BIOTECHNOLOGICAL APPROACHES FOR CEREAL CROPS IMPROVEMENT
PART II: USE OF MOLECULAR MARKERS IN CEREAL BREEDING

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ABSTRACT
Today the world population is increasing at the most rapid rate ever. It is fore cast that by the year 2050, the world's population will double to nearly 12 billion people. In fact it has been estimated that the world will need to produce more than twice as much food during the next 45 years as was produced since the beginning of agriculture 10 000 years ago. The continuous development of wheat, barley and maize varieties and lines with improved qualities to feed the world next future is of great necessity. At least for the foreseeable future, plant breeding will play a primary role. Still yet the conventional cereal breeding focuses on the selection of superior progeny from segregating populations, and selection is mostly based on phenotypic characters. Despite the use of many statistical and genetic tools to reduce the environmental effect on the selection of appropriate genotype, there is a confounding impact of environmental factors on phenotype. Breeding new variety takes between 8 to 15-20 years and the release of an improved variety cannot be really guaranteed and depend on the choice of the best parental combination. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient and reliable. Molecular marker technology and genetic engineering offer such a possibility by adopting a wide range of novel approaches to improving the efficiency of selection strategies in cereal breeding. This review aims at providing an overview of the state of art of the application of molecular marker techniques in molecular breeding of cereal crops and how this information could be used to increase the efficiency of plant breeding programs. It is also aimed to outline the recent developments of new improved varieties and lines by genetic engineering worldwide and in Bulgaria by the contribution of AgroBioInstitute.

Introduction
The extensive development of molecular techniques for genetic analysis in the last 2 decades has led to the increase of the knowledge of cereal genetics and our understanding of the architecture and behaviour of cereal genomes. Molecular techniques, in particular the use of molecular markers, have been used extensively to monitor DNA sequence variation in and among species and create new sources of genetic variation by introducing new and

favourable traits from landraces and wild grass species. Molecular markers bring new useful information on the determinism of trait variation and the organization of genetic diversity within cereals species of agricultural interest. This information can be used for efficiently managing and exploiting cereal genetic resources.

Three factors are required for the effective implementation of molecular markers in breeding programmes: (i) the availability of "user-frendly" markers (cheap, easy and reliable); (ii) the validation of markers across different genetic backgrounds; (iii) the possibility of implementing them within a breeding programme (1).

Improvements in marker detection systems and in the techniques used for identification of markers linked to useful traits, has enabled great advances to be made in recent years. While RFLP markers have been the basis for the development of the first genetic maps and identification of loci for agronomicaly important traits in cereals, valuable markers have also been generated from RAPDs and AFLPs. These 3 types of markers do not fit the first requirement for the effective implementation of markers in breeding programmes. However techniques are available to turn them into user-friendly markers such as STSs or SCARs. Simple sequence repeats (genomic SSR, EST-SSR) or microsatellite markers have been developed more recently for major crop plants and this marker system has been proven to be more effective for both mapping and implementation of breeding programs.

An increasing amount of sequence information and the determination the gene function in cereal crops will lead to the preferred use of new marker types, such as Single Nucleotide Polymorphisms (SNPs). High throughput genotyping methods, including DNA chips, allele specific PCR and primer extension approaches make SNPs especially attractive as genetic markers in trait association studies. The last marker system allows direct screening for the haplotype caring the desirable allele at gene /locus/ of interest.

The application of the molecular markers for genetic studies of cereals is too various but the main uses include:

- Assessment of genetic variability and characterization of germplasm collections;
- Variety fingerprinting for identification, accelerating the development of individuals that combine favourable alleles, contributing to hybrid performance prediction, establishment the distinctiveness of new cultivars prior to registration and protection;
- Estimation of genetic distances between populations, inbreds and breeding material;
- Facilitation the introgression of chromosomal segments from alien species and even tagging of specific genes;
- Detection of monogenic and qualitative trait loci (QTLs);
- Purity and stability of the seed and plant material
- Identification of sequences of useful candidate genes, etc.

Identification of markers linked to the trait of interest has been based on complete linkage maps and bulk segregant analysis (BSA). Alternative methods based on the construction of partial maps and combination of pedigree and marker information has also been proved useful in identifying marker/trait associations. The experimental data now confirm the efficiency of these approaches and the necessity of a revision of current breeding methods by utilizing molecular markers in breeding programmes (2, 3).

Genotype identification and large scale genetic diversity studies: searching for new sources of diversity and favourable alleles for variety improving

Genetic resources as new source for improving of elite material

Genetic erosion and habitat destruction by modern agriculture has increased the importance of germplasm characterization of plant material. At present time it is imperative to rationalize conservation and use of genetic resources to guide in the establishment of strategies that ensure the mantainance of genetic variability that is essential in plant breeding. Therefore, much attention was given to characterization of genetic resources and elite material at molecular level for better understanding and exploitation of genetic diversity.

The development of molecular marker techniques allowed an effective analysis of the global organization of genetic diversity within species, and evaluation of distance/similarity between individuals and populations. Identification of favourable alleles presently absent in a given breeding program, is therefore of key interest. Genetic resource collections are expected to possess such favourable alleles. The use of molecular markers allows assembling favourable alleles for quantitative traits in new developing varieties.

The characterization of elite material was started since 1985s because of its strategic interest for breeder's right protection and analysis of uniformity and distinctiveness of the material. These studies have confirmed that a high similarity for molecular markers is always associated with a high co-ancestry (4).

At present it is necessary for plant breeding programmes to have sufficient diversity available to allow the production of new varieties with improved productivity and abiotic and biotic stress tolerance. In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by measurement of genetic similarity (GS) of genetic distance (GD) between parents, since the later can be used as an estimation of expected genetic variance in different sets of segregating progenies derived from different crosses using a range of molecular markers.

