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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Seventh Session
Hanover, Germany, November 21 to 23, 2001

REPORT

prepared by the Office of the Union

1. The Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (hereinafter referred to as "BMT") held its seventh session in Hanover, Germany, from November 21 to 23, 2001, under the chairmanship of Mr. Michael Camlin, United Kingdom. The list of participants is reproduced in Annex I to this report.

2. Mr. Johann Habben, on behalf of Mr. Udo von Kröcher, President of the Federal Office of Plant Varieties (*Bundessortenamt*), welcomed the participants to Hanover.

3. Mrs. Beate Rücker, Federal Office of Plant Varieties, provided a brief overview of the work of the Federal Office of Plant Varieties. It was reported that iso-enzymes were used systematically as biochemical characteristics for DUS testing of maize and potato varieties in Germany. In the case of maize, this was according to the Annex to UPOV Test Guidelines for Maize and, in the case of potato, according to the national list of iso-enzyme characteristics. A copy of the presentation made by Mrs. Rücker is attached to this report as Annex II.

4. The experts from Ukraine, participating for the first time in a meeting of the BMT, made a brief presentation on the recent development of the plant variety protection system in Ukraine.

Adoption of the Agenda

5. The Working Group unanimously adopted the Agenda as reproduced in document BMT/7/1 Rev.

Report of Discussions and Developments in UPOV Regarding Possible Use of Molecular Techniques in DUS Testing (Document BMT/7/3)

6. The Office of the Union introduced document BMT/7/3, which summarized discussions and developments regarding biochemical and molecular techniques within UPOV. The BMT noted that the task of the BMT should be reconsidered as a result of the recent establishment of the *Ad hoc* Crop Subgroups and the *Ad hoc* Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques (hereinafter referred to as "BMT Review Group"), established by the Technical Committee (TC) and the Administrative and Legal Committee (CAJ). It considered that document BMT/7/3 provided a basis for discussion of agenda item 7 "Development of guidelines on the availability and suitability of different methods of DNA-profiling techniques" and agenda item 11 "Future program, date and place of the next session."

7. In response to questions concerning the organization of the BMT Review Group, the Office of the Union explained that its membership should be decided by the TC and the CAJ, and its first meeting would be convened once a specific proposal for the use of biochemical and molecular techniques was made. The expert from ASSINSEL, supported by experts from France and the Community Plant Variety Office (CPVO), considered that the first meeting should be convened rather urgently in order to give guidance for the use of biochemical and molecular techniques, before an inappropriate position on the use of such techniques for individual crops is taken by different groups. Furthermore, it was noted that there were sufficiently developed models prepared by the *Ad hoc* Subgroups.

8. The Office of the Union emphasized that the task of the BMT Review Group was clearly defined in its terms of reference, reproduced in paragraph 16 of document BMT/7/3, and in particular in its subparagraphs (a) and (b).

9. The BMT emphasized that the BMT should be composed of breeders and biochemistry and molecular experts and should be kept informed of new developments in biochemical and molecular techniques.

New Developments in Biochemical and Molecular Techniques

10. No report was presented under this agenda item. The BMT noted that Single Nucleotide Polymorphisms (SNP's) were being investigated by some molecular experts. However, it was generally recognized that microsatellite markers were now the most widely used technique in the characterization of plant varieties, and this was likely to remain the situation for the foreseeable future.

Reports of the *Ad hoc C*rop Subgroups for Maize, Oilseed Rape, Rose, Tomato and Wheat (Document BMT/7/2)

11. The BMT noted document BMT/7/2, containing an interim report of the *Ad hoc* Crop Subgroups on molecular techniques, which met in February and March 2001, and Annex III to document BMT/7/3, which was a reproduction of Annex IV to document CAJ/43/2 and contained a summary of the outcome of the *Ad hoc* Crop Subgroups. The BMT noted further short oral reports made by the chairmen of the *Ad hoc* Crop Subgroups as follows:

12. Mrs. Beate Rücker (Germany, Chairman of the *Ad hoc* Subgroup for Maize) reported on the work of her Subgroup. She emphasized the importance of molecular techniques for the management of reference collections and the opportunity to increase the efficacy of DUS testing by replacing environment-influenced morphological characteristics with molecular characteristics, which were not influenced by the environment. She also noted its possible use for the identification of varieties to improve the enforcement of plant variety protection. She reported that microsatellite markers were currently the most appropriate molecular technique for DUS testing for maize and a large number of microsatellite markers were now publicly available. It was also thought to be appropriate to consider how to reduce costs incurred in the uniformity assessment using microsatellite markers and that the use of a small number of markers and the possibility of using bulk samples should be explored further. Quantitative trait loci (QTL) could be used for predicting morphological variation. For the Options contained in Annex III to document BMT/7/3, there were no major concerns regarding Option 1 (Molecular Characteristics as Predictors of Traditional Characteristics) whilst Option 3 (Development of a New System followed by Impact Analysis) was considered not to respond to the interest of breeders.

13. Mr. Michael Camlin (United Kingdom, Chairman of the *Ad hoc* Subgroup for Wheat) reported on the work of his Subgroup. He noted that the coordination of reference collections in different countries needed to be pursued and use of molecular techniques would facilitate this task. The SNP's techniques as reported by the expert from Canada was promising as a new tool for variety identification and should be further studied in terms of its applicability for DUS testing. It was hoped that a ring test could be set up on the basis of a common microsatellite marker set for characterization of wheat.

