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Molecular markers for increasing efficiency of wheat genetic resources utilisation in breeding. Wheat genome origin according to protein markers.

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INTRODUCTION

To serve effective basis for improvement of cultivated plants stored in the gene banks genetic diversity should be carefully and comprehensively evaluated and characterised (investigated). **Collections should be rationally organised. Each accession should be identified and registered.**

Molecular markers (MM) are successfully used on the following steps of working with plant collections (In VIR – protein markers are mainly used)[1-7]:

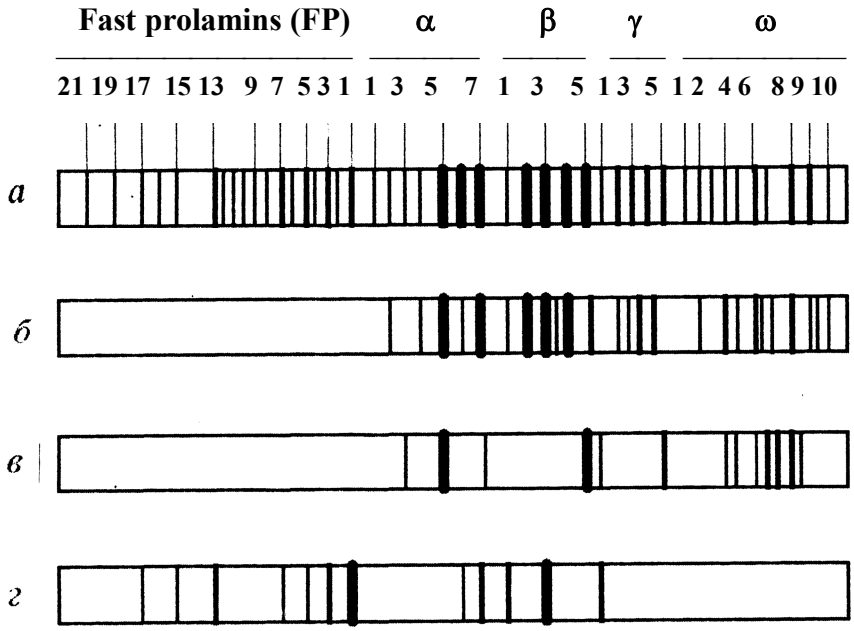
a) **search of new allelic diversity for gene banks *in situ* (exploration of wild-growing diversity);**
b) **originality testing of new accessions before the entry in collections;**
c) **the degree of similarity or difference among individual genotypes in an accession or among accessions in collection;**
d) **structure of collections: number of genetic variation present, intraspecies relations and interspecies relationships (genome analysis);**
e) identification and registration of genetic diversity (accessions, genotypes) and preparation catalogues and data bases on MM;
f) identification of duplicates, very similar accessions and various mistakes in collections;
g) **development of core collections;**
h) analysis of accessions genetic constitution (after reproduction and under various storage conditions) – genetic integrity control ;
i) **identification of alien genetic material;**
j) **authorship rights control (for gene bank problems);**
k) **other aspects of activity with plant genetic resources … .**

1. SEED PROTEINS IN IDENTIFICATION AND REGISTRATION OF A TRITICEAE GENE POOL [1,2].

The significant element in the work of botanist, geneticist or breeder is identification of species, varieties, biotypes (accessions in gene bank).

In order to develop a flexible and reliable nomenclature and system of pattern recording, practically all intraspecific (or intrageneric) variability of a given protein marker should be investigated. **Due to this almost all possible locations of protein (or DNA) components in electrophoretic patterns can be identified. Cultivars, wild growing populations, landraces from world collections have to be analyzed. I**n the N.I.Vavilov Institute this principle was laid in the base of nomenclatures and systems of recording electrophoretic components for many crops. This approach was first developed for wheat and then spread on all *Triticeae* and other crops**.**

Wheat seed storage protein (gliadin) pattern was taken as a basis. The pattern was divided into four zones. Within a zone the main possible positions of components were numbered to the start. By means of such etalon pattern, any variety or biotype of *Triticeae* may be recorded in the form of prolamin formula.