A special attention has also received the use of molecular markers for evaluation of genetic diversity in wild crop species under different climatic conditions (5) and the organization of genetic diversity within large collections of genetic resources (6). Such information about the allele variation in wild cereal crops under stress conditions could be used in breeding programs for improving the stress tolerance of elite material (5). The information about the genetic structure of the collections can be extremely helpful for identification of representative accessions for breeding purposes. Two approaches have been found to be effective for selection of such accessions:

The first approach is based on the selection of a set of populations so called "Core-Collection" representing small number of populations which contains most (>90%) of the alleles and numerous traits of key interest presenting in a large number of populations. This "core-collection" could be used from breeders to improve elite material. Such concept has been applied to barley, maize and other crop species. The Barley Core Collection (BCC) which is consisting of a limited sample of accessions considered to represent the spectrum of genetic diversity available in the genus *Hordeum* (the primary gene pool - H. vulgare spp. vulgare and H. vulgare spp. spontaneum; the secondary gene pool – H. bulbosum and the tertiary gene pool – other *Hordeum* species) has been established (7). In maize a sub collection of 400 populations has been determined based on collecting site information, phenotypic traits etc. from a set of 2900 maize traditional open pollinated varieties from EU countries. A representative "core collection" of 96 populations characterizing with numerous traits of key interest for breeding (silage digestibility, drought tolerance, etc.) has been extracted using the information from 23 RFLP markers (6) and MSTRAT

software. This new collection contains 93% of the alleles present in the collection of 400 maize populations.

The second approach is to use marker data on both genetic resources and elite material in order to identify a limited collection of genetic resources that contains alleles not presenting in the collection of elite material. The selected small number of populations containing most of alleles not detected in elite varieties can be used as parent of interest, to be crossed with elite material to develop new breeding populations. This approach has been used for selection of a set of small number of maize population (10) which display alleles not presenting in elite inbreds, based on the SSR data from comparison of 276 maize populations and 90 elite inbred European and American lines.

Recently, a large scale genotyping and gene diversity studies of wheat, barley and maize genetic resources and elite material in Bulgaria was initiated by means of morphological and molecular markers (Todorovska et al., non published data).

Application of molecular markers in gene diversity studies of cereals

DNA fingerprinting of cereals species and cultivated varieties has a long scientific history. RFLP was the first marker system used in genotyping and gene diversity studies in wheat, barley and maize, which has been considered as state-of- art for a long time but with improving of marker technologies in the last decade new marker types such as RAPDs, AFLPs, SSRs and SNPs were considered to be more effective, cheap and informative.

Gene diversity assessment and determination of exotic germplasm introgression in hexaploid wheat

In wheat RFLPs have been used for fingerprinting of wheat cultivars (8), assessment of genetic diversity (9, 10, 11, 12, 13) and mapping. RAPDs (14) have been used in the study of intervarietal relationships among hexaploid wheats. However, a relatively low level of polymorphism has been observed among cultivated lines and /or cultivars with both markers. According to Siedler (9) RFLP analysis revealed 4.7 polymorphisms per probe/enzyme combinations among 81 European cultivars, whereas RAPDs primers generated only 1.8 polymorphisms/primer among 15 wheat cultivars (14). RFLPs were proven to be more informative in determination the introgression of alien germplasm into hexaploid wheat (Alexandrova et al., non published data). Markers for 1U chromosome introgression that carries a new gene for resistance to powdery mildew in relation to loci Glu-1, Gli-1, Xpsr 688, Xpsr 634, Xpsr 162, as well as the microsatelite primer pair WMS 135 were obtained. These markers for intergeneral transfer of genetic material from *Aegilops kotschyi* are necessary for speeding up the further breeding process in Bulgaria, directed towards developing of translocation lines (15). The SSR analysis has been shown to be efficient for marker identification in the case of further distinguishing of lines with identical genomic constitution. In addition to molecular analyses the study of the locus Glu-U1 provided with information for the presence of two high molecular subunits in the profile of the derivative lines, which correlated with the dough quality improvement (16).

AFLPs have been extensively used in genetic diversity assessment among wheat cultivars from the Pacific Northwest (17, 18).

In wheat, two independent studies showed that SSR markers provide a greater level of intraspecific polymorphism than RFLP and RAPD (19, 20) and prompted the development of more than 500 SSR markers (19, 21, 22, 23, 24, 25, 26). At present, 862 new wheat SSR markers were developed and mapped by Génoplante. In addition, the SSR consensus map constructed by Somers (27) by fusing several genetic maps maximizes the integration of

genetic mapping information from different sources thus increasing the usefulness of SSR markers in genotyping and mapping analysis. Independently or in combination with AFLP markers, SSRs have been extensively used in a number of gene diversity studies in wheat (21, 28, 20, 29, 30, 31) Assessment of genetic diversity in 31 hexaploid wheat (*Tr. aestivum* L.) from Bulgarian germplasm collection (DZI - G.Toshevo) and 9 wheat varieties originating from Belgium (CRAW, Gembloux) was initiated using a set of 24 wheat microsatellite markers (Todorovska et al., non published data). A total of **202** alleles with an average **7.48** alleles per locus were detected at **27** microsatellite loci in this study. The average number of alleles per locus in the present study is much higher than the reported by Plaschke (20) (**6.2 alleles/locus**); Stachel (32) (**4.8 alleles/locus**). Similar results have been reported from Prasad (29) (**7.4 alleles/locus**) for 55 wheat genotypes, originating from 29 countries, representing six continents. Much more alleles/locus (**10.5**) have been reported by Roder (33) for 500 European wheat varieties and Huang (30) for 998 accessions (**18.1 alleles/locus**) and this is probably due to the large number of wheat genotypes included in both studies.

The largest number of alleles per locus and gene diversity content in studied 40 bred wheat from Bulgarian and Belgian germplasm collections occurred in B genome, compared to A and D genomes respectively. The results are comparable to those obtained by Huang (30) for 998 accessions of hexaploid bread wheat (*T. aestivum* L.), from IPK, Gatersleben originating from 68 counties of five continents. Mean gene diversity for the three genomes A, B and D in this study was **0.578**, **0.669** and **0.667** respectively. The highest gene diversity content among all 7 homeologous chromosome groups in 31 wheat varieties from Bulgarian collection was observed in 7th chromosome, following by 5th and 6th chromosomes. The lowest genetic variation exists in the chromosomes of homeologous group 4 as it have been also reported by Boyko (34), Ma (35) and Huang (30) for the chromosome 4D of *Aegilops tauschii*, the short arm of chromosome 4R of rye and 4 chromosome group of hexaploid bread wheat respectively. Different levels of gene diversity content among chromosomes of South-Eastern European and Western and Central European wheat was observed and this is probably due to different geographical origin of Bulgarian and Belgian collections **(Fig. 1).**

The gene diversity for 27 microsatellite loci in 40 wheat varieties (Bulgarian and Belgium germplasm collections) varied from 0.31 to 0.90 with an average **GD=0.638**. A relatively large genetic diversity has been detected in wheat germplasm from the gene bank at Gatersleben (0.77) (30). Few rare alleles (with frequency lower than 0.05) were also detected at several loci in some elite Bulgarian varieties which could be exploit for broadening the allelic diversity and as a potential for cultivar improvement of wheat in future breeding programmes.

