD*, $2n=6x=42$

New exotic alleles of *Glu-D1* locus transferred to wheat from wild relatives



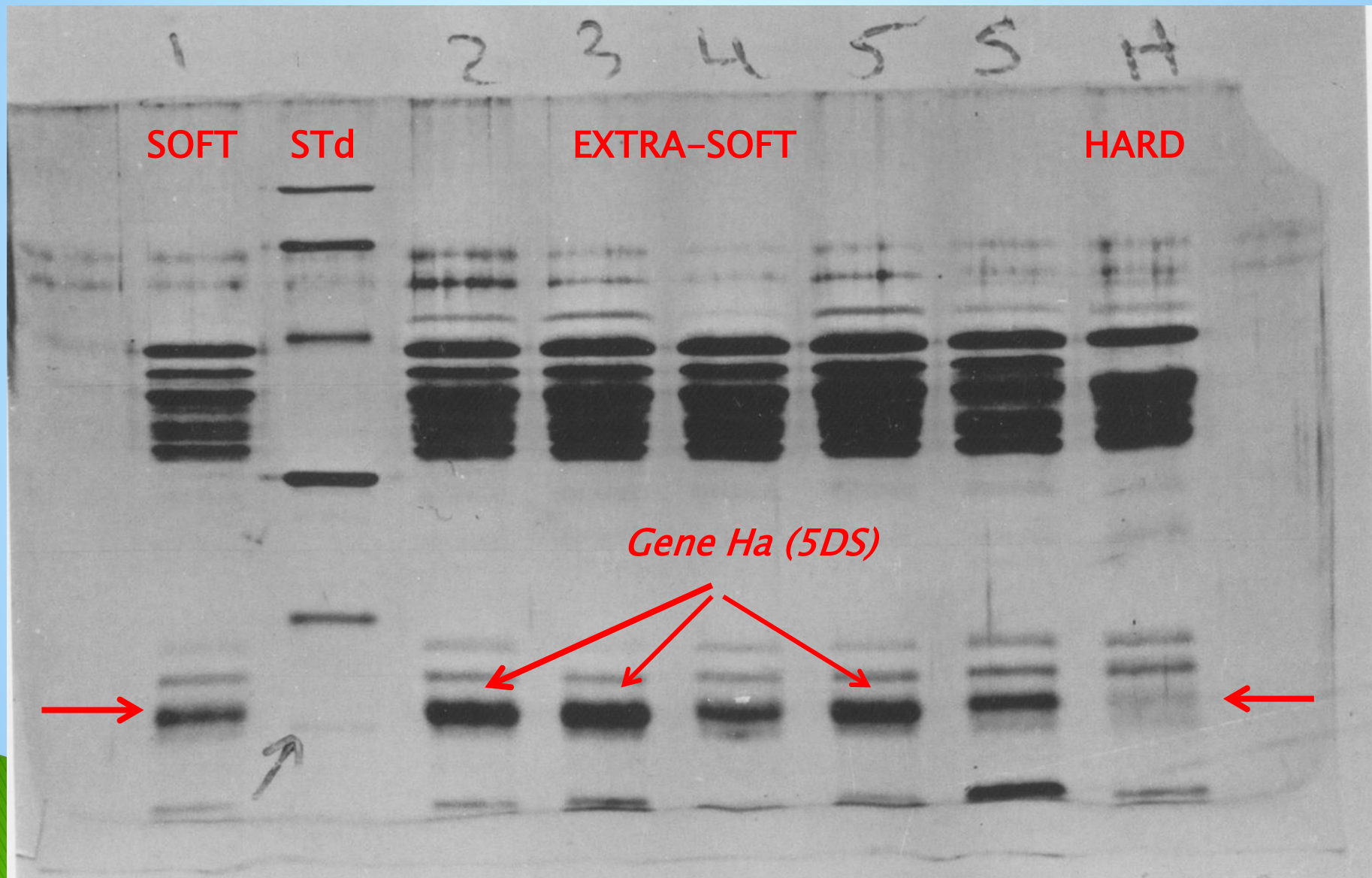
ts - *Ae. tauschii*; *ven* - *Ae. ventricosa*; *var* - *Ae. variabilis*; *R* - recombinant

Locus *Gli-D1cyl* transferred to wheat from *Ae. cylindrica* with positive effect on BM-quality and endosperm texture (**elevated hardness**)

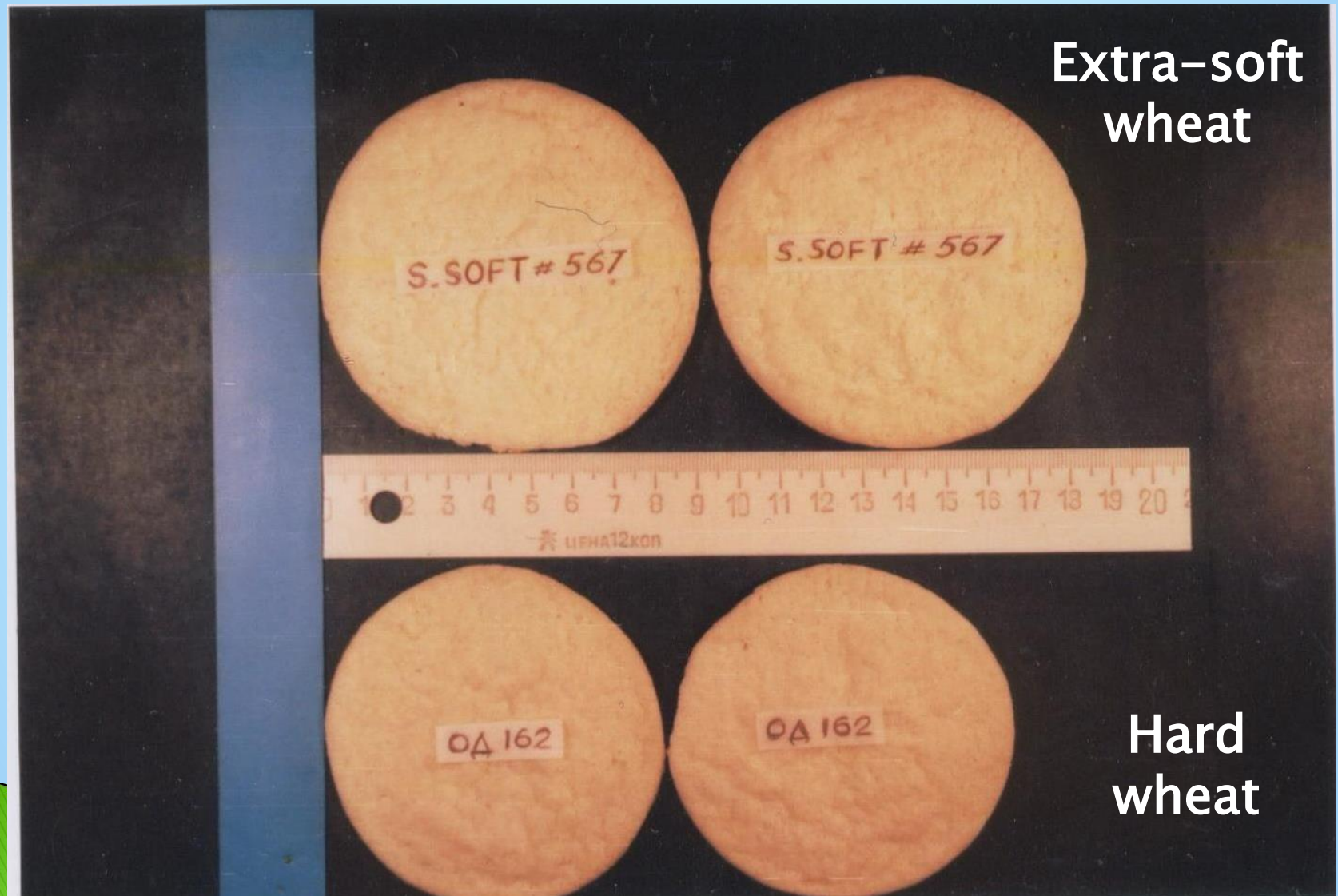


1-5,7 combination of two alien translocations

Exotic friabilin responsible for extra-soft endosperm texture transferred to wheat from aegilops *Ae. tauschii* (DD, $2n=2x=14$)



Extra-soft textured biscuit wheat (diameter, h/d ratio and surface aspect)
produced on the base of the *Ha* (hardness) gene from *Ae. tauschii*



Biscuit red and white wheat varieties developed on the base of *Ha* (hardness) gene derived from *Ae. tauschii*

var. Oksana
extra-soft **red** winter wheat
listed 2007

var. Byljava
extra-soft **white** winter wheat
listed 2014

ОКСАНА

озима екстра-м'якозерна пшениця *кондитерського використання*



- Сорт створено вперше в Україні, в реєстрі з 2006 р;
- Борошно має спеціальні характеристики (ВПС, консистенція) для бісквітних виробів;
- Зерно червоне, екстра-м'яке за консистенцією;
- Стебло 100-105 см, не полягає;
- Борошно як добавка (50%) поліпшує об'єм та якість хліба з борошна з тугою клейковиною;
- Екстра-м'яке зерно – відмінна сировина для годівлі птиці;
- Придатний для органічного пророщування з метою виготовлення дієтичних соків проростків.