а - etalon electrophoretic banding pattern and protein varietal **formulae**:

б – **Wheat (Var. Mir.808)** $\alpha\bar{2}\,\underline{4}\underline{5}\underline{6}\underline{7}_1\ \beta\bar{1}\,\underline{2}\underline{3}_2\bar{3}_3\,\underline{4}\underline{5}_2\ \gamma 2_1 2_3\underline{3}\underline{4}\ \omega\bar{2}4_2\underline{5}\underline{6}_2\bar{6}_3\,\bar{7}_1 8_1 9_1 9_3 10_2$

в – **Barley (Var. Krinichni)** $\alpha\bar{3}\bar{5}\bar{7}_2\ \beta\underline{5}_1\bar{5}_2\ \gamma\bar{\bar{5}}\ \omega 4_1 4_3\bar{6}_2\underline{6}_3 7_2\underline{8}_1\bar{8}_2$

г – **Avena (Var. Astor)** **FP** $\bar{\bar{1}}7\ \bar{\bar{1}}5$ **13** $\bar{7}5\underline{3}\underline{1}\ \alpha\bar{6}7_1\ \beta\underline{1}\underline{3}_2\underline{5}_2$

Tabl. Identification, Registration and Studying of Plant Genetic Resources by Seed Protein Markers (N.Vavilov Institute of Plant Industry)

	Studied and registered:		**Issued:**	
Genera	***Species***	***Accessions***	***Catalogs***	***Manuals***
Triticum	**20**	**5300**	**14**	**5**

Hordeum	**17**	**355**	**1**	**4**
Secale	**7**	**280**	**1**	**1**
Avena	**19**	**515**		**4**
Triticale	**1**	**600**	**1**	**1**
Aegilops	**25**	**2080**	**4**	**1**
Zea	**1**	**510**	**2**	**1**
Oryza	**17**	**1776**		**1**
Sorghum	**28**	**155**		
Elytrigia	**40**	**120**		**1**
Elymus	**33**	**68**		**1**
Agropyron	**4**	**25**		**1**
Leymus	**8**	**17**		
Festuca	**50**	**320**		**1**
Lolium	**9**	**168**		**1**
Dactylis	**3**	**173**		**1**
Poa	**30**	**120**		
Other cereals	**131**	**1260**		**1**
Pisum	**88**	**102**		**1**
Glycine	**118**	**510**		**1**
Solanum	**44**	**300**		
Beta	**13**	**300**		**2**
Brassica	**20**	**209**		**1**
Allium	**27**	**115**		**1**
Amaranthus	**5**	**18**		
Heliantus	**30**	**700**	**2**	**2**
Linum	**6**	**40**		
Fagopyrum	**4**	**150**		
Fruits	**33**	**333**		
Berries	**21**	**160**		
Cuphea	**26**	**36**		
Jojoba	**1**	**50**		
Citrus	**13**	**47**		