Gene diversity assessment in maize

In maize, restriction fragment length polymorphisms (RFLPs) have long been used in estimation of genetic diversity (36, 37, 38, 39). The greatest advantage of RFLPs in maize analysis is the large number of polymorphic loci found in breeding materials (38). Studies with elite lines from U.S. Corn Belt and European maize inbred lines showed that RFLPs are suitable for defining heterotic groups, assigning inbred lines to such groups, in revealing genetic relationships among lines and identifying diverse germplasm sources. Higher level of polymorphism has been reported for American population in comparison to European ones (respectively, 12.3 and 9.6 alleles per locus) and only few alleles were found to be specific for European populations (40). Furthermore, a simplified Bulk-

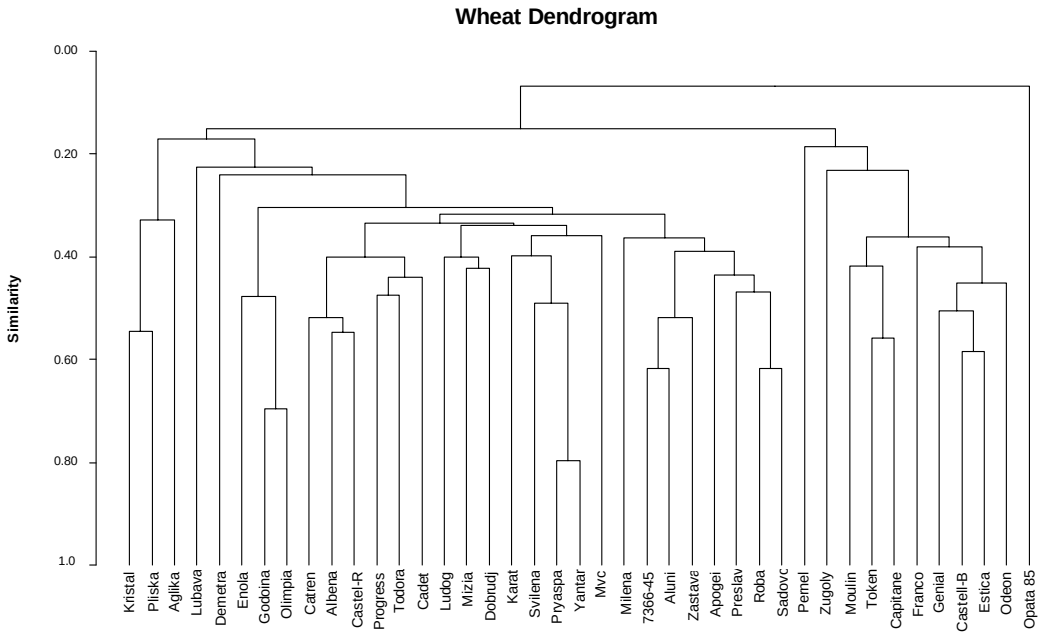

Fig. 1. Phenogramme of 40 hexaploid wheat varieties from South-Eastern and Western Europe (DZI- G. Toshevo collection, Bulgaria), genotyped at 27 nuclear microsatellite loci.

RFLP method (41) has been developed in order to analyze large set of maize populations. The analysis of a representative sample of the French INRA-PROMAIS gene bank has been performed first (42) followed by the analysis of more than 450 European maize populations (43, 6). These studies lead to classification of populations in genetic groups and showed a clear difference according to latitude, suggesting several independent introductions of maize in Europe. However, RFLP assay is labor intensive and time consuming and, therefore, increasingly substituted by marker techniques based on PCR such as RAPD, AFLP, SSR and SNPs.

RAPD markers have been used in the analyses of genetic distance among segregant lines (44) to predict the best crosses among lines for hybrid development (45), to assess genetic diversity among collection of native maize (46) and genetic relationships among 81 maize accessions (47).

Amplified Fragment Length Polymorphism markers (AFLPs) have been used in investigation the correlations between genetic distance and heterose for profit (48), genetic variability among U.S. dent lines (49), diversity among inbred lines in temperate climates (50) and relationships among precocious European maize lines (51) and tropical inbred lines (52).

Recently developed SSR markers have been extensively used in maize genetic studies such as construction of linkage maps and QTL mapping (53, 54) or the analysis of genetic diversity and evolution (55, 49, 56, 57).

Genetic diversity assessment among 56 Bulgarian maize inbred lines from the collections of gene banks of IPGR–Sadovo and Institute of maize-Kneja representing valuable source of genes for breeding programmes based on both phenotypic charac-