БІЛЈВА

озима екстра-м'якозерна білозерна пшениця *кондитерського використання*



- Сорт створено вперше в Україні, переданий у Держсорт випробування у 2010 р;
- Борошно підвищеної білизни, високий вихід при помелі, має спеціальні характеристики (ВПС, консистенція), призначене для бісквітних виробів;
- Зерно біле, прекрасно виповнене екстра-м'яке за консистенцією;
- Стебло 95-100 см, не полягає;
- Борошно як добавка (50%) поліпшує об'єм та якість хліба з борошна з тугою клейковиною;
- Екстра-м'яке зерно – відмінна сировина для годівлі птиці;
- Придатний для органічного пророщування з метою виготовлення дієтичних соків проростків.



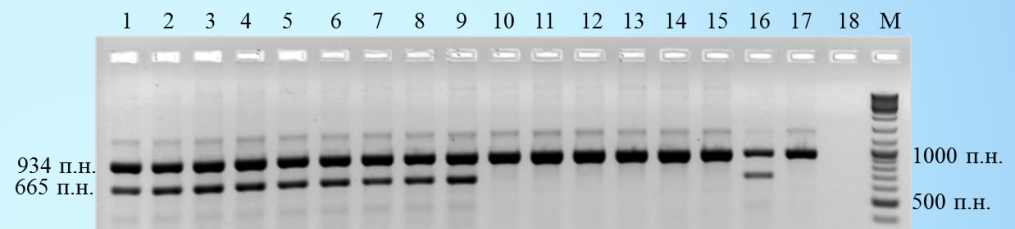
Co-author: *Alain Bonjean*

Author: *Alexander Rybalka*

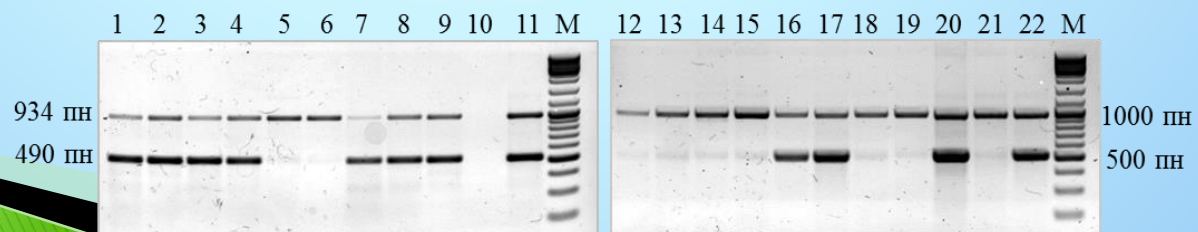
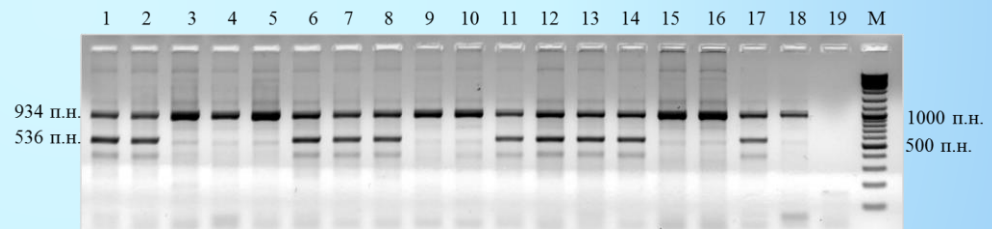
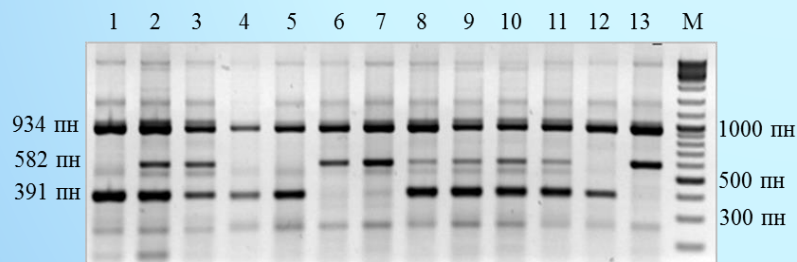
White wheat pre-breeding program



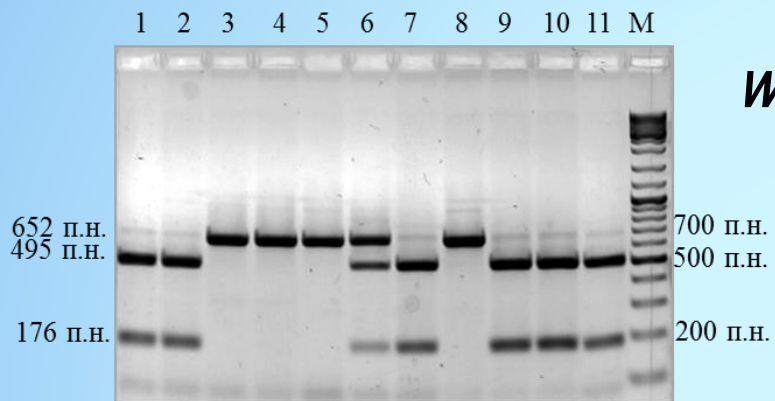
Screening for R-genes red color



Polyphenoloxydase *Ppo-A1, Ppo-D1* detection

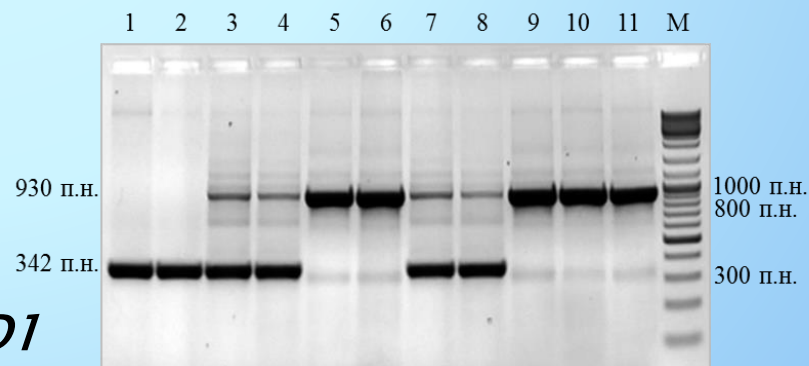
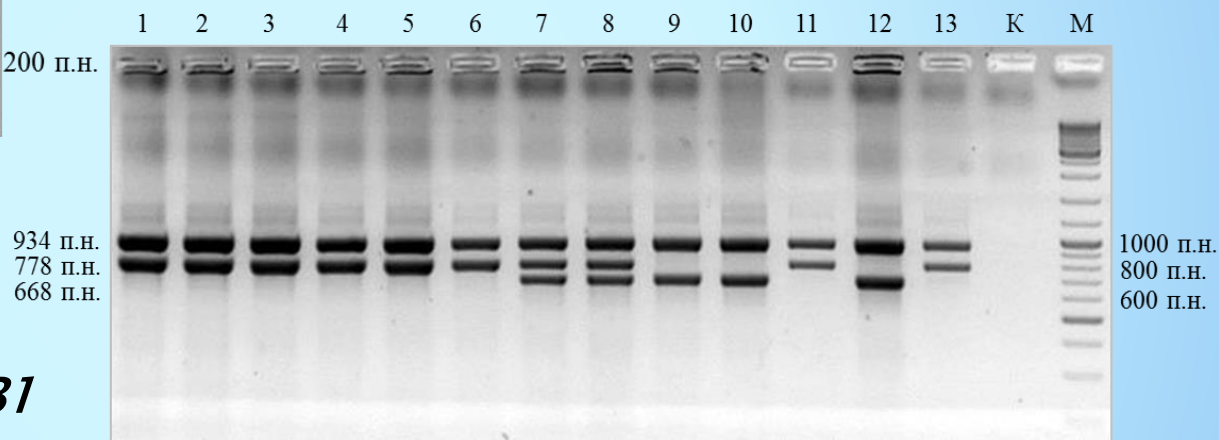


Waxy wheat pre-breeding program (molecular detection of *Wx-A1*, *Wx-B1*, *Wx-D1* alleles)