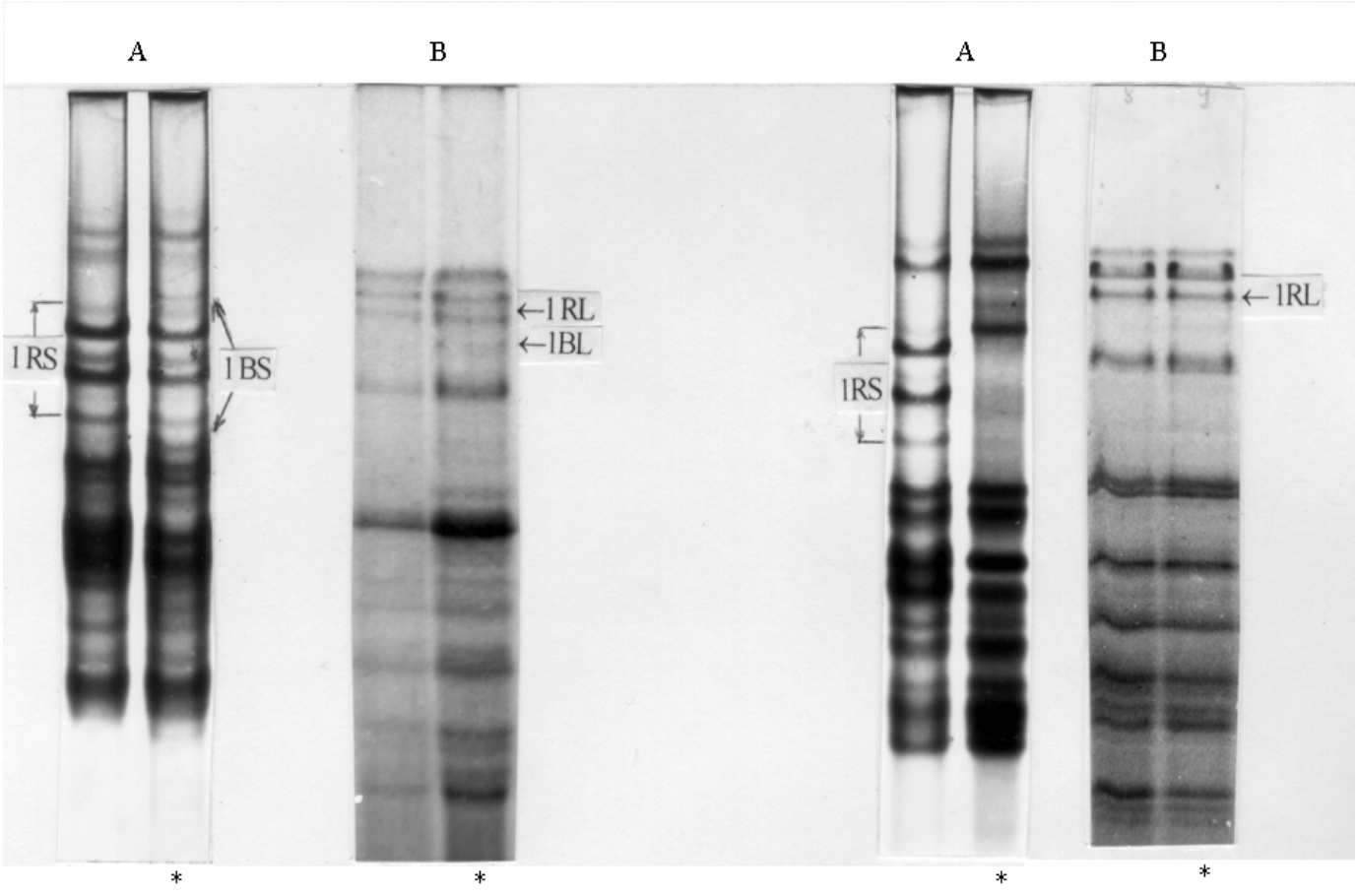
3. USAGE OF PROTEIN MARKERS FOR TESTING OF GENETIC CONSTITUTION OF *EX SITU* COLLECTION

In collection of N.I. Vavilov institute are stored a lot of accessions of old varieties (winter bread wheat) and forms with a high level of population polymorphism as compared with modern varieties. The diversity of old varieties and forms is an important source of genetic variability and respectively of valuable traits for wheat improvement. The problem of gene banks is to identify, register and to store all richness of genetic variability of these unique forms. It is known that in course of long-term storage and reproduction, original populations lose a part of their

genotypes. One of our goals was to estimate gliadin polymorphism of old wheat varieties and to deter-mine the level of populations's dynamics during reproduction and storage. Changes in genotype composition for some bread wheat landraces during seed increase in 1989-1991 were shown. Simultaneously it was discovered that separate genotypes of these old varieties lose their germination ability with different rate after storage during 2 to 8 years [3]. Practically, heterogenic populations (landraces, old varieties and original forms) require identification of separate genotypes and organization of their individual storage.