14. Mrs. Françoise Blouet (France, Chairman of the *Ad hoc* Subgroup for Oilseed Rape) reported on the work of her Subgroup. She noted that a study using 15 microsatellite markers for 10 varieties was underway in the United Kingdom and that it would be worthwhile to enlarge the study by involving more laboratories. Furthermore, she noted that few microsatellite markers were currently publicly available. For Option 1 of Annex III of document BMT/7/3 (Molecular Characteristics as Predictors of Traditional Characteristics), a strict relationship between traditional characteristics and molecular characteristics could be expected and the former could be replaced by the latter. For Option 2 (Calibration of Molecular Characteristics against Traditional Characteristics), a triangular shaped distribution of the distances measured by traditional characteristics and molecular characteristics would lead to different decisions on distinctness. Care would be needed for selecting an appropriate threshold. There was no study undertaken on the judgment of stability on the basis of biochemical and molecular techniques. The expert from ASSINSEL emphasized the importance of assessing the stability by applying molecular techniques to seeds from different generations.

15. Mr. Joost Barendrecht (Netherlands, Chairman of the *Ad hoc* Subgroup for Rose) reported on the work of his Subgroup, emphasizing the difficulty in the management of reference collections and the high costs incurred in the conduct of DUS testing for rose varieties. The Subgroup concluded that microsatellite markers were the most appropriate molecular techniques for DUS testing for rose and considered that one different band might be enough to clearly distinguish varieties. Mr. Barendrecht introduced the Subgroup's proposal for the use of microsatellite markers in the judgment of distinctness in rose varieties as given in Box 4 of document BMT/7/2 and reproduced below:

(1) Examination of distinctness

(a) Use of seven polymorphic STMS markers to establish distinctness between a candidate variety and other varieties

⇒If there are still some varieties which cannot be distinguished from the candidate variety, the second set of seven STMS markers will be used to examine distinctness between the candidate variety and the remaining varieties.

⇒If there are still some varieties which cannot be distinguished by the second set, those varieties that could not be distinguished by molecular characteristics (these varieties will be possibly identical varieties, sports or other genetically close varieties) will be included in the field trial together with the candidate variety to examine distinctness.

(2) Examination of uniformity and stability

Uniformity and stability of the candidate variety are examined in the field trial.

16. Mr. Richard Brand (France, Chairman of the *Ad hoc* Subgroup for Tomato) reported on the work of his Subgroup, referring to the expectation from professional circles for the introduction of molecular techniques to overcome the difficulty in handling over 10,000 tomato varieties. He also emphasized the intensive activities undertaken by private companies. He repeated the recommendation of the Subgroup that new subgroups be established for melon and lettuce.

17. During the general discussion, some experts noted that the concept of "pre-screening" should be considered separately from distinctness and would be better expressed by the word "grouping" or "management of reference varieties." The BMT recalled, however, the conclusion of the *Ad hoc* Crop Subgroups that the "pre-screening is a part of the process of examining distinctness, establishing distinctness between a candidate variety and others prior to a growing trial." The introduction of molecular characteristics for pre-screening could use a greater difference between varieties than differences required for a final decision of distinctness, to ensure an appropriate safety margin for molecular characteristics.

Report of Work on Molecular Techniques on a Crop-by-Crop Basis (Documents BMT/7/4, BMT/7/5, BMT/7/6, BMT/7/7, BMT/7/8, BMT/7/9, BMT/7/10, BMT/7/11, BMT/7/12, BMT/7/13, BMT/7/15, BMT/7/17)

18. An expert from the United Kingdom introduced document BMT/7/4 "Comparison of Anonymous and Genic Microsatellites for Variety Discrimination in Wheat."

19. The BMT noted that the study had been conducted with a view to improving the discriminating power of microsatellite markers by using microsatellite markers derived from expressed sequence tags (EST) rather than anonymous microsatellite markers. However, it noted that, on the basis of this study, there appeared to be no advantage, in terms of establishing distinctness, in using EST-derived microsatellite markers. An expert from Germany observed that the uniformity level measured by EST-derived microsatellite markers should be examined in comparison to that measured by anonymous microsatellite markers. An expert from France, whilst noting that it would not be within the scope of the BMT, suggested that anonymous microsatellite markers might be suitable for the investigation of essential derivation.

20. An expert from the United Kingdom introduced document BMT/7/5 "Development of Microsatellite Markers for DUS Testing in Wheat and Oilseed Rape." The objectives of the study were to develop a test set of DNA microsatellite primer pairs for oilseed rape and wheat, to evaluate their application in DUS testing and to devise an operational system for its use. The study indicated that microsatellite markers could readily discriminate between varieties and that it was possible to select microsatellite primer pairs that were robust and repeatable between laboratories. It was emphasized that a "parallel running" exercise would be conducted where the results of microsatellite markers would be compared to those obtained with conventional characteristics. As the next step the study would consider various remaining questions including the number of markers needed, different interpretation of mapped versus unmapped markers, the importance of distribution of markers, and interpretation of markers related to expressed regions.

21. The expert from Japan introduced document BMT/7/12 "Distinctness and Uniformity Based on DNA Markers in Soybean Varieties." The study showed that DNA-based markers detected intra-varietal genetic variation in an autogamous crop, such as soybean, even in cases where phenotypic differences were not apparent.

22. In the absence of the expert from Argentina, the Chairman introduced document BMT/7/13 "Application of Microsatellite Markers for the Assessment of Distinctness, Uniformity and Stability (DUS Testing) of Commercial Soybean Varieties." The Chairman drew the attention of the BMT to the conclusion of the study, which was that the 100 soybean varieties could be discriminated using 30 microsatellite markers and suggested that microsatellite markers could be used to complement the present system used for the assessment of distinctness. He noted that a lack of uniformity was observed in some cases. In particular it was noted that 9 microsatellite markers, of 32 observed, had shown variations over the period of four years. It might be necessary to select appropriate microsatellite markers to avoid uniformity and stability related difficulties.

23. In response to the observation made by the expert from ASSINSEL, that the intentional selection of microsatellite markers to ensure sufficient uniformity levels in the protected varieties might not be appropriate, the Chairman clarified that the objective of selecting markers was to establish a marker set appropriate to discriminate varieties which would not, at the same time, cause any problem for uniformity.