Wx-A1

Wx-B1



Wx-D1

Waxy wheat pre-breeding program

var. **Sophia**
waxy winter wheat, listed 2014

С О Ф І Й К А ваксі

озима пшениця з генетично модифікованим складом крохмалю

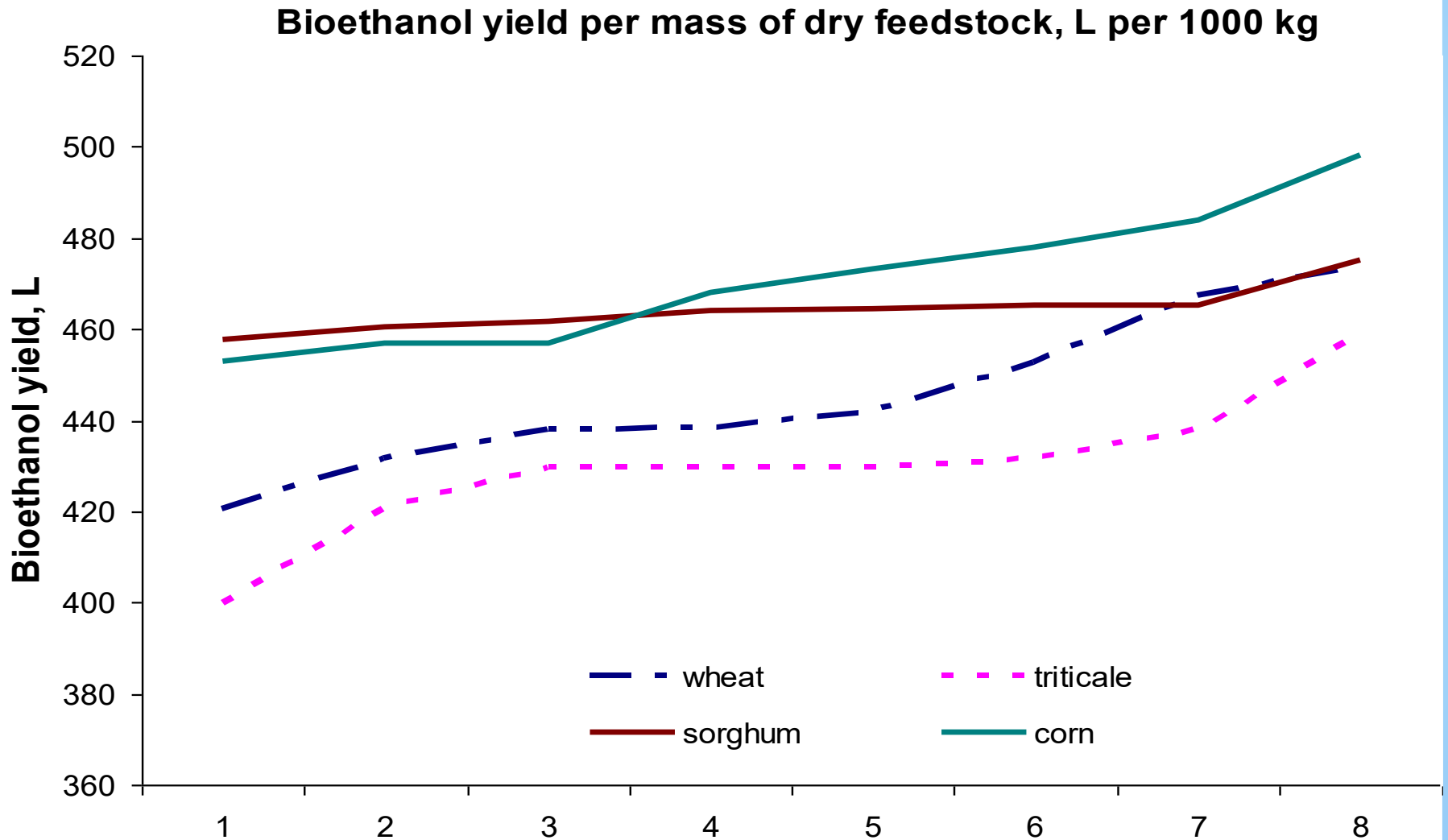


- Сорт створено вперше в Україні, переданий у Держсортвипробування у 2010 р;
- Технологічне призначення: локшина, заморожене тісто, харчові згущувачі, хліб та борошняні вироби, спирт;
- Борошно має аномальні технологічні властивості: екстра-висока ВПС (до 72%), екстра-низьке ЧП (60-70 сек.);
- Тісто має аномально високу газо-генеруючу спроможність;
- Борошно для випікання хліба у чистому вигляді не придатне, як добавка (15-30%) поліпшує хлібопекарські властивості борошна з високим ЧП (350-400 сек.) характерним для південних регіонів (див. ілюстрацію нижче).