In VIR collection are stored the original cultivars of winter bread wheat (Mironovskaya 10, Soladin, Burgas 2, Orlandi, Neuzucht 14/14, WRN 48/49, Aurora, Caucasus, Bezostaya 2, Lovrin 10, Hamlet, Linos etc.) carrying a genetic material of chromosome 1R from rye and their reproductions. Gliadin and glutenin PAG electrophoresis was used to test the genetic stability of such cultivars. Gliadin components $\omega\underline{2}\ 3_1\ \underline{4}\ \gamma 5$ encoded by a polygenic *Sec l* locus were used for detection of 1RS chromosome part. Glutenin components controlled by the *Sec 3* locus are used as markers of 1RL (Fig.1). Above mentioned markers of 1RS and 1RL were discovered simultaneously in Mironovskaya 10, Soladin, Burgas 2, Orlandi, Neuzucht 14/14, WRN 48/49. That means substitution of 1B for 1R. The others are characterized by translocation T1BL-1RS. Cultivars Mironovskaya 10, Feldkrone, Perseus, Aurora, Caucasus, Bezostaya 2, Lovrin 10, Burgas 2, Saladin were also stable and corresponded to originals on 80-90%. However, some cultivars undergone the considerable changes (on the data of protein markers) after reproduction. These changes may be as the following: elimination of 1RS, structural modification of wheat and rye chromosomes, mechanical admixtures and so on. Our results showed necessity of strong control for originality and integrity of such accessions stored and reproduced in gene banks and efficiency of usage of protein markers for these purposes.

Fig.1. Gliadin (A) and glutenin (B) electrophoretic pattern var. Winneton and Burgas 2 (1R from Rye).
*** – gliadin anf glutenin pattern untypical for var.**



Winneton **Burgas 2**

In grain var. Burgas 2 "1RS" was lost with presence 1RL. In grain Winneton markers 1RS,1RL and 1BS 1BL was identified.

4. ANALYSIS OF *TRITICEAE* GERMPLASM BASED ON PROLAMIN POLYMORPHISM

Genetic diversity of *Triticum, Aegilops, Erytrigia, Secale, Hordeum* collections stored in VIR were characterized by prolamin polymorphism. The main goals were: identification and registration of diversity in form of prolamin formulae, identification of duplicate accessions, formation of core-collections, differentiation of biodiversity. Genetic differentiation of *Triticum spelta* L. germplasm based on gliadins polymorphism is one of the last examples of these investigations.

5. GENETIC DIFFERENTIATION OF *TRITICUM SPELTA L.* GERMPLASM BASED ON GLIADIN POLYMORPHISM

Germplasm collection of spelt wheat maintained at the VIR consists of 170 accessions originated from all principal regions of its cultivation in modern and

former times. Polymorphism of gliadins was used for characterisation of spelt wheat genetic diversity.

Spelt collection was registered in form of database of the protein formulae (Tabl. 1).

We identified some accessions, which can be attributed to doublets (duplicate accessions) or to "genetically very close accessions" (Tabl. 2) . The methods of cluster (Fig.2) and principal component analyses divided spelt collection into the some genetic groups. Accessions from Germany and from other European countries, from Spain and Tadjikistan were classified most precisely. In cluster analysis four Iranian accessions have formed a subgroup and have been combined with accessions from Tadjikistan and Morocco. The groups of accessions identified based on gliadins mainly correspond to the ancient centres of spelt wheat cultivation or global centres of spelt diversity (Zhukovskii, 1972).