24. An expert from Ukraine made a brief presentation on "the identification of cereal crop varieties in Ukraine using the Simple Sequence Repeat (SSR) approach." The presentation and other related papers are attached to this report as Annex III.

25. An expert from France introduced document BMT/7/8 “Use of ISSR to Study the Genetic Variability of Poplars, Hydrangea and Peas Varieties.”

26. Regarding the intra-variety variability of ISSR markers in vegetatively propagated varieties, such as poplar and peach, an expert from the Netherlands observed that the quality of DNA extracted for testing might influence the results. It was noted that somatic mutation, which was DNA-methylation-sensitive but did not cause any change at the DNA sequence level, could not be detected by microsatellite markers. The BMT considered that it would be appropriate to investigate the uniformity of vegetatively propagated varieties with molecular markers.

27. An expert from France introduced document BMT/7/9 “Development of SSR Analysis Strategy for Varietal Identification in Sunflower.” The study was undertaken as a three-year project, starting in 1999, with a view to establishing a microsatellite marker analysis system in sunflower to improve the efficacy and the accuracy of sunflower DUS testing in France.

28. An expert from Belgium introduced document BMT/7/10 “Pre-Screening of Sugar Beet Varieties Using Microsatellite Markers.” Some experts considered that the stability should be assessed on the basis of seed lots over several years.

29. An expert from the United Kingdom introduced document BMT/7/7 “Microsatellites for Variety Discrimination in Potatoes.” He concluded that a panel of microsatellite markers, consisting of five microsatellite markers, provided a reliable method for potato variety identification which represented a “down-stream” application of microsatellite markers where markers could be used throughout the production chain.

30. An expert from Germany mentioned that, in Germany, electrophoresis had been used for more than 30 years for the identification of potato varieties and reminded the BMT that some existing UPOV Test Guidelines documents, e.g. for Barley and Sunflower, contained a list of electrophoresis characteristics in an annex. The BMT observed that it might be useful to examine the need for such a list in the revised UPOV Test Guidelines document for Potato, which was now under revision by the Technical Working Party for Agricultural Crops (TWA).

31. The expert from Australia introduced document BMT/7/6 “DNA Profiling in Sugarcane: Implications for Varietal Protection.” It was noted that microsatellite markers could be used for clear identification of sugarcane varieties. The International Society of Sugar Cane Technologists, with the participation of its 10 members from eight countries, was now cooperating with the aim of generating a worldwide standardized protocol for the use of DNA profiling in sugarcane identification.

32. Some experts raised questions on the relationship between the distance measured on the basis of morphological characteristics and that calculated on the basis of molecular characteristics. The BMT thought that the consideration of this relationship could lead to the possibility of an Option 2 approach and might be developed further.

33. The expert from Japan introduced document BMT7/11 “Development of SSR Markers and Identification of Pears.” He noted a high level of uniformity in the “Choujuurou” variety when assessed by microsatellite markers, indicating the possibility of using microsatellite markers for the assessment of uniformity of Japanese pear varieties.

34. An expert from the Netherlands introduced document BMT/7/15 “DNA Profiling and Protection of Mushroom Varieties.” He noted that after the release of first hybrid varieties of *Agaricus bisporus* in 1980, varieties which had since been released commercially were rather similar. Molecular techniques (RFLP and AFLP) supported this observation. As a result, it was difficult to distinguish new varieties using morphological characteristics. The expert from the Netherlands concluded thatthe application of molecular techniques for DUS testing, because of its greater capacity of discrimination, and the notion of essential derivation would strengthen breeders’ rights for mushroom varieties and thus contribute to the promotion of substantial breeding.

35. Other experts considered that it was a matter of variety identification rather than DUS testing and the owner of a plant variety protection title could defend his variety right by using the DNA profile to show that the variety in question was his protected variety.

36. The expert from ASSINSEL noted that Test Guidelines for Mushroom needed to be established to provide protection for mushroom breeders. Some experts questioned whether agronomic characteristics, such as yield and disease resistance, which were the objectives of mushroom breeding, might be used for DUS testing, although they observed that the conduct of DUS testing on these characteristics would be difficult and time consuming.

37. The BMT was informed that the Technical Working Party for Vegetables (TWV) planned to discuss Test Guidelines for Mushroom at its thirty-sixth session to be held in September 2002 in Tsukuba, Japan, and suggested that the TWV establish a crop subgroup for mushroom and hold its meeting in conjunction with the next TWV meeting.

38. The expert from Italy introduced document BMT/7/17 “Fingerprinting Peach Varieties Using Molecular Markers.” She concluded that microsatellite markers were a powerful tool to distinguish peach varieties although it had been reported that some peach varieties discriminated by morphological characteristics could not be distinguished by microsatellite markers.

39. Discussions concentrated on the possibility of distinguishing mutants using molecular techniques. The expert from ASSINSEL suggested that molecular techniques should be used in conjunction with morphological characteristics, otherwise a number of mutant varieties would not satisfy the distinctness criterion. The procedure proposed for rose varieties might be appropriate. An expert from France mentioned that, in general, in the case of some fruit species, where most of varieties were mutants from existing varieties, molecular data could be useful for the management of reference collections by ensuring that the candidate mutant varieties would be compared with relevant initial varieties.

40. An expert from the Netherlands noted that there were other cases where molecular techniques alone could not discriminate varieties, such as the case of tomato varieties bred by repeated backcrossing. The BMT noted that molecular techniques were unlikely to detect point mutations, which were frequently used for fruit breeding, but would be useful for establishing the origin of mutant varieties.

Stability of Molecular Markers

41. Some experts considered that, at least in some crops such as some fruit trees, there could be some degree of instability as measured by molecular markers. Other experts considered that this was not the case and that the data suggesting such instability was probably due to methodological problems. It was concluded that this should be investigated further to clarify the situation.