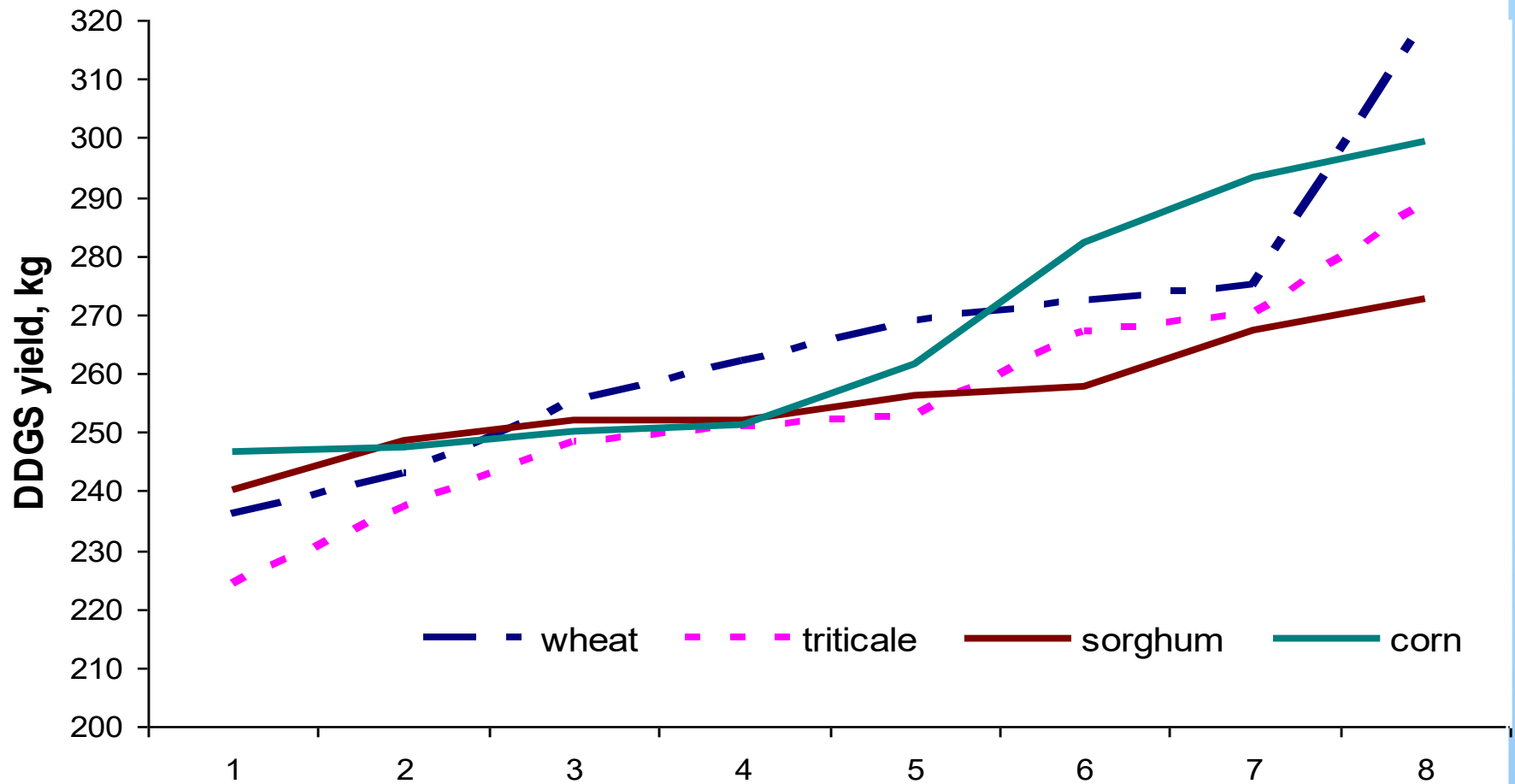
Author: Alexander Rybalka

Industrial crops pre-breeding program

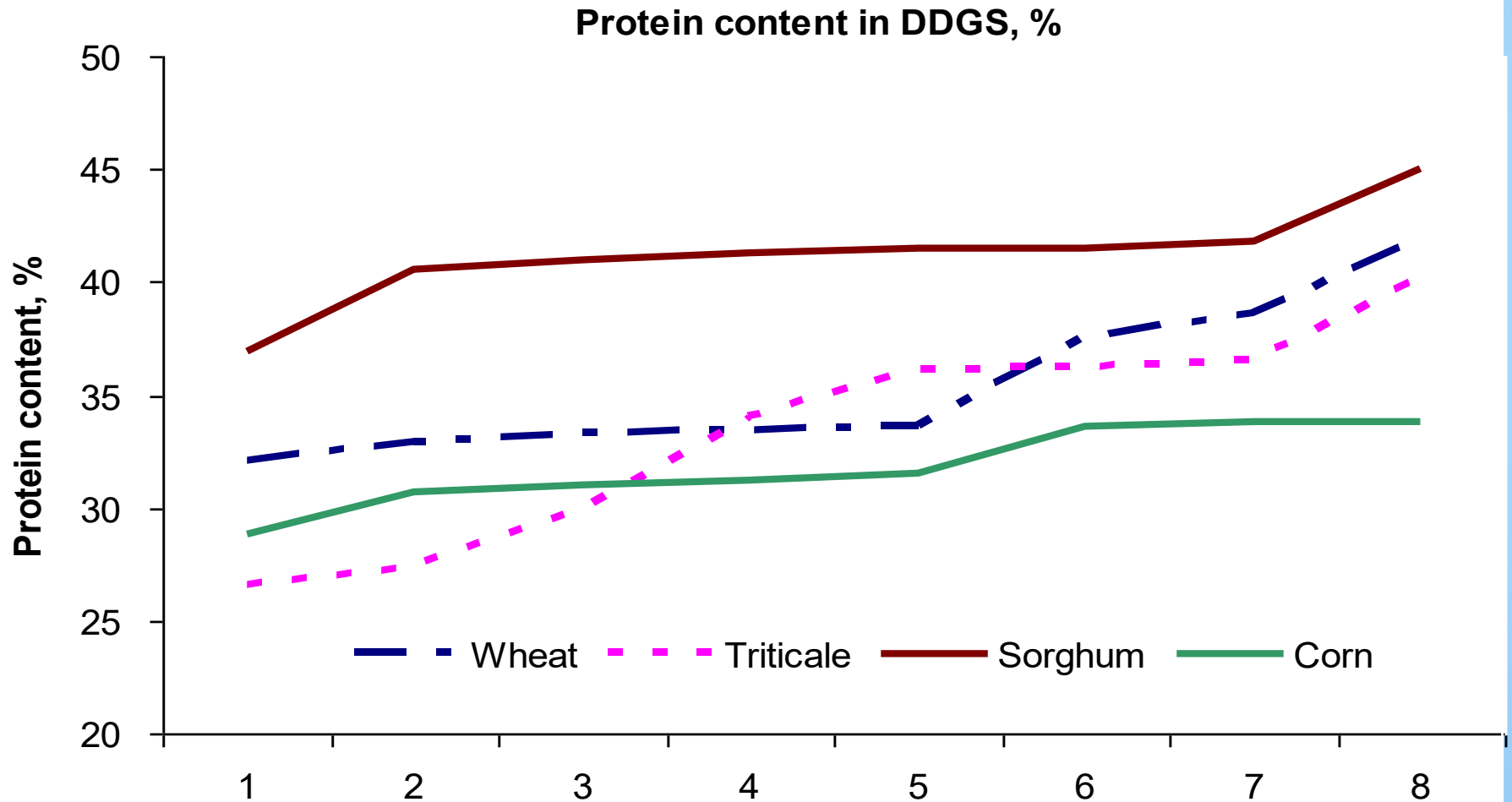


DDGS yield: Kg / 1000 kg dry feedstock

DDGS yield of 1000kg feedstock, kg



DDGS: protein content, %

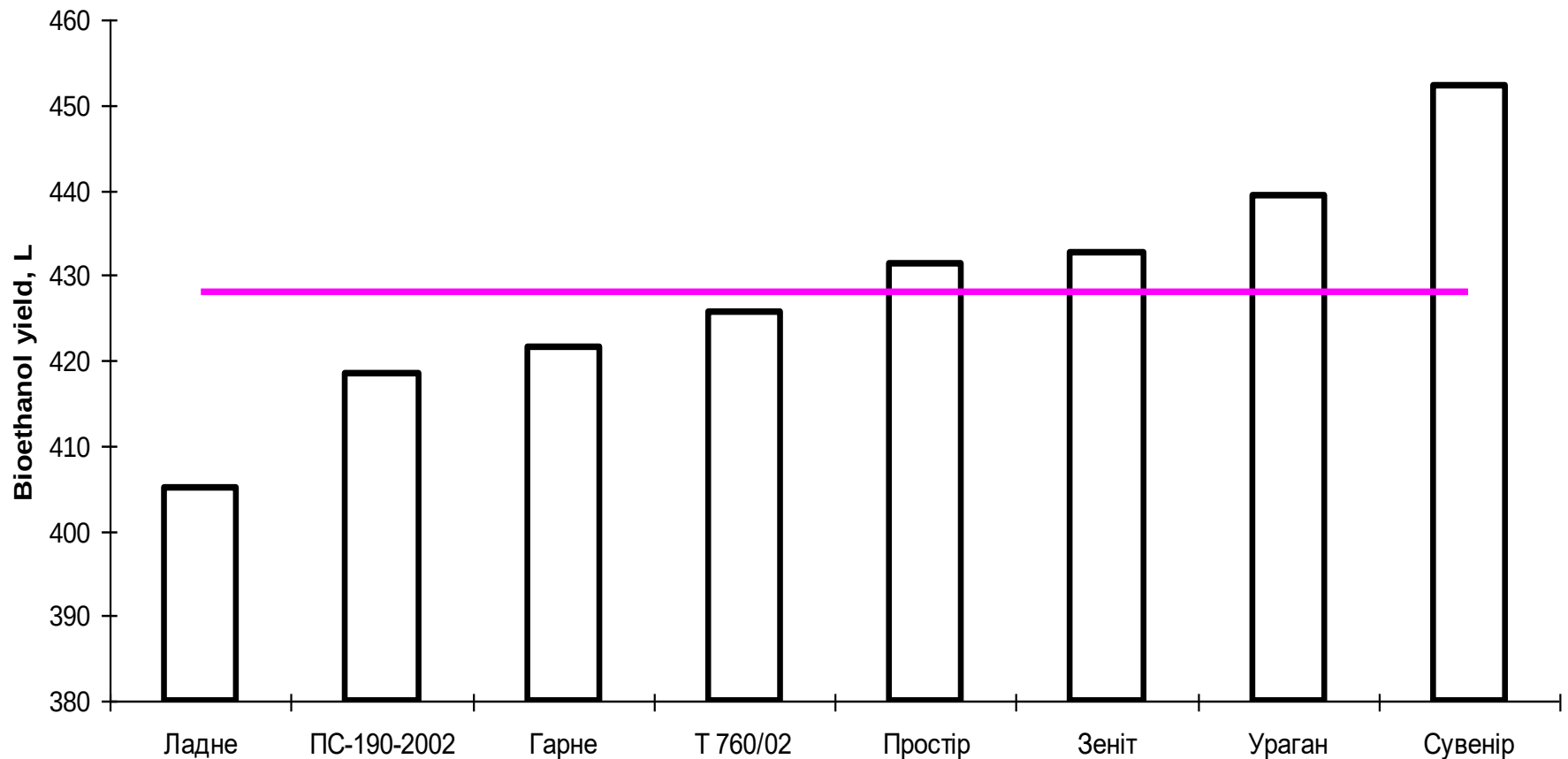


TRITICALE PRE-BREEDING PROGRAM

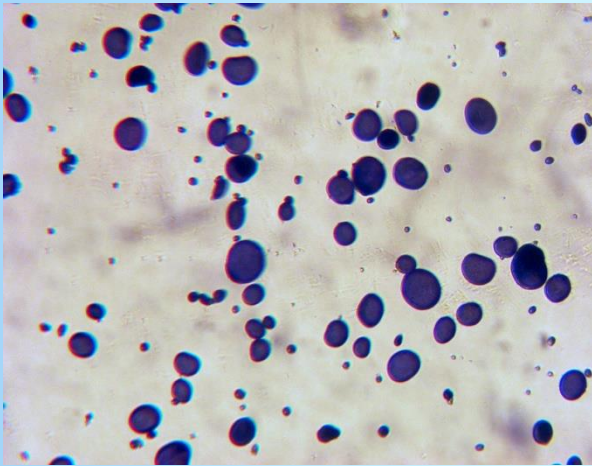
- Based on the use (5B)5D chromosome substitution allows to get wheat-rye chromosomes recombination and wide genetic variability
- Aimed on the development of triticale varieties for edible ethanol and bioethanol end-use
- The best winter triticale breeding lines are superior to winter wheat in hardiness, disease resistance and grain yield (max. yield 14 tons/ha)
- Development of triticale waxy as a factor positively correlated with triticale fermentability

TRITICALE: bioethanol yield L/1000kg feedstock

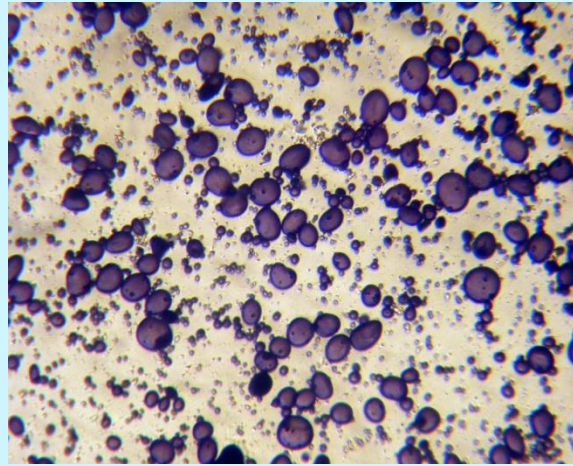
**Bioethanol yield per mass of dry feedstock, L per 1000 kg
(triticale varieties)**



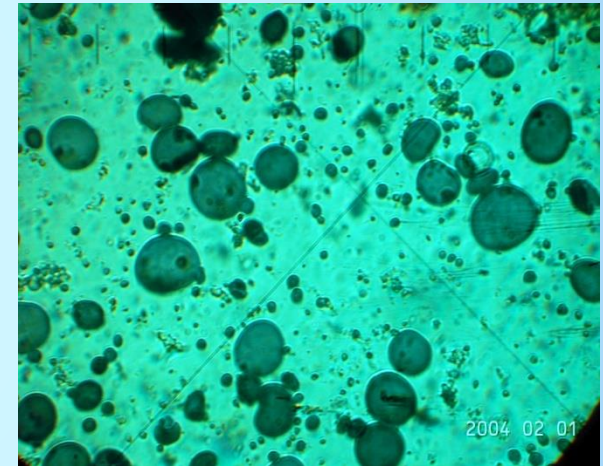
Starch granulometry



**Triticale
var.
BOGDAN**



**Wheat
extra-soft
var. OKSANA**



**Extra-strong
bread wheat var.
KUJALNIK**

Healthy grain program



Jean Anthelme Brillat-Savarin
1755–1826

*Dis-moi ce que tu manges,
je te dirai ce que tu est*

Cereals as a food source:

- *Unique dietary fiber (particularly soluble)*
- *Antioxidants*
- *Essential minerals*

What is wrong with wheat modern varieties?

