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PROTEIN MARKERS FOR INCREASING EFFICIENCY OF *TRITICEAE* DUM. GENETIC RESOURCES UTILISATION IN BREEDING

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To serve by effective basis for improvement of cultivated plants, genetic diversity stored in the gene banks, should be carefully and comprehensively evaluated and characterised (investigated). Collections should be rationally organised. Each accession should be identified and registered. The preservation of genetic constitution of accessions is included also into the category of basic problems. The objective of gene bank is to maintain the accession without change as regards its genetic constitution. It means preservation not only sample as such, but with its valuable properties, in particular adaptive etc. Understanding of genetic structure of biodiversity (relationships inside genepool or between structural elements of genetic diversity) is the important goal of gene bank activity. All above mentioned directions of activity should be developed to facilitate the use of germplasm for improvement of cultivars. Protein markers (PM) are successfully used in VIR since 1969 for increasing of utilisation efficiency of *Triticeae* Dum. genetic resources in plant breeding. Genetic resources of *Triticeae* are studied in following aspects: a) structure of biodiversity (intraspecies relations and interspecies relationships, genome analysis); b) identification and registration of genetic diversity and preparation of catalogues and data bases based on protein formulae; c) identification of duplicates, development of core collections; d) genetic integrity control; e) authorship rights control (for gene bank). Serological markers have been successful in genome analysis of *Triticum* L., *Aegilops* L., *Elytrigia* Desf., *Elymus* L., *Agropyron* Gaertn. Genetic differentiation of wheat, oat, rye biodiversity based on prolamin polymorphism was carried out. Genetic diversity of the most economically important *Triticeae* species was registered on prolamin patterns, catalogues and data base of protein (prolamin) formulae were composed.

1. INTRODUCTION

The world (global) plant genetic resources are considered all over the world as the basic source of improvement of agricultural cultures the coming decades. The creation of sources and donors of the important attributes, i.e. organization of pre-

2. SEED PROTEINS IN IDENTIFICATION AND REGISTRATION OF A *TRITICEAE* GENE POOL.

The most significant element in the work of botanist, geneticist and breeder is an identification of species,

breeding work, is in most cases based on world genetic resources or collections of cultivated plants and their wild relatives.

The disclosing of potential of genetic resources on the basic biological and selection attributes provides genetic base for realisation of the selection programs of various directions. As a whole the pre-breeding work includes all stages of work with plant germplasm from collecting, maintenance and study up to legal aspects of authorship on the donors and sources of valuable characters.

Traditional approaches founded on morphological characters which possess by some limitations. Proteins as a primary products of gene expression reveal small changing (mutations and so on) inaccessible to visual analysis. Protein markers (PM) are as a rule inherited codominantly and analysis of genotype is possible immediately by protein phenotype. PM are successfully used in VIR for decision of theoretical and applied problems of introduction, study, storage, reproduction and identification and registration of *Triticeae* genetic resources. Special attention is given to development of effective tools for breeding, variety testing and seed production.

Wheat gliadin pattern was taken as a basis. Pattern was divided into four zones. Within zone the main possible positions of components are numbered to the start. By means of such etalon pattern any variety or biotype of *Triticeae* may be recorded in form of prolamin formula. At present time cultivar and wild growing gene pool of wheat, rye, barley and other representatives of *Triticeae* have been written down in form of protein formulae; catalogues and data base of such formulae were

varieties, biotypes and other biological systems. Identification simplifies documentation of a gene pool of cultivated plants and their wild relatives with the aim of registration, preservation and effective usage in breeding. It is necessary to reveal and isolate desirable genotypes from complex natural varietal and hybrid populations. Identification of varieties and lines is especially actual for intensification of breeding and seed production, which demand the high accuracy and efficiency in seed control (1).

In order to elaborate the flexible and reliable nomenclature and system of pattern recording practically all intraspecies (or intrageneric) variability of assumed protein marker should be investigated. Due to this total investigation of species and genera biodiversity, almost all possible positions of protein components may be identified. Cultivars, wild growing populations, landraces from world collections have to be analysed. It was realised in N.I.Vavilov institute and this principle was laid in the base of nomenclatures and systems of recording electrophoretic components for many crops. This approach was developed firstly for wheat and then spread on all *Triticeae* and other crops (2).