Work of the Review Group and *Ad hoc* Crop Subgroups

42. The BMT considered that it was important for the BMT Review Group to consider models for the use of biochemical and molecular techniques in DUS testing and make recommendations on the acceptability of these models, before the *Ad hoc* Crop Subgroups take their work further. ASSINSEL advised that it would like to be invited to participate in this subgroup as an observer.

43. The BMT proposed that recommendations be sought on the basis of selected proposals developed in the Crop Subgroups, as reported in document BMT/7/3, Annex III. In particular, it suggested that models should beproposed for :

Option 1: "Molecular Characteristics as Predictors of Traditional Characteristics"

(a) Gene specific markers: the BMT Review Group would be asked to consider the acceptability of gene specific markers for predicting individual phenotypic characteristics. The characteristic of herbicide tolerance, introduced by genetic modification, is to be given as the example. The recommendation would need to be on the basis that there was reliable linkage between the marker and the expression of the characteristic. In considering this proposal, the BMT Review Group would be requested to make a recommendation on the acceptability of differences arising from different markers developed for the same expression of a characteristic.

(b) The use of a set of molecular characteristics to estimate a traditional characteristic: a model based on this approach would not be proposed at this time but it was emphasized that work on this approach was ongoing.

Option 2: "Calibration of Molecular Characteristics against Traditional Characteristics"

A model would be presented on the basis of information from oilseed rape, maize and rose. This option would be proposed on the basis of a genetic distance assessment, rather than a characteristic-by-characteristic approach, and would be presented for use in the management of reference collections.

Option 3: "Development of a New System"

This option would be presented on the basis of the model proposed in the Rose Crop Subgroup and a model that will be developed on the basis of the information available from wheat. This option will be based on the use of molecular characteristics in the same way as existing non-molecular characteristics.

44. It was clarified that the three options developed by the Crop Subgroups related to the options for distinctness, including management of reference collections, and that it was equally important for the BMT Review Group to consider the uniformity and stability issues outlined in document BMT/7/3, Annex III.

45. The BMT Review Group would be asked to consider these models on the basis of certain assumptions, which would need to be made, regarding information which is not yet available for the crops used in the illustrations.

46. The BMT emphasized that the use of biochemical and molecular techniques in any of these proposals should not be interpreted as the complete replacement of non-molecular characteristics and that these methods should be considered in conjunction with non-molecular characteristics, for example, in the management of reference collections.

47. The following general schedule was then envisaged:

(a) The BMT Review Group to make recommendations to the Technical Committee and Administrative and Legal Committee, on the models outlined above.

(b) The Office of the Union to produce a document, containing these recommendations and the considerations of the Technical Committee, for circulation to the Technical Working Parties (TWPs).

(c) The TWPs to consider this document and to consider detailed reports of the work of Crop Subgroups.

(d) Where possible, the Crop Subgroups to meet after the next meeting of the relevant TWP to enable the views of the relevant TWP to be presented at the meeting.

48. The BMT recommended that the Crop Subgroup meetings should, in general, be held in association with meetings of relevant TWPs.

49. The BMT suggested the following approach for the existing Crop Subgroups:

(a) Maize: no future meeting planned at this stage, subject to consideration by the Technical Working Party for Agricultural Crops (TWA);

(b) Oilseed Rape: to meet sometime before the next TWA meeting, not necessarily at the same time as the TWA meeting;

(c) Rose: to meet before the next Technical Working Party for Ornamental Plants and Forest Trees (TWO) meeting;

(d) Tomato: no future meeting planned at this stage, subject to consideration by the Technical Working Party for Vegetables (TWV);

(e) Wheat: to meet immediately after, and in association with, the next TWA meeting.

50. The BMT suggested the establishment of new Crop Subgroups as follows:

(a) Sugarcane: to hold its first meeting immediately after, and in association with, the next TWA meeting;

(b) Potato: to hold its first meeting immediately after, and in association with, the next TWA meeting;

(c) Mushroom: to hold its first meeting immediately after, and in association with, the next TWV meeting;

(d) Soybean: to hold its first meeting immediately after, and in association with, the next TWA meeting, if there is sufficient interest amongst experts.

51. The BMT noted that its proposals, regarding existing and new Crop Subgroups, would be considered by the TC in April 2002. It also noted the large number of Crop Subgroups associated with the TWA and recognized the time pressures this would place on this Technical Working Party.

52. The proposed Chairman of a Peach/Citrus Crop Subgroup (Mr. Schulte, Germany), concluded that, on the basis of presentations at the session, there was insufficient basis for the creation of such a Crop Subgroup at this time. However, he would report on the BMT session to the next Technical Working Party for Fruit Crops (TWF) to ensure that it was fully informed of the current situation, since the TWF wished to be involved in this work.

Future Role of the BMT

53. In response to developments in UPOV, regarding biochemical and molecular techniques, and in particular the establishment of the BMT Review Group and the Crop Subgroups, the BMT clarified its understanding of the role it should perform as follows:

The BMT is a group open to DUS experts, biochemical and molecular specialists and plant breeders, which considers its role to be to:

- Review general developments in biochemical and molecular techniques;
- Maintain an awareness of relevant applications of biochemical and molecular techniques in plant breeding;
- Consider the possible application of biochemical and molecular techniques in DUS testing and report its considerations to theTechnical Committee ;
- If appropriate, establish guidelines for biochemical and molecular methodologies and their harmonization and, in particular, contribute to the preparation of document TGP/15, “New Types of Characteristics”. These guidelines to include methods for analysis of data resulting from such methods, to be developed in conjunction with the Technical Working Party on Automation and Computer Programs (TWC);

- Consider initiatives from Technical Working Parties, for the establishment of crop specific subgroups, taking into account available information and the need for biochemical and molecular methods;

- Develop guidelines regarding the management and harmonization of databases of biochemical and molecular information, in conjunction with the TWC;

- Receive reports from Crop Subgroups and the BMT Review Group;

- Provide a forum for discussion on the use of biochemical and molecular techniques in the consideration of essential derivation and variety identification.