Is wheat an unhealthy product?

1. Poor protein amino acid balance
2. Proline/Glutamine-rich proteins difficult intestinal assimilation
3. Immunoreactivity of peptides related to **CD** (celiac disease)
4. **IBS** (irritation bowel syndrome)
5. **NCGS** (non celiac gluten sensitivity)
6. **ATIs** – nutritional activators of innate immunity
7. Low minerals
8. **FAW** (food allergy to wheat)
9. Very high **GI** (glycemic index)
10. Post prandial **glycemic** and **insulinemic** response
11. Relation to **metabolic syndrome/diabetes mellitus type 2**
12. Gluten-free products
13. Gluten-free wheat production
14. High amylose wheat production (**RS starch** elevation)
15. Spelt wheat the world's growing popularity

High nutrition black wheat and black spelt wheat pre-breeding program

var. **Chornobrova**

- Developed first variety in Ukraine, listed 2014
- Elevated vitamin/mineral content
- High protein (15–16%) and protein solubility
- Grouts, flakes, snacks, organic sprouting end-use
- Sprouted grain bread end-use

Author: Alexander Rybalka



Sprouted wheat grain bread



Wheat berry



Sprouted grain bread

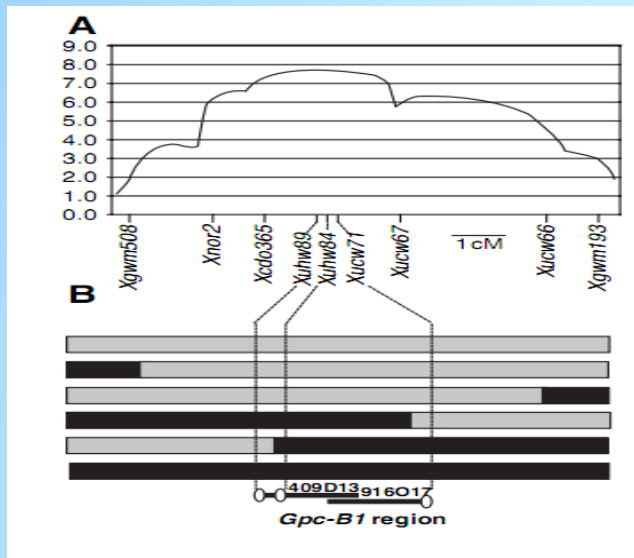
Wheat varieties for organic sprouting (wheat grass juice)



Spelt wheat grain modification from red to black



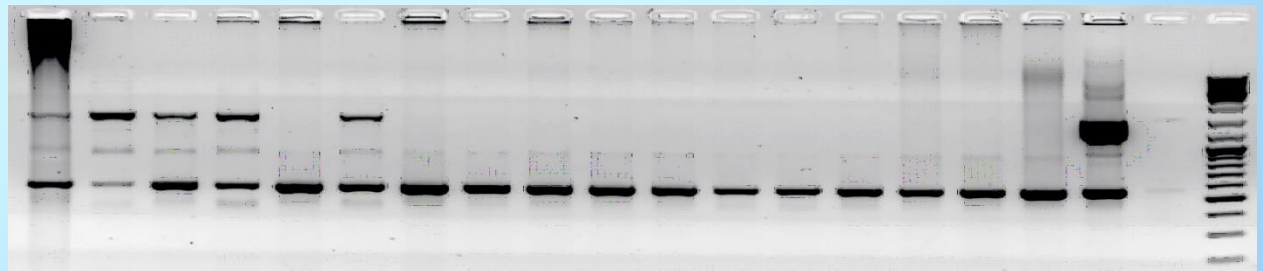
High protein (minerals) wheat pre-breeding program (QTL factor *Gpc-B1*)



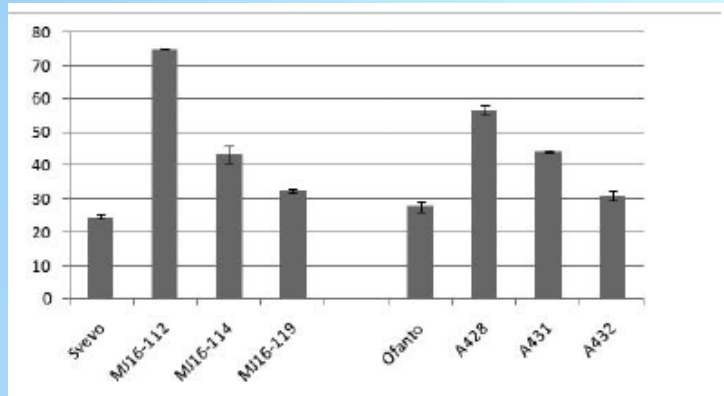
A. Distelfeld et al., 2006

Use of 6B(6B) 6x substitution line with chromosome 6B from *T. turgidum* var. *dicoccoides*

Molecular detection of QTL factor *Gpc-B1*

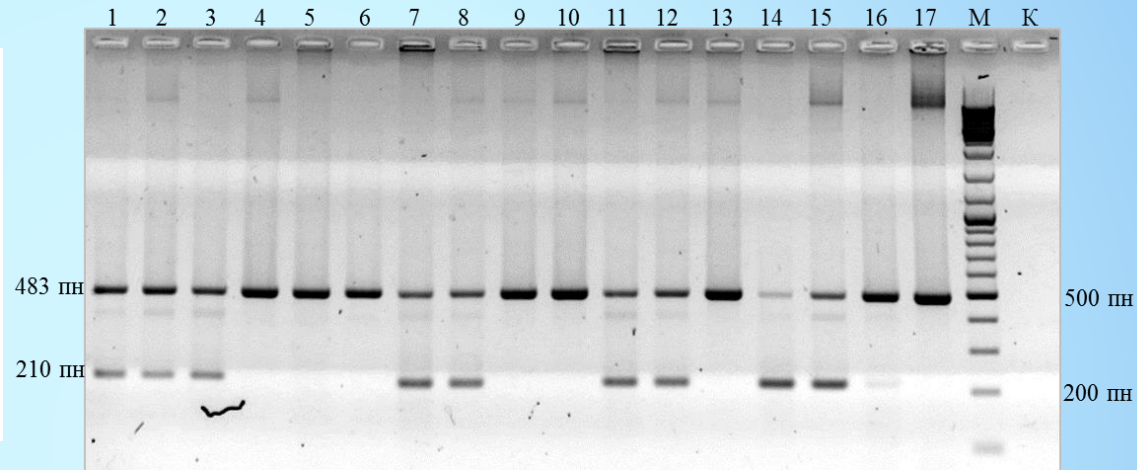


High amylose wheat pre-breeding program

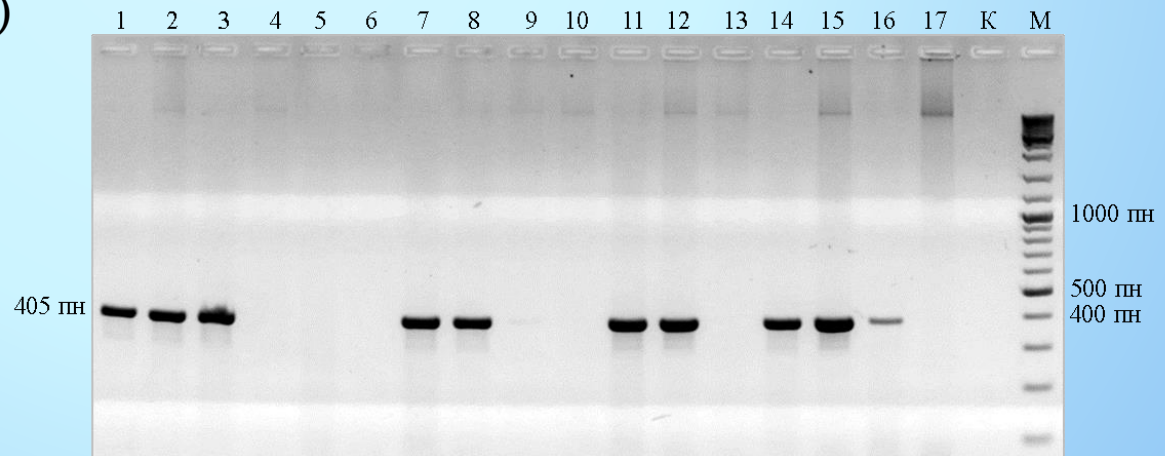


F. Sestili et al., 2010, Univ. Tuscia, Viterbo

**T. durum line (70% amylose)
in use as a source of high
amylose content**



***SBEIIa* gene detection in hybrid populations**



***bar* gene detection in hybrid populations**

Hull-less (naked) barley – renewed healthy food pre-breeding program



Hulled barley



Hull-less barley

Hull-less barley healthy grain program objectives:

The largest in Ukraine food end-use hull-less barley pre-breeding program:

- Waxy barley
- High amylose barley
- Low phytate barley
- Grain color, grain and germ morphology
- Threshing ability
- Protein content and solubility in 50% 1-propanol
- β -Glucan content
- Oil content
- Oil fatty acid composition
- DPPH free radical scavenging capacity (antioxidant activity)
- Grain hardness
- Flour extraction and flour mixing properties
- Grouts extraction and grouts culinary properties
- Flakes quality
- Molecular markers for target characters
- High RS starch barley Himalaya 292 (BARLEYmax)

Hull-less barley program mainly focused on two barley head morphology: 2-rowed awned and 2-rowed awnless



Barley seed color (black, blue, purple) as an indicator of particular pigments (antioxidants)

The purple barley has the highest DPPH radical and superoxide radical scavenging capacity, black barley rich in delphinin 3-glucoside



Var. **ACHILLES** the first in Ukraine high protein naked
spring 2-rowed barley for food end-use (listed 2014)



Author: Alexander Rybalka



The “healthy bowel” breakfast development (the everyday breakfast of Alexander Rybalka)



Porridge composed of:

Naked barley grouts + Black wheat bran + Flaxseed flour



Fruit smoothie composed of:

Grapefruit + Kiwi + Banana + Apple

Health benefits of the “healthy bowel” breakfast

- 100% natural product
- Optimal balance of cereal and fruits health promotion compounds
- Cereal + fruit prebiotics balance for gut microbiota

– Naked barley grouts:

(soluble dietary fiber, antioxidants, minerals, vitamins)

– prevents cardiovascular disease, diabetes mellitus type 2, cancer

– Black wheat bran:

(rich in fiber, antioxidants, minerals, vitamins)

– excellent colon cleaner

– Flax flour:

(rich in ω -3 α -linolenic, lignans, antioxidants, minerals, vitamins)

– flax lignans are unique polyphenolic compounds 3 in 1:

phytoestrogen, antioxidant, dietary fiber

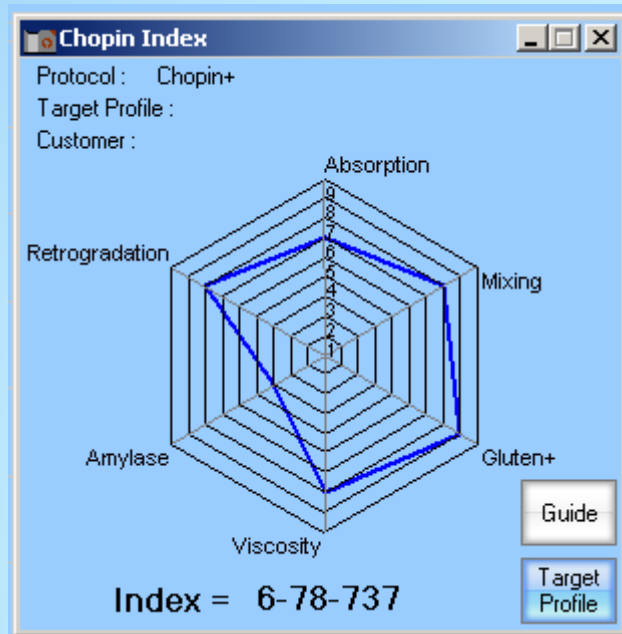
– prevents of women breast and ovarian cancer, man's prostate tumor

Fruits are fruits

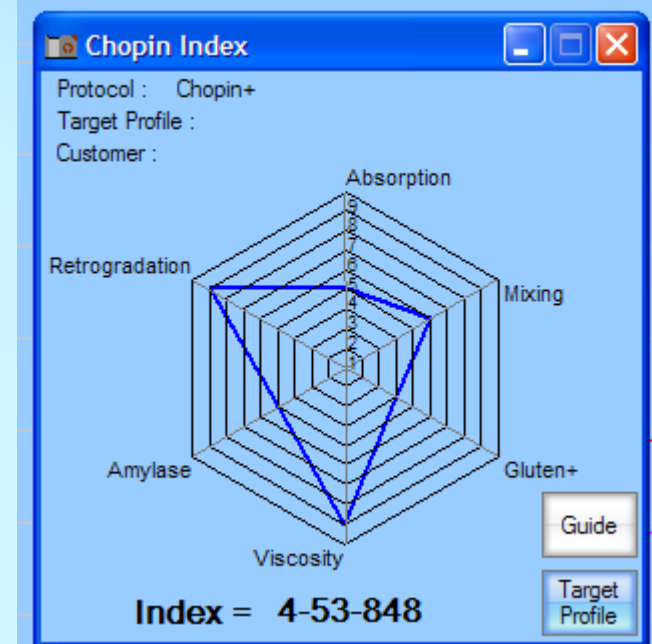
LABORATORY PROCEDURES DEVELOPMENT and SEED PURITY TESTING

MIXOLAB profiles of extra-strong wheat variety Kujalnik damaged by sun pest (*Eurygaster integriceps*)