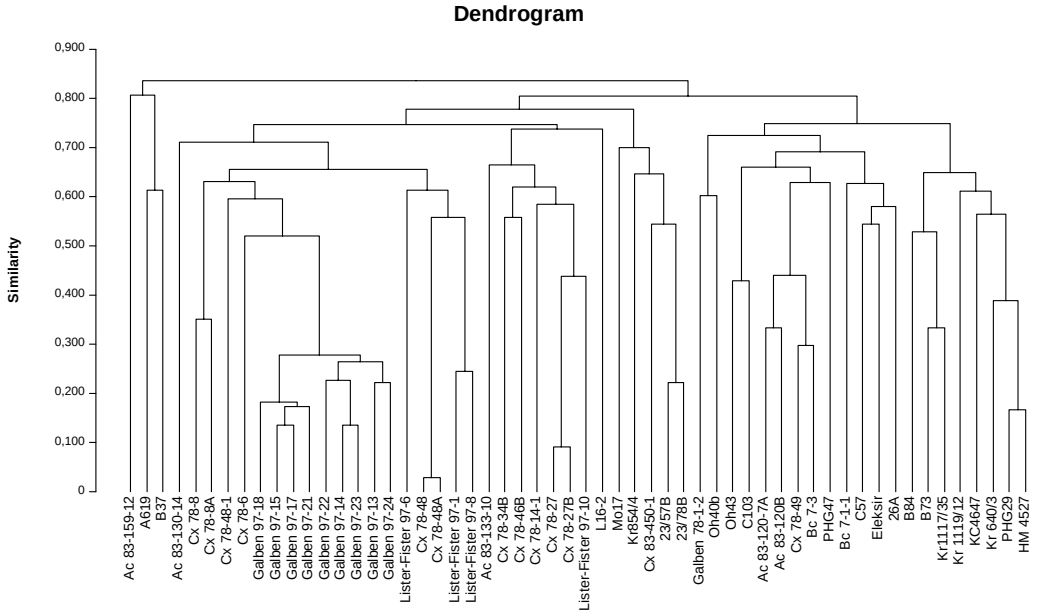

Fig. 2. Phenogramme of 57 maize inbred lines (from the gene bank of IPGR –Sadovo and Instiute of Maize - Kneja, Bulgaria) genotyped at 18 microsatellite loci.

ters and microsatellite analysis was initiated (Kostova and Todorovska, non published data). Eighteen microsatellite markers distributed throughout the maize genome were chosen based on their PIC and localization near to loci previously described (58, 59, 60) to be involved in drought tolerance. A total of 173 alleles with an average 9.61 alleles per locus were detected is study. The average number of alleles /locus obtained in this study is higher compared to those previously reported in maize inbred diversity studies. Lu & Bernardo (56), characterizing 40 US inbreds lines with 83 SSR markers reported 4.9 alleles per SSR locus, Senior (55) found 5.0 alleles/locus for 94 elite US maize inbreds with 70 SSR markers, and Vaz Patto (61) reported 5.33 alleles per locus using 104 Portuguese and US inbreds with 15 SSR. In addition, Pejic (49) using 33 inbreds from the US cornbelt and 27 SSRs, reported 6.8 alleles/locus and Enoki (62), studying 65 inbred lines adapted to cold regions of Japan and imported American materials with 60 SSRs reported 7.3 alleles per locus. The higher values in the last two reports can probably be explained by a large percentage of direpeats used in both studies (31 out of the 60 SSR in Enoki's study (62)) that are known for their potential to generate large number of alleles in plants.

The obtained mean He value (**0.733)** for Bulgarian inbreds is higher than those reported from previous studies of genetic diversity between US maize inbreds (63, 55, 61) but similar to those obtained from Pejic (49) in US cornbelt (0.72), **(Fig. 2).** The preliminary exclusion of SSR primers with low discriminatory power done by Enoki (62) seems to be a cause of the higher He value detected (0.69) in 65 inbred lines. No significant correlation between the repeat size and the number of alleles was found herein.

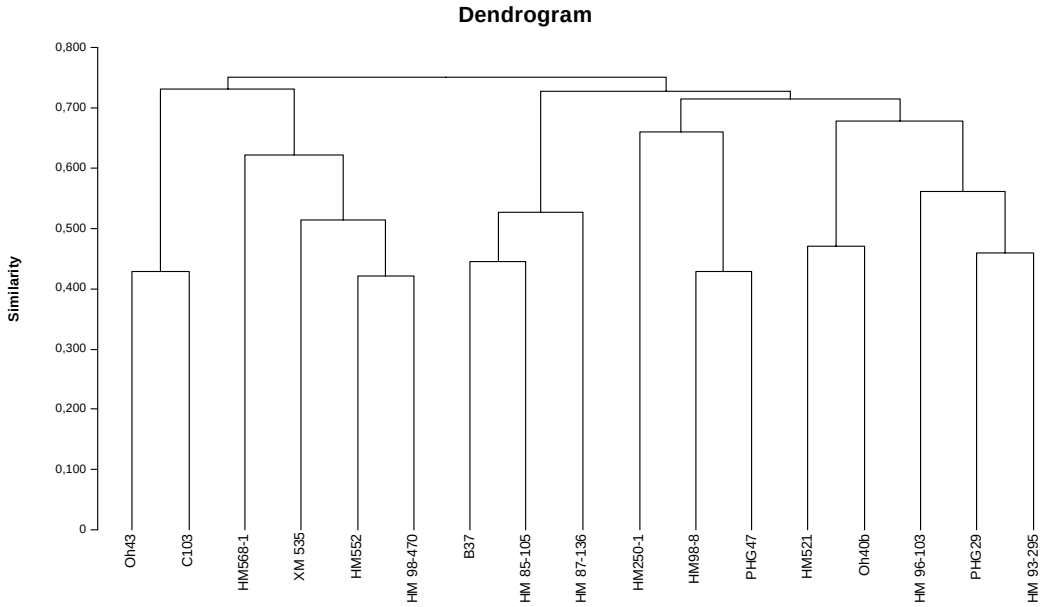

Fig. 3. Phenogramme of 11 maize mutant lines and their initial lines genotyped at 18 microsatellite loci.

Chemical mutagenesis induced diversity in elite maize germplasm currently grown in Bulgaria (64) was assessed using microsatellite and PCR–based DNA markers. Several mutant inbred lines with improved GCA and SCA for grain yield, proven by their predominance in Bulgarian breeding programmes and shifts in flowering time as compared to the initial inbred Iowa Superstiff Stalk (SSS) lines - B37, B73, B84 and Lankaster inbred line – Oh43 were selected. The data showed mutagenesis induced polymorphism in the coding sequences of two important for determination of flowering time transcription factors (*dwarf* 8, chromosome1, 198.5cM and *indeterminate* 1, chromosome 1, 175.0cM). In addition, microsatellite analysis at 18 genomic loci distributed over all 10 chromosomes confirmed the usefulness of chemical mutagenesis for generation of genetic diversity within elite maize germplasm (Kostova, Todorovska, Christov, non published data), **(Fig. 3).**