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

released.

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

3.1. 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 germi-nation 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.

3.2. In collection of N.I.Vavilov Institute are stored the original cultivars of winter bread wheat (Mironovskaya 10, Soladin, Burgas 2, Orlandi, Neuzucht 14/14, WRN 48/49, Aurora, 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.

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

4.1. Genetic diversity of *Triticum, Aegilops, Erytrigia, Secale, Hordeum* collections stored in VIR were characterised 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.

4.2. Spelt wheat is the hexaploid wheat with genome composition AABBDD. Germplasm collection of this crop maintained at the VIR consists of 170 accessions originated from all principal regions of its cultivation in modern and former times. Polymorphism of seed storage proteins (gliadins) was used for characterisation of spelt wheat genetic diversity. From 170 analysed accessions 71 were characterised by one specific type of a gliadin electrohporetic pattern and they were identified as monomorphic. Other 99 accessions comprised from two up to eight biotypes with different gliadin patterns and they were

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 ω2 3_1 4 γ5 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. 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 characterised by translocation T1BL-1RS. Cultivars Hamlet and Linos were stable after reproduction as it was shown by protein markers. Cultivars Mironovskaya 10, Feldkrone, Perseus, named as global centres of spelt diversity. Eco-geographical classification of this crop was proposed by P.M.Zhukovskii (4). V.F.Dorofeev et al. (5) distinguished European (subsp. *spelta)* and Asian (subsp. *kuckuckianum)* subspecies of *T.spelta*. The first subspecies comprises two eco-geographical groups - proles *bavaricum* (accessions from Germany and Switzerland) and proles *ibericum* (accessions from Spain). These researchers did not divide the Asian subspecies into distinctive groups. Revealed in the work by gliadin analysis German and Spanish genetic groups correspond to the above mentioned eco-geographical groups of subsp.*spelta.* The differentiation European and Asian spelt revealed on gliadins may serve as a basis for more detailed eco-geographical and taxonomic classification of this crop. We identified some accessions, identified as polymorphic. Totally 42 gliadin pattern types were revealed for 86 accessions from different European countries and 29 pattern types – for 50 accessions from Azerbaijan and Central Asia (Tadjikistan, Turkmenistan, Uzbekistan). Spelt germplasm collection maintained at the N.I.Vavilov institute was registered in form of database of the protein formulae. The methods of cluster and principal component analyses have allowed to divide spelt germplasm collection into the some genetic groups. Groups of accessions from Germany and from some other European countries, and also groups from Spain and Tadjikistan were classified most precisely. Iranian accessions have not formed distinctive group based on the analysis of principal components. 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 gliadin markers mainly correspond to the ancient centres of spelt wheat cultivation, which were

Aegilops L. and *Elytrigia* Desf. A correspondence was established between the number and quantity of specific antigens of GSP and genetic interrelationships of cereal species or genomes (6). 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***

which can be attributed to doublets or to "genetically very close accessions". In case of confirmation of their resemblance by other methods, these accessions can be transferred in a rank of accessions with more rare reproduction cycle.

5. GENOME ANALYSIS OF WHEAT AND ITS RELATIVE CEREALS

5.1 Advantage of the immunological technique has been explored in a study of the phylogenetic relations between different genomes belonging to *Triticum, Aegilops, Elytrigia, Elymus and Agropyron.* Non-prolamin seed proteins of alcohol extract were successfully used as serological markers in genome analysis of wheat and its relative cereals (2,6).

5.2. 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 **group of wheat species, while *T.boeoticum* was the donor for the first genome of *timopheevii* group. A.V.Konarev et al. (7) 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 (5), morphological (5) and molecular (8) 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* (1,2). 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 (1,2,6).