V.F.Dorofeev et al. (1972) distinguished European (subsp. *spelta)* and Asian (subsp. *kuckuck.)* subsp. of *T.spelta*. The first subsp. comprises two eco-geographical groups: accessions from Germany and Switzerland and accessions from Spain. These researchers did not divide the Asian subsp. into distinctive groups. Revealed by gliadin analysis German and Spanish genetic groups correspond to the eco-geographical groups of subsp.*spelta.* The differentiation European and Asian spelt revealed on gliadins may serve as a basis for more detailed classification of this crop.

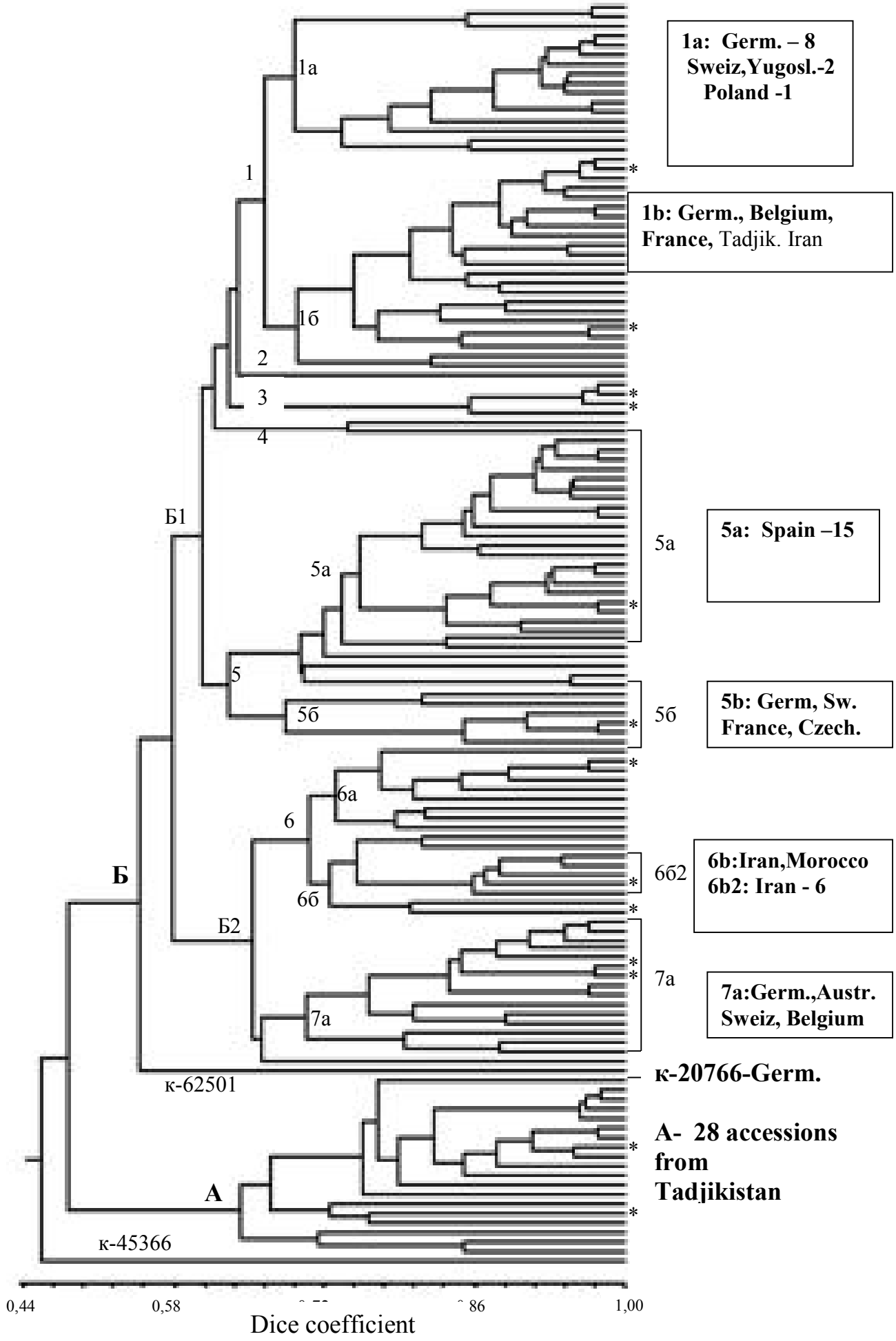
Tabl. 1. Formulae of some types of electrophoretic spectra of *Triticum spelta* prolamines