Gene diversity assessment in barley

In barley, RFLPs have been extensively used for study genetic diversity among European spring and winter barley (65, 66). RAPDs have been used in the studies of genetic diversity of wheat cultivars but the level of the obtained polymorphism was too lower to differentiate them (67). In comparison to wheat RAPD markers are more polymorphic in barley. Tinker (68) have reported that all 27 inbred barley lines could be distinguished by 9 RAPD polymorphisms using a total of 7 arbitrary primers. Since such polymorphism has been found between lines with the value of *r* as high as 0.92, RAPD polymorphisms might be useful in distinguishing among barley lines that share a high degree of similarity. RFLPs in combination with RAPDs and biochemical markers have been used for genotyping and studying the genetic diversity of Bulgarian barley cultivars and their somaclonal variants (69, 70). High level of genetic similarity among

studied Bulgarian cultivars (**0.93**) was obtained based on both RFLP and RAPD markers. The study showed that RAPD markers are more suitable for discrimination of elite barley varieties with high coancestry. Overall a total of 15 polymorphic phenotypes were found among Bulgarian cultivars using 11 out of 25 tested RAPD primers (70). RAPD assay proved also to be more useful in determination the genetic variability induced in tissue culture derived (TCD) lines of barley (69). Tissue culture method was chosen as one of the approaches applied for diversification of elite material in cereals. Four previously characterized cultivars (70) were subjected to tissue culture using high concentration of 2.4D in the callus induction medium (71). Some of the selected TCD lines were evaluated by field test and molecular markers were used as measures of the frequency and inheritance of the induced alterations. Heritable polymorphic RAPD fragments were obtained in few TCD lines deriving from cvs. Ruen and Karnobat. In addition, RFLPs was also detected in sequences coding C hordeins in TCD line of cv. Jubiley. For the first time in the literature was reported the usefulness of RAPD markers in discrimination of barley TCD lines possessing valuable agronomic characters. Both studies showed that the combination of few marker systems and phenotypic trait evaluations in gene diversity studies can better reveal the level of genetic variation into the genome of cereals and could be effectively used for selection of the best parental combination for the purposes of cereal breeding.

AFLPs has also been used in genotyping and gene diversity studies in barley (72).

In barley, SSR markers have been extensively used in a number of studies including genotyping (73, 74, 75, 76) genetic diversity assessment (77, 78, 79,) and mapping (80, 81, 82). An extensive genotyping and gene diversity studies of Bulgarian and Cypriot barley germplasm collections was initiated using SSR markers (Todorovska et al., non published data). 61 accessions covering a wide spectrum of genetic diversity of cultivated barley gene pool from different agro-geographical areas representing part of Bulgarian (Institute of Agriculture, Karnobat, Bulgaria) and Cypriot (Agricultural Research Institute, Nicosia, Cyprus) collections were characterized at 11 genomic and 3 EST-derived SSR loci. In total, 90 alleles with an average 6.43 alleles per locus were identified at 14 polymorphic SSR loci distributed on all barley chromosomes (HVM03, HVM27, HVM30, HVM 36, HVM40, HVM43, HVM49, HVM60, HVM62, HVM67, HVM74, GBM1022, GBM1045 and GBM1060).

Gene diversity estimated for all 14 microsatellite markers varied from 0.00 for HVM30 to 0.955 for GBM 1022 with a **mean GD=0.601**. Site-of-origin ecogeographic variation in the level of genetic diversity was observed. European and Cypriot accessions possessing rare alleles at many different loci were detected and could be effectively used for broadening the allelic diversity and the potential for cultivar improvement of barley, **(Fig. 4).**

SSR markers have been used for estimation of the genetic diversity in 163 barley genotypes from the collection of the IPK Genebank, Germany (78). The authors reported mean value of genetic diversity in the wild form and landraces 0.74 and extremely high gene diversity (GD=0.72) for cultivated barley. Balvinskaja (76) reported a mean GD=0.415 for 32 South-Ukrainian winter barley using 20 genomic SSR markers. Similar results have also been obtained for 26 Tunisian winter barley using 17 microsatellite markers (79), (MGD=0.45). Genetic diversity study in three groups of barley germplasm: 22 Hordeum vulgare spontaneum accessions, 32 Hordeum vulgare vulgare and 96 elite varieties and lines with 42 SSR markers showed a total of 687 alleles (117 unique alleles) with an average 16.3 alleles /locus

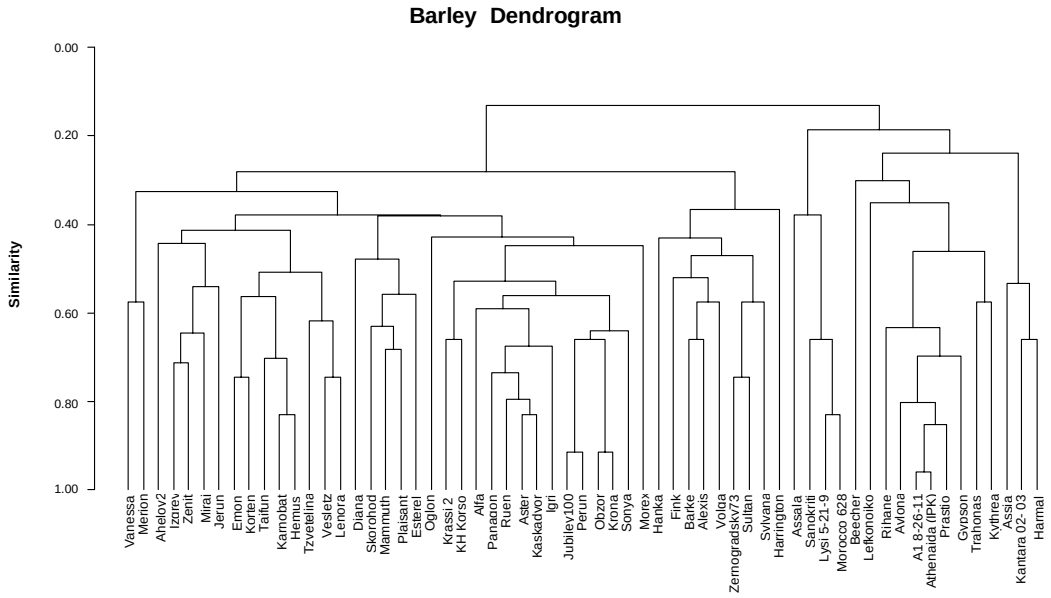

Fig. 4. Phenogramme of 61 European (collection of the Institute of Agriculture- Karnobat, Bulgaria) and Cypriot (ARI –Nicosia, Cyprus collection) barley varieties genotyped at 11 nuclear and 3 EST-SSR loci.

and (83). The authors reported different level of mean GD for the three groups of barley. The highest level of MGD=0.79 has been obtained for Hordeum vulgare spontaneum accessions, following by Hordeum vulgare vulgare (MGD=0.75) and elite material (MGD=0.43). Few studies have proved that wild barley could be effectively exploit for broadening the genetic variation in cultivated barley and especially recently developed elite varieties and lines (5, 83).