Development of Guidelines on the Availability and Suitability of Different Methods of DNA-Profiling Techniques

54. The Office of the Union explained that the TC was currently revising the General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants. The new General Introduction would be complemented with a series of associated documents, of which document TGP/15 "New Types of Characteristics," if appropriate, would contain general guidance of the application of molecular techniques for DUS testing.

55. The BMT noted that general guidance on the application of molecular techniques, once established, would serve as a platform to enable the harmonized application of such techniques to different crops and should cover the areas indicated in the first part of the table in Annex II of document BMT/7/3. It was agreed that Mr. Vosman (Netherlands), in conjunction with Mr. Reeves (United Kingdom), would prepare draft guidelines on the suitability and application of different biochemical and molecular methods for variety characterization. The first draft would be circulated for comment by the BMT, by December 2002, and a revised document produced for discussion at the next session of the BMT.

Construction and Standardization of Databases of DNA Profiles of Plant Varieties (Document BMT/7/16)

56. An expert from the Netherlands reported on the EU Demonstration Project "Molecular Markers for Variety Testing," which was carried out within the European Union Biotechnology program with the aim of demonstrating the technical viability of the microsatellite markers for identification and discrimination of tomato and wheat varieties.

57. The BMT agreed to retain this agenda item for its future sessions pending further developments in the work currently underway in the EU.

Statistical Methods

58. The Chairman observed thatthe elaboration of statistical methods for the interpretation of molecular data was important and was linked to the work of the Technical Working Party on Automation and Computer Programs (TWC). In the past several measures, such as Rogers' distance and dendrograms, had been used to indicate genetic distance. The BMT considered that, if molecular techniques were accepted for DUS testing, statistical methods would need to be developed. Statistics would be needed both for computerization of data and for the process of decision making.

59. In the light of the wide use of dendrograms as a tool to indicate the genetic distance between varieties, the BMT thought it necessary to review the relevance of this technique in relation to DUS testing.

60. It was agreed that Mr. Grégoire (France) and Mr. Law (United Kingdom) should coordinate the development of papers on statistical methods for data produced by biochemical and molecular techniques. The topics addressed should include, in particular, band scoring, calculation of distances between varieties, uniformity assessment and the development of databases of variety information. Members of the TWC could contribute to the development of these papers.

Costs of Biochemical and Molecular Techniques

61. The BMT noted the observation made by the expert from ASSINSEL that a new cost estimation of biochemical and molecular techniques should be provided in the light of rapid development in the equipment used.

The Use of Molecular Techniques in Examining Essential Derivation (Document BMT/7/14)

62. An expert from the United Kingdom introduced document BMT/7/14 "Molecular and Other Markers for Establishing Essential Derivation in Crop Plants." The main purpose of the study was to identify the degree of relatedness within a given species for a variety to be judged as being essentially derived.

63. The BMT agreed to retain this item for discussion at its future sessions.

Discussions on Phenotype

64. During discussions on the draft report of the conclusions, regarding models to be presented to the Review Group (see paragraphs 4-8), there were suggestions that the term "traditional characteristics" should be replaced with "phenotypic characteristics", since the current UPOV characteristics are phenotypic. However, the Office of the Union noted that the titles of the options, including the term traditional characteristic, were taken from document BMT/7/3, Annex III and BMT/7/2, and it would, therefore, be inappropriate to seek to change the use of the term in these titles. It also noted that ploidy level, which is an existing UPOV characteristic, is not a truly phenotypic characteristic. Nevertheless, the delegations which expressed an opinion agreed that the existing UPOV characteristics should be recognized as phenotypic characteristics. To reflect this agreement it was decided that the

first sentence of Option 1 (a) “Gene specific markers” should be reported as: “The Review Subgroup would be asked to consider the acceptability of gene specific markers for individual phenotypic characteristics.”

Future Program, Date and Place of Next Session

65. At the invitation of Japan, the BMT agreed to hold its eighth session in Tsukuba, Japan, in 2003. The BMT noted that Japan had already invited the TWA to hold its thirty-second session in Japan. It was anticipated that these two sessions could be held in consecutive weeks to facilitate the attendance of the members of the TWA if the TWA meeting was scheduled for May or June.

66. The following provisional program was agreed:

1. Opening of the Session
2. Adoption of the agenda
3. Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, and plant breeders
4. Reports from the BMT Review Group, Technical Committee and Crop Subgroups
5. Report of work on molecular techniques on a crop by crop basis, including methods to assess the potential impact on the strength of variety protection
6. Development of guidelines on the availability and suitability of different biochemical and molecular techniques for variety characterization
7. Review of the costs of molecular techniques
8. Construction and standardization of databases of molecular characteristics of plant varieties
9. Statistical methods for data produced by biochemical and molecular techniques
10. The use of molecular techniques in examining essential derivation
11. Future program, date and place of the next session
12. Report of the conclusions of the session

67. This report has been adopted by correspondence.

[Annex I follows]

ANNEX I

LIST OF PARTICIPANTS

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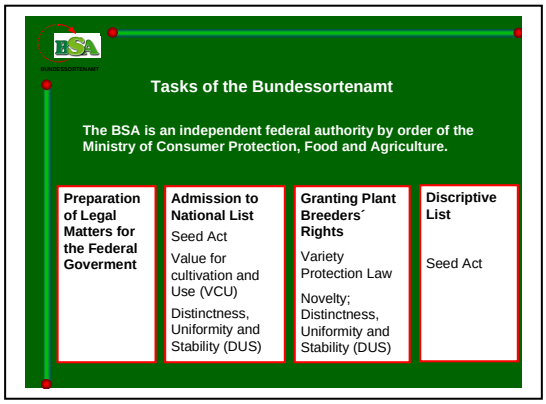
[Annex II follows]