Catalog №	Origin	Prolamine fractions and formulae				Frequency of occurrence (%)
		α	β	γ	ω	
1724	Germany	5 $\underline{6_1}6_2\underline{7_1}$	12 $3_2\underline{4}$ $5_2\underline{5_3}$	$\underline{2_1}$ $\underline{34}$	2 4_2 $\underline{6_2}$ 7_1 $8_1\underline{8_2}\underline{9_2}$	100
20384	"	$\underline{6_1}$ $\underline{7_1}7_2$	$3_2\underline{4}$ 5_2	1 $2_3\underline{34}$	34_1 6_2 7_1 $8_2\underline{9_2}$	88
"	"	$5\underline{6_1}$ $\underline{7_1}$	12 $3_2\underline{4}$ $\underline{5_3}$	$\underline{2_1}$ $\underline{3}$ $\underline{5}$	$4_1\underline{4_3}5$ $7_1\underline{8_1}\underline{8_2}\underline{9_2}$	8
"	"	$6_1\underline{6_2}$ $\underline{7_1}$	2 $3_2\underline{4}$ 51	$2_3\underline{34}$	$\underline{4_1}$ 6_3 $8_2\underline{9_2}$	4
20539	Spain	24 $\underline{6_1}\underline{6_2}$ $\underline{7_1}$	12 $3_2\underline{4}$ $\underline{5_2}$	1 $\underline{2_2}$ $\underline{3}$ $\underline{5}$	$4_1\underline{4_3}5$ $8_2\underline{9_2}$	87
"	"	24 $\underline{6_1}\underline{6_2}$ $\underline{7_1}$	12 $3_2\underline{4}$ $\underline{5_2}$	$\underline{2_1}$ $\underline{3}$ $\underline{5}$	$4_1\underline{4_2}$ 8 $\underline{9_2}$	13
45818	Iran	6_1 $6_3\underline{7_1}7_2$	2 $3_2\underline{4}$ 5_2	$\underline{2_1}$ $\underline{34}$	3 $5\underline{6_2}7_1\underline{8_1}\underline{8_2}\underline{9_2}$	55
"	"	6_1 $6_3\underline{7_1}7_2$	2 $3_2\underline{4}$ 5_2	$\underline{2_1}$ $\underline{34}$	3 4_2 $\underline{6_2}$ 8_1 9_2	45
45366	Azerbaidjan	$\underline{5}$ 62 $\underline{7_1}7_2$	12 $\underline{3_2}\underline{4_1}5_1\underline{5_2}\underline{5_3}$	$2_3\underline{34}$	23 $\underline{6_3}$ $8_2\underline{9_2}$	100

Tabl.2 List of *Triticum spelta L.* accessions with identical and closely related gliadin patterns

Groups	Catalog № of N.I.Vavilov Institute (VIR) Wheat Collection, origin of accessions
	Identical monotypical accessions
1	к-1724 (Germany) =к-1727 (Germany) =к-1941 (Germany) = к-19097 (Switzerland)
2	к-20382 (Germany) =к-20383 (Germany) =к-20392 (Germany)
3	к-20568 (Spain)=к-20579 (Spain)
4	к-24724 (Yugoslavia) =к-6535 (Germany)
	Identical polytypical accessions
5	*к-29607 (Czechoslovakia before 1992) =*к-1734 (Germany)
6	*к-20543 (Spain) =*к-20558 (Spain)
	Closely related accessions
7	к-20760 (Germany) = predom. biot. of *к-40828 (Austria)
8	к-56591 (Turkmenistan) = predom. Biot. of *к-52440 (Tadjikistan)
9	к-52443 =к-52457 = predom. biot. of *к-52437 =*к-52442 =*к-52444 =*к-52445 =*к-52466 (Tadjikistan)