III. Molecular mapping, trait tagging and marker–assisted selection in cereals - the present

A large number of cereal studies have used molecular markers as a tool to identify major genes, QTLs, or to introduce new characters in elite germplasm. In wheat, for example molecular markers have been identified that are associated with more than 40 traits of economic importance such as grain protein content, preharvest sprouting tolerance, vernalization response, dwarfing genes, bread-making quality, leaf rust resistance, cereal cyst nematode resistance, etc. (2).

Knowing the location of these genes/traits it is possible to apply marker-assisted selection (MAS) in cereal crops, because one of the main objectives of plant breeding is the introgression of one or more favourable genes from a donor parent into the background of an elite variety. Marker–assisted selection provides a potential for increasing selection efficiency by allowing for earlier selection and reducing plant population size used during the selection. The predictive value of genetic markers used in MAS depends on their inherent repeatability, map position and linkage with economically important traits (quantitative or qualitative). The presence of a tight linkage (<10cM) between qualitative trait/s/ and a genetic marker/s/ may be useful in MAS to increase gain from selection.

For simply inherited traits such as desease resistance the conventional PCR,

which requires a small amount of DNA for screening of large populations of segregating progenies became useful. The application of closely linked PCR-based markers for the transmission of resistance gene(s) against one of the most important diseases of winter barley in Europe -*Barley yellow mosaic virus* is now successful and efficient (84). Molecular markers closely linked to the mayor QTL involved in FHB (*Fusarium* head blight) resistance have been found by Buerstmayer (85) and raised possibility of using MAS for introducing alleles into elite wheat varieties. A major MAS focus in the Monsanto winter wheat programmes is concentrated on the transmission of resistance to FHB from Chinese wheat variety Sumai 3 into elite varieties with microsatellite markers (sequence tagged microsatellite sites, STMS). Other current MAS targets of this programme are Lr37 (a single gene encoding resistance to leaf rust), the wheat/rye translocation 1BL.1BRS, and the resistance to BYDV (barley yellow dwarf virus) introgressed from the related grass species *Thinopyrum intermedium*.

At present one of the most important MAS focus in the Bulgarian winter wheat programmes is the transmission of resistance to FHB from cv. Sumai 3, resistance to leaf rust by introducing a single gene Lr19 from cv. Super seri into elite varieties and wheat /rye translocation 1BL.1BRS by means of SSR markers.

Conclusions

Recent development of DNA marker based technologies together with the concept of marker–assisted selection provides one of the most powerful genomics tool for new solution for selecting and maintaining desirable genotype. Once molecular markers closely linked to the trait of key interest are identified, marker–assisted selection can be performed in early segregating populations and at juvenile stage from an early generation. Marker-assisted selection can be used to pyramiding genes, and especially resistance genes, with the ultimate goal of producing varieties with several desirable traits. Thus, with marker-assisted selection it is now possible to reduce the process of selection of a population with desirable character/s/ from segregation progenies. However at present MAS is a capital–intensive endeavour and needs of high investments. The success of MAS to improve the efficiency of conventional breeding systems is thus largely dependent on the development of automation platforms which will reduce the cost of the unit cost per assay, and so allow an increase in the number of assays possible.

With the development of automatable assays is expected MAS to be increasingly applied (1) for traits which are difficult to manage via phenotype, because of low penetrance and/or complex inheritance; (2) for maintenance of recessive alleles in backcrossing pedigrees; and for (3) making choices of parents in crossing programmes, in order to assure minimal levels of duplication of alleles across sets of genes targeted for selection, and fixation in those for which no variation is preffered.

REFERENCES

1. **Langridge P., Chalmers K.** (1998) Techniques for marker development. In: Proc. 9th International Wheat Genetics Symposium (A.E. Slinkard, Ed.), Saskatoon. 2–7 Aug. 1998. Univ. Extension Press, Saskatoon, SK, Canada, 107–117.

2. **Gupta P.K., Varshney R.K., Sharma P.C., Ramesh B**. (1999) Plant Breed., **118,** 369-390.

3. **Korzun V.** (2003) Molecular markers and their application in cereals breeding. In: Marker Assited Selection: A fast track to increase genetic gain in plant and animal breeding Session I: MAS in plants (http://www.fao.org/biotech/docs).

4. **Melchinger A.E.** (1999) Genetic diversity and heterosis. In: The genetic and exploitation of heterosis in crops, (C.J.G.P.S., Ed), American Society of Agronomy Crop Science Society of America, Madison, Wisconsin, USA, 99-118.

5. **Forster B.P., Ellis R.P., Thomas W.T.B., Newton A.C., Tuberosa R., This D., El-Enein R.A.,**