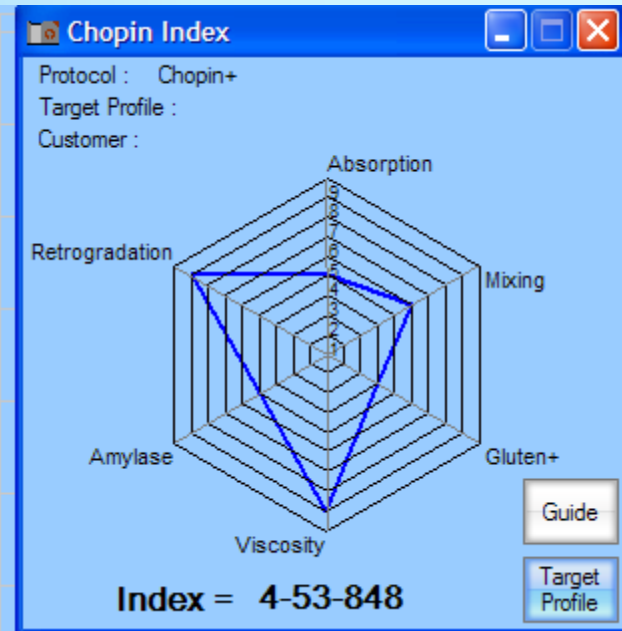
0%



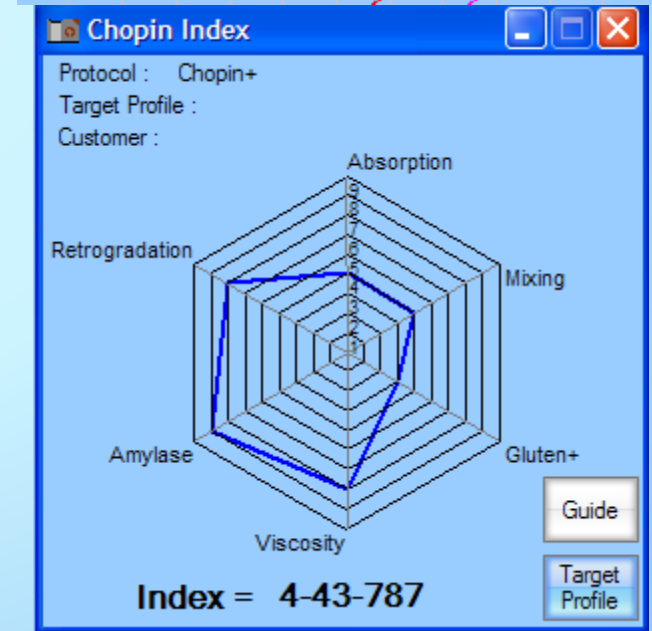
4%



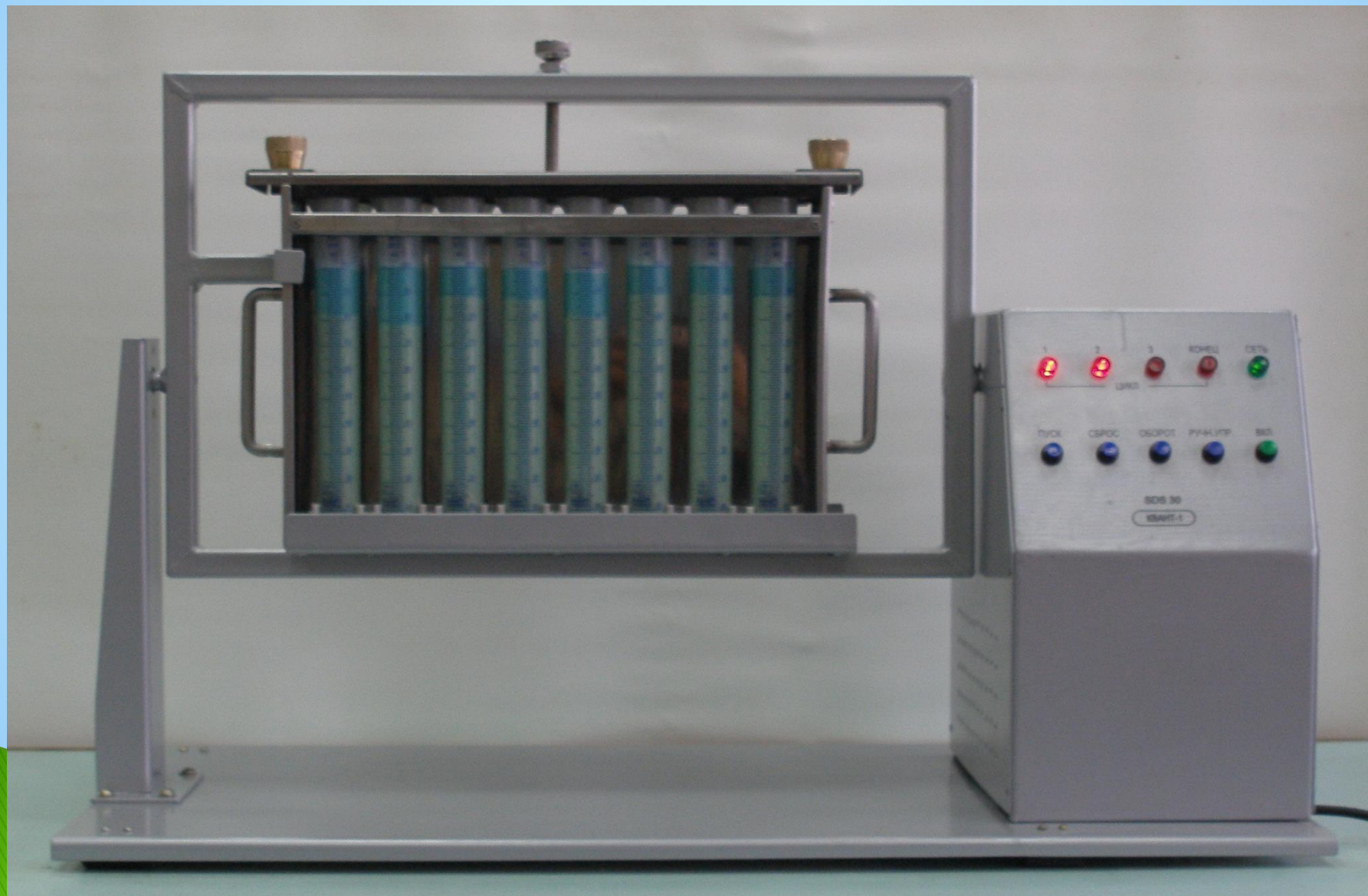
6%



8%

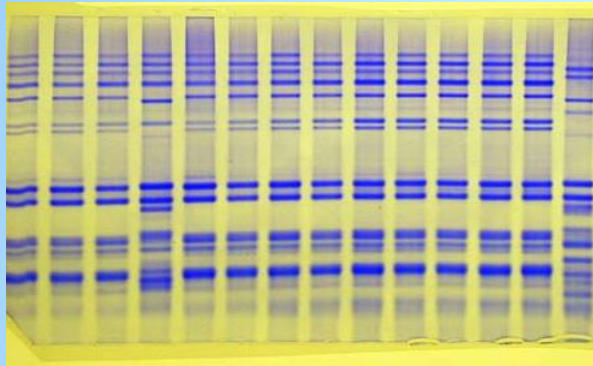


SDS-30 two-step sedimentation method and semi-automatic device for wheat BM quality evaluation (to be applied in breeding and bread-making)

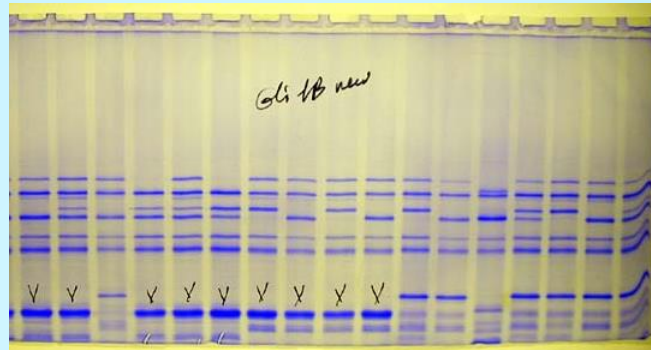


Seed purity testing (wheat, barley, corn, sunflower)

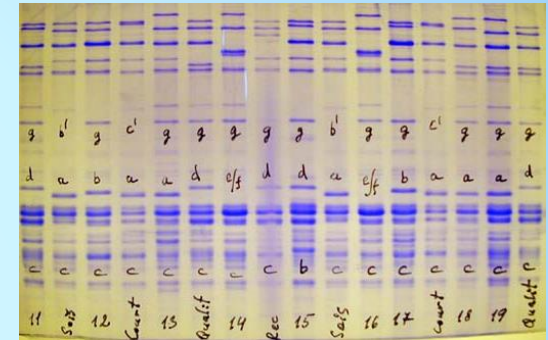
Wheat gliadin



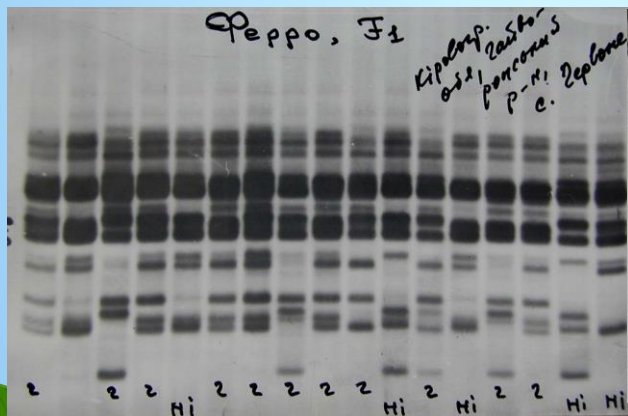
HMW-glutenin



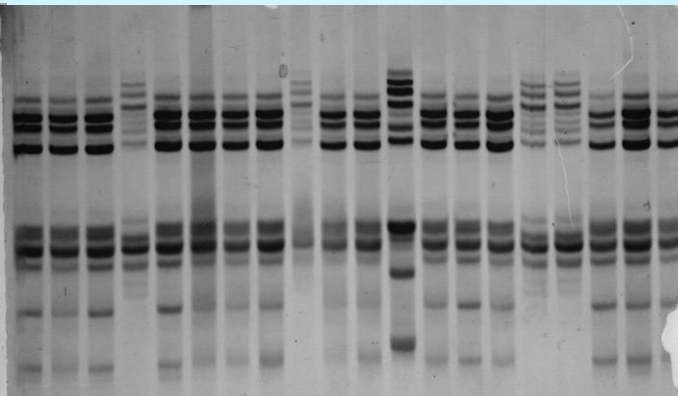
HMW+LMW glutenin



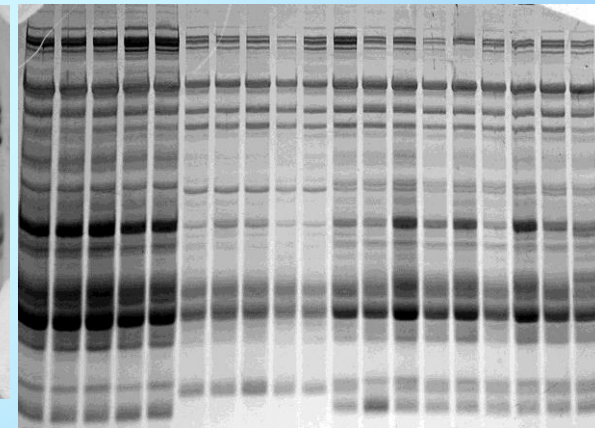
Corn zein



Barley hordein



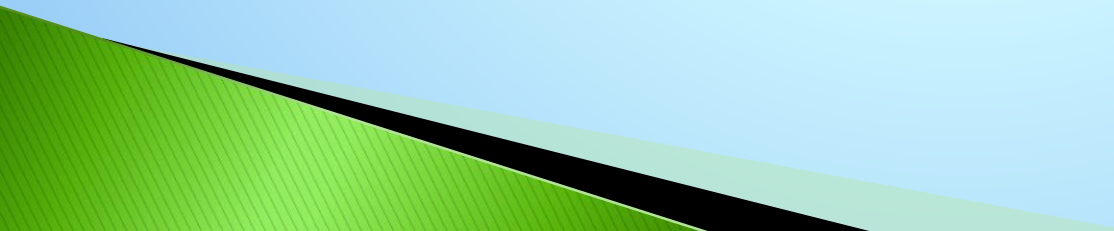
Sunflower heliantin



We provide seed purity service for Limagrain group in Ukraine

Cooperation with Limagrain Group

(Jayne Stragliai team 1996–2006)