*** - polytypical (polymorphic) accessions**



Distribution of clusters on the phenogram of 180 spelta accessions based on prolamine patterns

6. GENOME ANALYSIS OF WHEAT AND ITS RELATIVE CEREALS

Modern varieties of cultivated wheat belong mainly to two species: *T.durum* and *T.aestivum*. Polypliod wheats are traditionally divided into two evolutionary groups: *turgidum* group with genome formula AABB and *timopheevii* group (AAGG). Up to the present, there has been no single opinion on the origin of these genomes, especially of genome A. Originally, *T.monococcum L.* was considered as the donor of the first genome of polyploid wheat. Later it was assumed that wild einkorn *T.boeoticum* Boiss. is the source of genome A, whereas *Aegilops speltoides* Tauch. or another species of the *Sitopsis* section is the source of genome B. The problem of wheat genomes has been discussed by many workers, but remains unsolved. In genome analysis of wheat and closely related cereals we used as serological markers a fraction of wheat albumins accompanying prolamins in alcohol extract. This albumin fraction of seed proteins was a peculiar concentrate of genome specific proteins (GSP). Methods of electrophoresis, immunodiffusion, affinity immune chromatography, enzyme-dependent immunosorbent test, thin-layer chromatography and others have been used for fractionation, purification and study of the component composition and nature of *Triticum L.*, *Aegilops* L. and *Elytrigia* Desf. It was shown that the most active GSP antigens of cereal seeds are lipoproteins of cell membranes.

Analysis of polyploid and diploid *Triticum* and *Aegilops* GSP showed that wild einkorn *T.urartu* Thum. was the phylogenetic donor of genome A in *turgidum-aestivum* group of wheat species, while *T.boeoticum* was the donor for the first genome of *timopheevii* group. A.V.Konarev et al. were the first to publish information on the relationship of *T.aestivum* and *T.durum* genome A to wild einkorn *T.urartu*. Later this was confirmed by immunological, morphological and molecular methods. The proteins of wheat species from the *turgidum-aestivum* group revealed antigens typical for the genome of *Ae.longissima,* while the proteins of wheat with genome G revealed antigens typical for *Ae.speltoides.* It seemed likely, therefore, that *Ae.speltoides* (genome B^{sp}) could be the source of genome G, whereas *Ae.longissima* could be source of genome B [1,2,4,5,7].