Bahri M.H., Salem M. Ben (2000) J. Experimental Botany, **51,** 19-27.
6. **Gautheir P., Gouesnard B., Dallard J., Redaelli R., Rebourg C., Charcosset A., Boyat A.** (2002) Theor. Appl. Genet., **105,** 91-99.
7. **Hintum ThLJ van** (1992) The barley core collection, workshop summary. In: Barley genetics 6, vol 2. (Proc 6th Int Barley Genet Symp.), (L. Munck, Ed.), Munksgaard International Publishers Ltd, Helsinborg, Sweden, 703-707.
8. **Vaccino P., Accerbi M., Corbellini M**. (1993) Theor. Appl. Genet., **86,** 833-836.
9. **Siedler H., Messmer M.M., Schachermayr G.M., Winzeler H., Winzeler M., Keller B.** (1994) Theor. Appl. Genet., **88,** 994-1003.
10. **Kim H.S., Ward R.W.** (1997) Theor. Appl. Genet., **94,** 472-479.
11. **Bryan G.J., Stephenson P., Collins A., Kirby J., Smith J.B., Gale M.D**. (1999) Theor. Appl. Genet., **99,** 192-198.
12. **Kim H.S., Ward R.W.** (2000) Euphytica, **115,** 197-208.
13. **Paull J.G., Chalmers K.J., Karakousis A., Krestcher J.M., Manning S., Landridge P.** (1988) Theor. Appl. Genet., **96,** 435-466.
14. **Joshi C.P., Nguyen H.T.** (1993) Plant Sci., **93**, 95-103.
15. **Todorovska E., Alexandrova N., Marinova E., Atanassov A.** (2001) Biotechnol.&Biotechnol. Eq., **15(2)** supplement, 14-16.
16. **Alexandrova N., Papasova N., Marinova E.** (1997) Selskostopanska Akademia, Nauchni trudove (in Bulg.), **4**, 44-46.
17. **Barrett B.A., Kidwall K.K** (1998) Crop Sci., **38,** 1261- 1271.
18. **Barrett B.A., Kidwall K.K., Fox P.N.** (1998) Crop Sci., **38,** 1271- 1278.
19. **Roder M.S., Plaschke J., Konig S.U., Borner A., Sorrells M.E., Tanksley S.D., Ganal M.W.** (1995) Mol. Gen. Genet., **246,** 327-333.
20. **Plaschke J., Ganal M.W., Roder M.S.** (1995) Theor. Appl. Genet., **91,** 1001-1007.
21. **Devos K.M., Bryan G.J., Collins A.J., Stephenson P., Gale M.D.** (1995) Theor. Appl. Genet., **90,** 247-252.
22. **Plaschke J., Borner A., Wendehake K., Ganal M.W., Roder M.S.** (1996) Euphytica, **89**, 33-40.
23. **Bryan G.J., Collins A.J., Stephenson P., Orry A., Smith J.B., Gale M.D.** (1997) Theor. Appl. Genet., **94,** 557-563.
24. **Roder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P., Ganal M.W.** (1998) Genetics, **149,** 2007-2023.
25. **Stephenson P., Bryan G., Kirbi J., Collins A., Devos K., Busso C., Gale M.** (1998) Theor. Appl. Genet., **97,** 946-949.
26. **Gupta P.K., Balyan H.S, Edwards K.J., Isaac P., Korzun V., Roder M., Gautier M.-F., Joudrier P., Schlatter A.R., Dubcovsky J., De la Pena R.C., Khairallah M., Penner G., Hayden M.J., Sharp P., Keller B., Wang R.C.C., Hardouin J.P., Jack P., Leroy P.** (2002) Theor. Appl. Genet., **105,** 413-422.
27. **Somers D.J., Isaac P., Edwards K.** (2004) Theor. Appl. Genet., **109,** 1105-1114.
28. **Lee S.J., G. Penner, Devos. K.** (1995) Genome, **38,** 1037 – 1040.
29. **Prasad M., Varshney R.K., Roy J.K., Balyan H.S., Gupta P.K.** (2000) Theor. Appl. Genet., **100,** 584 –592.
30. **Huang X.Q., Borner A., Roder M.S., Ganal M.W** (2002) Theor. Appl. Genet., **105**, 699-707.
31. **Manifesto M.M, Schlatter A.R., Hopp H.E., Suarez E.Y., Dubcovski J.** (2001) Crop Sci., **41,** 682-690.
32. **Stachel M., Lelley T., Grausgruber H., Vollmann J.** (2000) Theor. Appl. Genet., **100,** 242-248.
33. **Roder M.S., Wendehake K., Korzun V., Bredemeijer G., Laborie D., Bertrand L., Isaac P., Rendell S., Jackson J., Cooke R.J., Vosman B., Ganal M.W.** (2002) Theor. Appl. Genet., **106,** 67-73.
34. **Boyko E.V., Gill K.S., Mickelson-Young L., Nasuda S., Raupp W.J., Ziegle J. N., Singh S., Hassawi D.S., Fritz A.K., Namuth D., Lapitan N.V.L., Gill B.S.** (1999) Theor. Appl. Genet., **99,** 16-26.
35. **Ma X.F., Wanous M.K., Houchins K., Rodriguez Milla M.A., Goicoechea P.G., Wang Z., Xie M., Gustafson G.P.** (2001) Theor. Appl. Genet., **102,** 517-523.
36. **Melchinger A.E., Lee M., Lamkey K.R.** (1990) Crop Sci., **30,** 1033-1040.
37. **Melchinger A.E., Boppenmaier J., Dhilon B.S.** (1992) Theor. Appl. Genet., **84,** 672-681.
38. **Messmer M.M., Melchinger A.E., Boppenmaier J**. (1992) Crop Sci., **32,** 1301-1309.
39. **Gerdes J.T., Tracy W.F.** (1994) Crop Sci., **34**, 26-33.
40. **Rebourg C., Chastanet M., Gouesnard B., Welcker C., Dubreuil P., Charcosset A.** (2003) Theor. Appl. Genet., **106**, 895-903.
41. **Dubreuil P., Charcosser A.** (1999) Theor. Appl. Genet., **99,** 473-480.
42. **Rebourg C., Dubreuil P., Charcosset A**. (1999)