5.3. Like *Trriticum* and *Aegilops* the genera *Elytrigia, Elymus* and *Agropyron* include species of different ploidy levels: 2n=14,28,42,56,80. Serological markers (GSP) have been successful in analysing the interrelation of genomes belonging to genera *Triticum, Aegilops, Elytrigia, Elymus* and *Agropyron* (1,6). We used monospecific immune sera against following genome specific protein antigens: Antigen A^b (*T.boeoticum* genome), antigen A^u (*T.urartu*), antigen B^l (Ae.longissima), antigen D (Ae.taushii), antigen Albumin 0,19 (*Triticum, Aegilops* species except *T.boeoticum* and *T.monococcum*) and other antigens (1). It was shown that some *Elytrygia* species, including *E.elongata* (2n=56,70), *E.intermedia* (2n=42), *E.trichophora* (2n=42) and *E.iuncea* (2n=42) possess antigens common for all three genomes of *T.aestivum*. Antigen 0,19 was also typical for them. Other *Elytrigia* and *Agropyron* species have common

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.* Antigens marking genome E have been found in proteins of 28-56 and 70 chromosome E.elongate races and some of *Elytrigia* and *Elymus* species (1,6). Antigens marking genome S have been found in most of *Elytrigia* species, almost always where are no antigens of genome E.

Antigens of S genome are present in the proteins of all *Agropyron* and many *Elymus* species. It should be noted that S-antigens distinctly differentiate *Elymus* species into two groups. *Elymus* species with genome S stand closer to *Elytrigia* species possessing genome S than to the species of their own genus (*Elymus*) which do not have this genome. *Elymus* species with S-antigens are well compatible with diploid *Elytrigia* species carrying genome S. All this agrees with the results of cytogenetic analysis and supports the opinion of those researches (9, 10) who suggest that only the species with genome S should be attributed to the *Elymus* genus. It has been suggested that only those forms which cross with wheat should remain in the *Elytrigia* genus. Similar classification based on cytogenetic genome analysis was proposed (10), whereby *Elytrigia* antigens only as for as one of these genomes is concerned. A series of *Elytrigia* and *Agropyron* species do not have wheat genome antigens or have the most common antigens. The representative of these species appear to be incompatible of poorly compatible in crosses with wheat species. Small degree of homology between genomes of T.aestivum and Ag.yezoense was demonstrated. More than 80 species belonging to Elytrigia, Elymus and Agropyron were proved to be immunochemically distinctive from wheat genomes. It was shown that the genetic compatibility between these species and wheat ones is in correspondence with the presence of protein antigens marking wheat genomes (1,6).

5.4. Own genome-specific antigens have been identified in proteins of the following diploid *Elytrigia* species: *E.elongata* (antigen E), *E.stipifolia, E.ferganensis* (antigen S), *E.juncea* (antigen J).

determining the degree of relationships between cereal species and genomes by antigen markers. In the recent years we have managed to develop a method of applying ELISA and immunoblotting techniques to GSP of *Triticeae* (12). The data derived from ELISA correspond well with the results of double immunodiffusion. All this enhances the possibility of a more efficient genome analysis by GSP antigens and of solving the problems faced in the search for markers of valuable qualities and characters of cereals.

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species which could be crossed with wheat were separated from *Agropyron* genus. Antigens of genome J have been identified as traces in 28- and 42- chromosome races of *E.juncea* as well as in polyploid forms of *E.elongata* (2n=56,70), *E.intermedia and E.trichophora* (2n=42). Genome J unites the *Elytrigia* species closely related by their antigenic composition to wheat species, allowing easy crossability (6).

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5.5. Thus, comparative analysis of *Elytigia, Elymus and Agropyron* showed that their diploid species carried genomes E, S and J which had specific antigens-markers. Judging by protein antinens, in most cases these genomes show similarity with wheat genomes either by einkorns (A^u,A^b) or by *Aegilops* (B^l, D). Polyploid species may involve, together with above mentioned genomes E, S and J, the genetic material from "wheat" genomes. Thus, a comparative analysis of *Triticum, Elytrigia, Elymus* and *Agropyron* species by grain proteins-antigens gave a possibility to correct the genome composition and also to elucidate genomic interrelation between the species. The problem of relationship between *Elytrigia* and wheat species is of principal interest because *Elytrigia* is often involved in hybridisation to produce *Triticum* x *Elytrigia* hybrids. However, a set of *Elytrigia* species used in distant hybridisation is very limited. Most of *Elytrigia* and *Elymus* never were involved in crossing with wheat because of lacking information on genetic proximity of genomes. Our results (1) partially compensate for this deficiency.

5.6. Above mentioned data on genome origin have been obtained by

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