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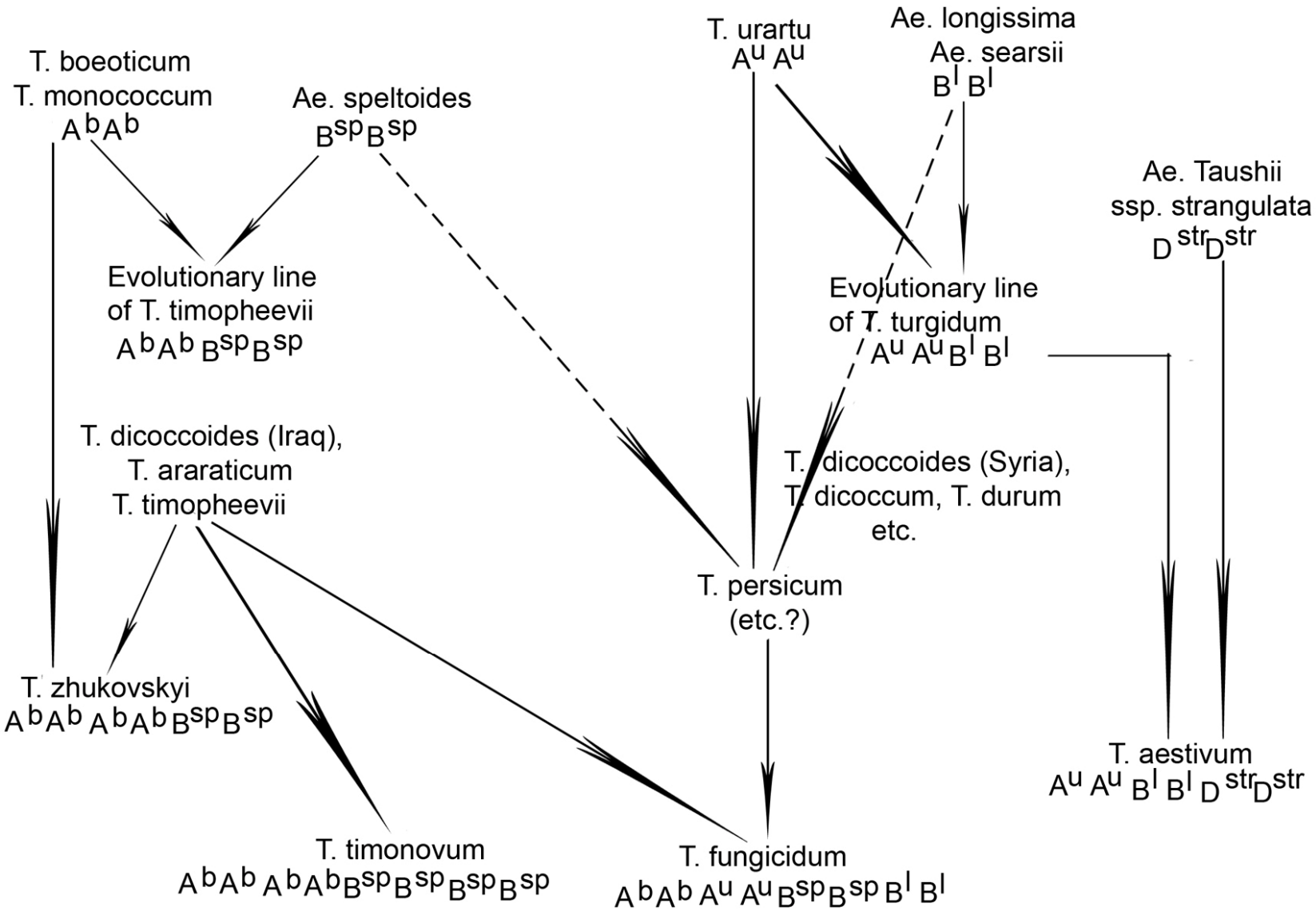
T. boeoticum
T. monococcum
A^bA^b
Ae. speltoides
$B^{sp}B^{sp}$
T. urartu
A^uA^u
Ae. longissima
Ae. searsii
B^lB^l
Ae. Taushii
ssp. strangulata
$D^{str}D^{str}$
Evolutionary line
of T. timopheevii
$A^bA^bB^{sp}B^{sp}$
Evolutionary line
of T. turgidum
$A^uA^uB^lB^l$
T. dicoccoides (Iraq),
T. araraticum
T. timopheevii
T. dicoccoides (Syria),
T. dicoccum, T. durum
etc.
T. persicum
(etc.?)
T. zhukovskyi
$A^bA^bA^bA^bB^{sp}B^{sp}$
T. timonovum
$A^bA^bA^bA^bB^{sp}B^{sp}B^{sp}B^{sp}$
T. fungicidum
$A^bA^bA^uA^uB^{sp}B^{sp}B^lB^l$
T. aestivum
$A^uA^uB^lB^lD^{str}D^{str}$