Maydica, **44,** 237-249.
43. **Rebourg C., Gouesnard B., Charcosset A**. (2001) Heredity, **86,** 574-587.
44. **Marsan P.A., Egidy G., Monfredini G., Disilvestro S., Motto M.** (1993) Maydica, **38,** 259-264.
45. **Lanza L.L.B., De Souza Jr.C.L, Ottoboni L.M.M, Vieira L.M.C., De Souza A.P.** (1997) Theor. Appl. Genet., **94**, 1023-1030.
46. **Moeller D.A., Schaal B.A** (1999) Theor. Appl. Genet., **99,** 1061-1067.
47. **Valdemar C., Ruas C., Ferreira J.** (2004) Genet. Mol. Biol., **27(2)**, 228-236.
48. **Ajmone-Marsan P., Castiglioni P., Fusari F., Kuiper M., Motto M.** (1998) Theor. Appl. Genet., **98**, 219-22.
49. **Pejic I., Ajemone-Marsan P., Mofgante M., Kozumplick V., Castiglioni P., Taramino G., Motto M.** (1998) Theor. Appl. Genet., **97,** 1248-1255.
50. **Chitto A., Bertolini M., Hartings H**. (2000) Maydica, **45,** 257-266.
51. **Lubberstedt T., Melchinger A.E., Duble C.** (2000) Crop Sci., **40,** 784-791.
52. **Oliveira K., Laborda P., Garcia A., Paterniani M., Souza A.** (2004) Hereditas, **140**, 24-33.
53. **Romero-Severson J.** (1998) Maize microsatellite-RFLP consensus map Maize DB(http://www.agron.missouri.edu).
54. **Frova C., Krajewski P., di Fonzo N., Villa Sari-Gorla M.** (1999) Theor. Appl. Genet., **99,** 280-288.
55. **Senior M.L., Murphy J.P., Goodmanb M.M., Stuber C.W.** (1998) Crop Sci., **38,** 1088-1098.
56. **Lu H., Bernardo R.** (2001) Theor. Appl. Genet., **100**, 552-556.
57. **Matsuoka Y., Mitchel S.E., Kresovich S., Goodman M, Doebley J.** (2002) Theor. Appl. Genet., **104,** 436-450.
58. **Quarrie S., Lanzic-Jancic V., Kovacevic A., Steed, Pekic S.** (1999) Journal of Experimental Botany, **50,** 1299-1306.
59. **Tuberosa R., Salvi S., Sanguineti M., Landi P., Maccaferri M., Conti S.** (2002) Annals of Botany, **89,** 941-963.
60. **Li Xin-Hai, Xian-de Liu, Ming-Shun Li, Shi-Huang Zhang** (2003) Acta Botanica Sinica, **45(7)**, 852-857.
61. **Vaz Patto M.C., Satovic Z., Peĝo S., Fevereiro P.** (2004) Euphytica, **137,** 63-72.
62. **Enoki H., Sato H., Koinuma K.** (2002) Theor. Appl. Genet., **104(8)**,1270-1277.
63. **Smith J.S.C., Chin E.C.L., Shu H., Smith O.S., Wall S.J., Senior M.L., Mitchell S.E., Kresovich S., Ziegle J.** (1997) Theor. Appl. Genet., **95,** 163–173.
64. **Christov N., Todorovska E., Fasoula D., Ioannides I., Atanassov A., Hristov K.** (2004) Acta Biologica Iugoslavica, Genetica, **36**, 47-60.
65. **Melchinger A.E., Graner A., Singh M., Messmer M.M** (1994) Crop Sci., **34,** 1191-1199.
66. **Graner A., Bauer E., Kellermann A., Kirchner S., Muraya J.K., Jahoor A., Wenzel G.** (1994) Barley Genetics Newsletter, **23,** 53-59.
67. **Devos K.M., Bryan G.D., Collins A.J., Stephenson P., Gale M.D.** (1995) Theor. Appl. Genet., **85**, 784-792.
68. **Tinker N.A., Fortin M.G., Miller D.E**. (1993) Theor. Appl. Genet., **85,** 976-984.
69. **Todorovska E., Trifonova A., Petrova M., Vitanova Z., Marinova E., Gramatikova M., Valcheva D., Zaprianov S., Mersinkov N., Atanassov A.** (1997) J. Plant Breeding, **116,** 511-517.
70. **Todorovska E., Trifonova A., Atanassov A.** (2003) Euphytica, **129,** 325-336.
71. **Vitanova Z., Vitanov V., Trifonova A., Savova D., Atanassov A.** (1995) Plant Cell Reports, **14,** 437-441.
72. **Hayes P.M., Cereno J., Witsenboer H., Kuiper M., Zabeau M., Sato K., Kleinhofs A., Kudrna D., Kilian A., Saghai-Maroof M., Hofman D.** & the North American Barley Mapping Project (1997) Characterizing and exploiting genetic diversity and quantitative traits in barley (*Hordeum vulgare*) using AFLP markers. J Quant Trait Loci 3, http://probe.nalusda.org:8000/otherdocs/jqtl.
73. **Russell J.R., Fuller J.D, Macaualy M., Hatz B.G., Jahoor A., Pawell W., Waugh R.** (1997) Theor. Appl. Genet., **95,** 714-722.
74. **Russell J.R., Fuller J.D., Young G., Thomas B., Taramino G., Macaulay M., Waugh R., Powell W**. (1997) Genome, **40,** 442-450.
75. **Pillen K., Binder A., Kreuzkam B., Ramsay L., Waugh R., Foster J., Leon J.** (2001) Theor. Appl. Genet., **101**, 652-660.
76. **Balvinskaya M.S., Roder M., Sivolap J.M.** (2001) Doklaki rosiiskoj akademii selskohozjaistvennih nauk, **5**, 3-7.
77. **Saghai-Maroof M.A., Biyashev R.M., Yang G.P., Zhang Q., Allard R.W.** (1994) Proc. Natl. Acad. Sci. USA, **91,** 5466-5470.
78. **Struss D., Plieske J.** (1998) Theor. Appl. Genet., **97**, 308 – 315.
79. **Hamza S., Hamida W.B., Rebai A., Harrabi**

M. (2004) Euphytica, **135**, 107-118.
80. **Becker J., Heun M**. (1995) Genome, **38**, 991-998.
81. **Liu Z.W., Biyashev R.M., Saghai-Maroof M.A**. (1996) Theor. Appl. Genet., **93**, 869-876.
82. **Ramsay L., Macaulay M., Ivanisssevich D.S., MacLean K., Cardle L., Fuller J., Edwards K.J., Tuvesson S., Morgante M., Massary A., Maestry E., Marmiroli N., Sjaktste T., Ganal M., Powell W., Waugh R.** (2000) Genetics, **156,** 1997-2005.
83. **Matus I.A., Hayes P.M.** (2002) Genome, **45**, 1095 -1106.
84. **Graner A, Streng S, Kellermann. A., Schiemann A., Bauer E., Waugh R., Pellio B., Ordon F.** (1999) Theor. Appl. Genet., **98**, 285-290.
85. **Buerstmaer H., Lemmens M., Hartl L., Doldi L., Steiner B., Stiechneider M., Ruckenbauer P.** (2002) Theor. Appl. Genet., **104**, 84-91.