1. Original interspecific lines with extra-strong gluten and extra-soft endosperm texture contribution to the Nickerson's wheat breeding program.
 2. Mini-PAGE electrophoresis protocols application to the Nickerson's wheat seed purification.
 3. Seed purity testing for Limagrain Group Ukraine.
- 

Cooperation with Limagrain Group (Jayne Stragliai team 1996–2006)

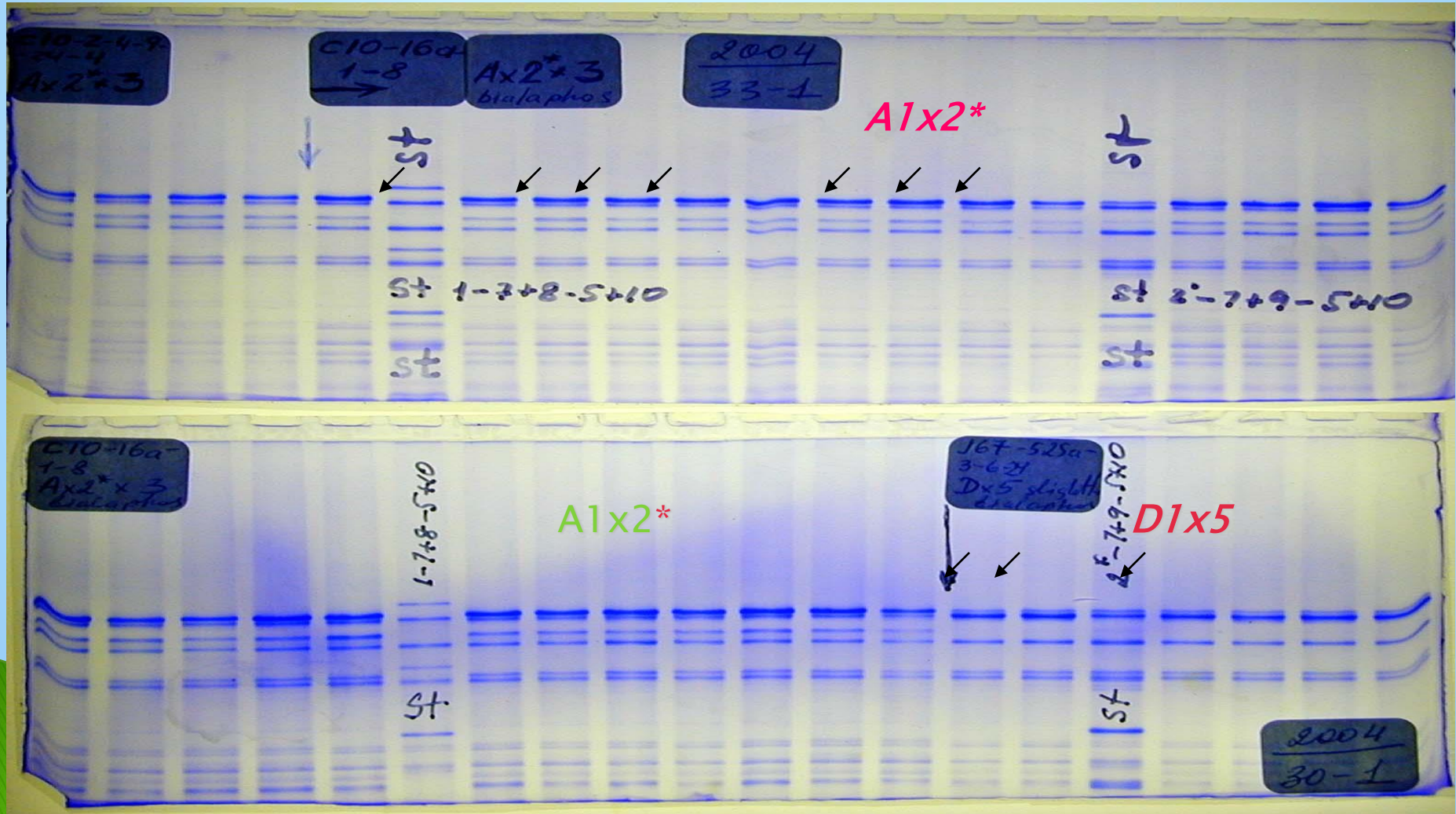
We have been working together 1996–2006



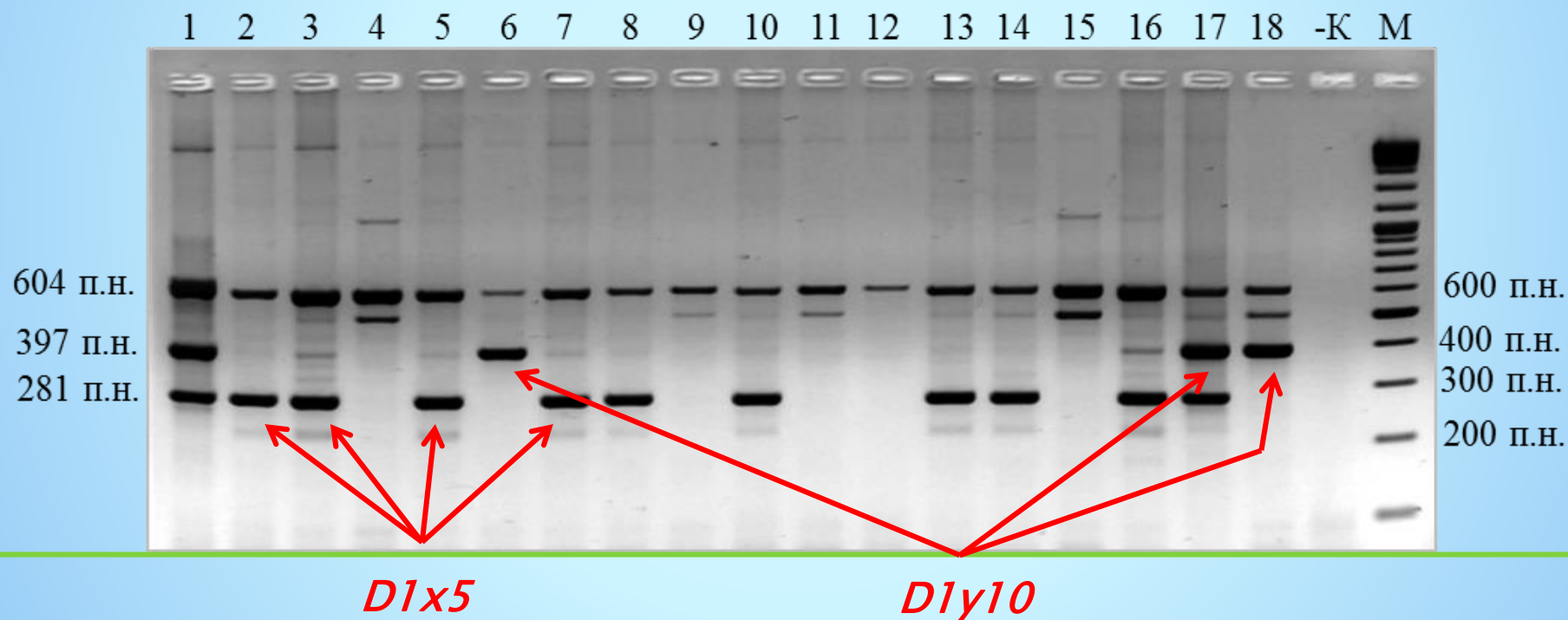
TRANSGENIC WHEAT AND BARLEY RESEARCH

- In wheat:** over-expression of the *Glu-D1x5* and *Glu-D1y10* (three-fold extra copies located in the same *Glu-D1* site), evaluation of over-expression on BM quality
- In barley:** expression in barley genome of wheat HMW-glutenin *Glu-D1x5*, *Glu-D1y10*, *Glu-D1x5+y10* evaluation of wheat proteins on mixing properties of barley flour with wheat flour

Over-expression of *Glu-A1x2** and *Glu-D1x5* in wheat



Molecular markers for wheat HMW-GS *D1x5* (281 bp) and *D1y10* (397 bp) in hull-less barley hybrid population



Alexander RYBALKA

is an author of 220 scientific publications,

two book–monographs:

1. Wheat quality and its amelioration (550 pages), 2011
2. Barley as a functional food product (670 pages), 2016
3. AUTHOR OF 5 VARIETIES (4 wheat and 1 barley) listed in Ukraine
and many patents

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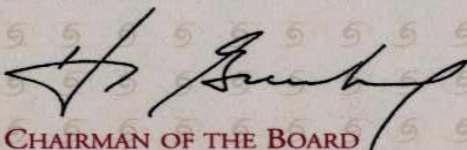
PRESENTED TO

Alexander I. Rybalka, Ph.D.

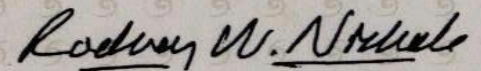
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