ANNEX II

Presentation of the Federal Office of Plant Varieties

Slide 1

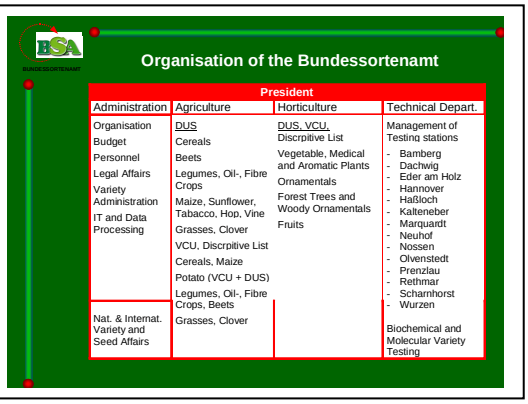
Slide 2



Slide 3

Slide 4

Slide 5



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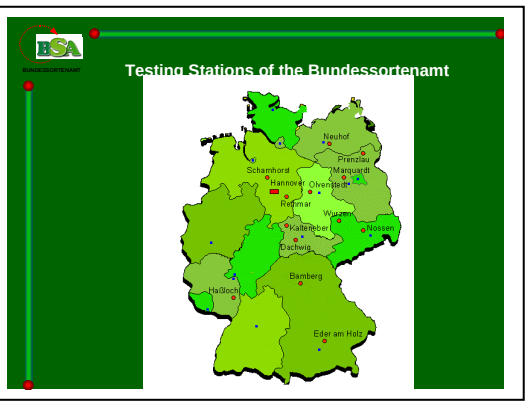

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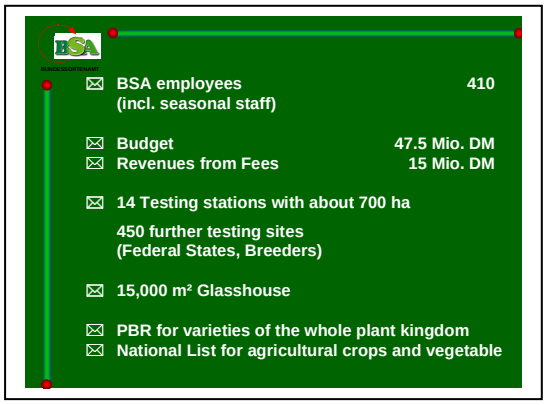

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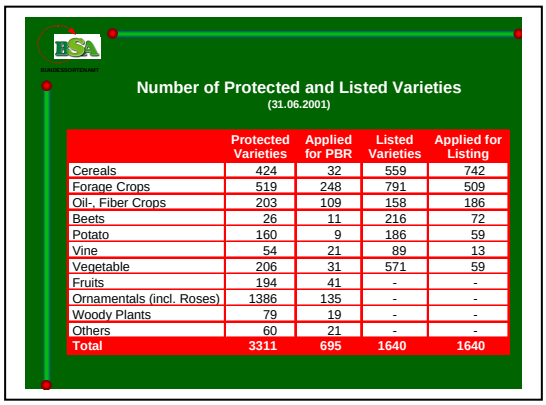
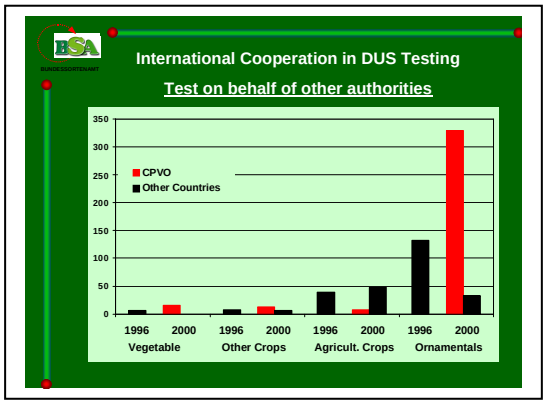


	Protected Varieties	Applied for PBR	Listed Varieties	Applied for Listing
Cereals	424	32	559	742
Forage Crops	519	248	791	509
Oil-, Fiber Crops	203	109	158	186
Beets	26	11	216	72
Potato	160	9	186	59
Vine	54	21	89	13
Vegetable	206	31	571	59
Fruits	194	41	-	-
Ornamentals (incl. Roses)	1386	135	-	-
Woody Plants	79	19	-	-
Others	60	21	-	-
Total	3311	695	1640	1640

Slide 9



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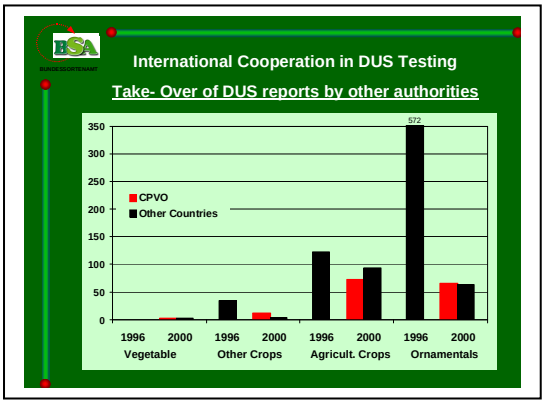

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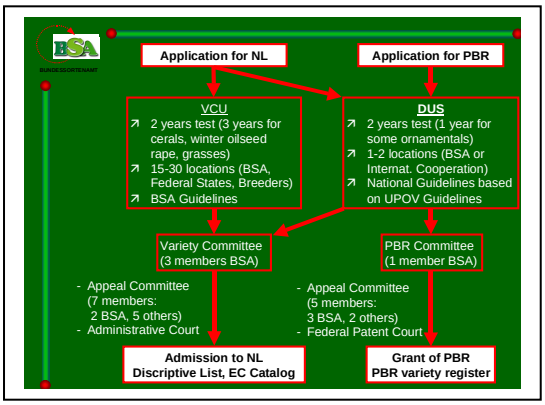

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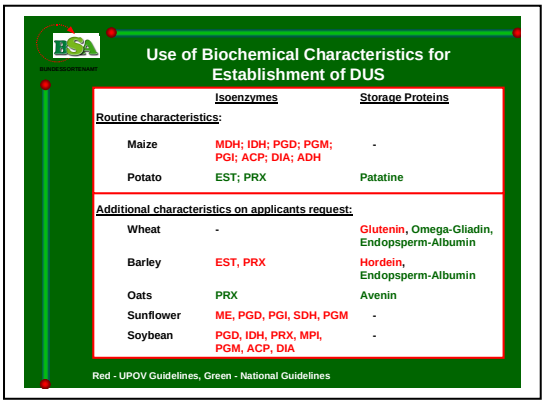

[Annex III follows]

ANNEX III[1]

IDENTIFICATION AND CERTIFICATION OF BARLEY VARIETIES OF THE SOUTH-UKRAINIAN BREEDING

Balvinskya M., Sivolap Yuri

1. Introduction

Nowadays DNA-technologies based on the analysis of DNA-polymorphism are widely used for the solving of theoretical and applied aspects of genetics and plant breeding. One of most urgent problems of modern plant breeding is identification and certification of genotypes of the most important crops. The precise differentiation and definition of plant variety is necessary in deciding a number of problems in plant breeding, and also for protection of the plant breeders and their establishments. At the present moment when identifying any variety one accentuate mainly on morphological methods, however a question of introduction of the molecular-genetic approaches into practical plant breeding is widely discussed. In this connection using of DNA-markers for the purposes of certification has a great practical interest. The significant role in studies on development of genetic resources catalogization system belongs to the SSRP-analysis. The special interest is caused by high resolution and informativeness of this type of analysis allowing to reveal polymorphism of specific sites of plant genome, containing high–variable tandem repeats, and first of all, by opportunity to use microsatellite loci for creation of the genetic passport of a plant variety. The genotype characterization using a set of microsatellite markers allows to carry out variety registration practically for all kinds of agricultural plants quickly and most precisely (Becker, Heun, 1995; Struss, Plieske, 1998).

The purpose of our study was the SSRP-analysis of spring barley varieties of the South-Ukrainian breeding for possible identification and certification of their genotypes.

2. Materials and methods

32 spring barley (*Hordeum vulgare* L.) varieties (breeding of PBGI, Odessa, Ukraine) were used in this study. Investigated genotypes are presented in Table 1. DNA was isolated from etiolated seedlings by CTAB - method according to a technique published earlier (Sivolap et al., 1998). Polymerase chain reaction with directed (sequence – targeted) primers (Liu, Biyashev, Saghai Maroof, 1996) was used for the analysis of molecular-genetic polymorphism (table 2); the main part of primers contained dinucleotide sequences $(GA)_n$ and $(CA)_n$ with different number of repeats *n*.

The reaction mix (volume 25 μl) providing PCR with directed primers contained: 50 mM KCl; 20 mM Tris-HCl, pH 8.4 (25^{o}C); 1-4 mM $MgCl_2$ (depending on primers); 0,01 % Tween-20; 0,2 mM every dNTP; 0,25 μM of primer; 100-150 ng of DNA and 2 U Taq-polymerase. 30 μl of mineral oil was piled up in every tube. Amplification was carried out using thermocycler "Terzik" ("DNA-TECHNOLOGY", Russia) under following conditions: 45 cycles; denaturation - 94^{o}C, 1,5 min (initial), 1 min (all next); annealing - 55^{o}C , 1 min; synthesis - 72 ^{o}C, 2 min, final elongation - 10 min at 72 ^{o}C.

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[1] The texts in this Annex were sent in by Dr. Yuri Sivolap, Director, South Plant Biotechology Center, Odessa, Ukraine, with his electronic letter of February 8, 2002.

Amplification products were separated in 10 % PAAG (denaturating) (gel sizes: 175 x 220 x 1 mm). Electrophoresis was carried out during 1-1,5 h at voltage 500 V in 1xTBE buffer (50 mM Tris-H_3BO_3; 2 mM EDTA, pH 8.0). Amplified DNA-fragments were stained by silver according to "Silver sequence TM DNA sequencing System Technical Manual" ("Promega", USA). The video image of gel and the sizes of amplified fragments were obtained using "Image Master VDS". The amplification fragments were designated as present (1) or absent (0) in order to register the genotypes by loci.

The level of polymorphism for every locus was calculated using an polymorphism index 1-ΣP_{ij}^2, where P_{ij} – phenotypic frequency of every ***j*** fragment for every ***i*** microsatellite locus (Struss, Plieske, 1998).

3. Results and discussion

The PCR-analysis 20 SSR-loci of 32 barley varieties has revealed 57 allelic variants with various molecular weight (Table 3). The total number of alleles detected in the sample investigated was as following: 1 allele for loci HVM9, HVM44, HVM77; 2 alleles for loci HVBKASI, HVM65 and HVM33; 4 alleles for SSR-loci HVM3, HVM40, HVM68, HVCSG; 5 alleles for HVM20 locus. 3 alleles were detected at 9 from 20 SSR-loci (Table 3).

The level of polymorphism for each analyzed SSR-locus varied in great extent (Table 3). The most of loci under study is characterized by an average degree of polymorphism. Four loci have shown a low degree of genetic variability (from 0.12 up to 0.34), for others the data varied in limits from 0.42 up to 0.70. The highest meaning of this parameter is revealed for HVM3 locus, and lowest for HVBKASI (with the exception of loci which had 1 allele).

The results of SSR-analysis allow to present genotypes of varieties as the genetic formulas (Table 4). A certain letter of the Roman alphabet with a digital index (according to the allele size, detected at a certain locus at each variety investigated) corresponds to a certain microsatellite locus.

Loci are designated accordingly: HVM3 – A, HHVM4 – B, HVM9 – C, HVM13 – D, HVM20 – E, HVM30 – F, HVM33 – G, HVM36 – H, HVM40 – I, HVM44 – J, HVM49 – K, HVM 54 – L, HVM62 – M, HVM65 – N, HVM67 – O, HVM68 – P, HVM74 – Q, HVM77 – R, HVBKASI – S, HVCSG - T. Using these microsatellite loci, all genotypes (within the limits of the investigated sample) were differentiated. For each variety the unique set of amplification band patterns is received and fixed in the genetic formula.

Thus the analysis of spring barley varieties using 20 SSR-loci was carried out. The genotypes of 32 varieties are identified. The results received can be added to the barley database. The used system of molecular-genetic markers has allowed to differentiate the investigated varieties of spring barley completely. This fact enables applying of markers (used in this study) for the purposes of certification and also for registration of genetic resources of barley.

4. References

Becker J., Heun M. (1995): Plant Molecular Biology 27, 835-845
Struss D., Plieske J. (1998): Theor. Appl. Genet. 97, 308-315
"Usage of PCR-analysis in genetics and plant breeding investigations" The technique manual. Edited by Sivolap Yuri M. (1998),Kiev, 156 p.
Liu Z. W., Biyashev R. M., Saghai Maroof M. A. (1996): Theor. Appl. Genet. 93, 869-876.

Table 1: Investigated barley genotypes

№	Variety name*	Origin
1	Ros	(Od.82 x Don.6) x NE2468
2	Pallidum 107	Pallidum 76 x Karagandinsky 5
3	Odessky 131	Od. 82 x Od.100
4	Odessky 151	(Nut.106 x 518) x Don.100
5	Prestizh	Itil x K-6823
6	Deribas	(Kosmos x Mirena) x Karlsberg 33787
7	Peremozhny	Romantik x Od.115
8	Gambrinus	(244 x Vizir) x Karlsberg 816
9	Edem	Yermak x GOH
10	Stalker	(Od.82 x Don.6) x Zernogradsky 86
11	Nexalezhny	(778 x Abava) x Nad 360
12	Adapt	(Chernomorets x Dn. 425) x Karlsberg x Preriya
13	Galateya	Od.11 x Itil
14	Galaktik	Itil x Od.115
15	Zoryaniy	(Od.100 x Dzhordzhiya) x Golf
16	Pivdenniy	(Od.111 x Preriya) x Od.163
17	Getman	87-188-8 x Prestizh
18	Obolon	Bogir x Galant
19	Druzhba	Trumpf x Ametist
20	Odessky 100	774/74 x Hml 36462
21	Odessky 115	Karlsberg 28771 x Od.100
22	Preriya	Od.100 x Don.9
23	Taifun	Hml 36462 x Nut.540
24	Eney	Nut.540 x Don.8
25	Romantik	(Pervenets x Trumpf) x Sandens
26	Itil	Don.8 x Vestnik
27	Pervenets	(ci 13664 x Don.4) x Od.36
28	Vestnik	Medicum $^{42}/_{76}$ (ci 13664 x Don.4) x Od.36 – sibs line
29	Nutans 778	Minerva x Union
30	Odessky 111	Don.6 x Elgina
31	Odessky 82	Chernomorets x Elgina
32	Gelios	(Medicum $^{32}/_{76}$ x Pallidum 129) x Atos

Table 2: Characteristics of 20 Barley Microsatellite Loci

№	Microsatellite locus	Microsatellite repeat sequence	Number of alleles	Allele size limits, b.p.
1.	HVM 3	$(AT)_{29}$	4	186-208
2.	HVM4	$(AT)_9$	3	198-202
3.	HVM9	$(TCT)_5$	1	230
4.	HVM13	$(GA)_6$, $(GA)_6$. $(GA)_6$	3	249-253
5.	HVM20	$(GA)_{19}$	5	133-157
6.	HVM30	$(CA)_8$	3	150-154
7.	HVM33	$(CA)_7$	2	157-163
8.	HVM36	$(GA)_{13}$	3	106-110
9.	HVM40	$(GA)_6$ $(GT)_4$ $(GA)_7$	4	144-164
10.	HVM44	$(GA)_8$	1	114
11.	HVM49	$(GA)_{12}$	3	99-117
12.	HVM 54	$(GA)_{14}$	3	149-161
13.	HVM62	$(GA)_{11}$	3	229-243
14.	HVM65	$(GA)_{10}$	2	129-132
15.	HVM67	$(GA)_{11}$	3	116-120
16.	HVM68	$(GA)_{22}$	4	190-214
17.	HVM74	$(GA)_{13}$	3	188-192
18.	HVM77	$(CA)_7$	1	199
19.	HVBKASI	$(C)_{10}(A)_{11}$	2	185-197
20.	HVCSG	$(CA)_4$, $(C)_{17}$	4	192-203

Table 3: Alleles of 20 SSR-loci, revealed in sample of barley varieties by Odessa breeding

№	Microsatellite locus (SSR-locus)	Allele sizes, b.p.	Number of genotypes possessing this allele	Allele frequency (for the sample under study)	Polymorphism level (genetic variability)
1.	HVM 3	186 188 190 208	6 7 14 5	0.1875 0.21875 0.4375 0.15625	0.70
2.	HVM4	198 200 202	3 12 17	0.09375 0.375 0.5375	0.56
3.	HVM9	230	32	1.00	
4.	HVM13	249 251 253	8 21 3	0.25 0.65625 0.09375	0.50
5.	HVM20	133 151 153 155 157	1 4 5 16 6	0.03125 0.125 0.15625 0.5 0.1875	0.52
6.	HVM30	150 152 154	7 23 2	0.21875 0.71875 0.0625	0.43
7.	HVM33	157 163	25 7	0.78125 0.21875	0.34
8.	HVM36	106 108 110	9 20 3	0.28125 0.625 0.09375	0.55
9.	HVM40	144 146 152 164	1 17 6 8	0.03125 0.5375 0.1875 0.25	0.61
10.	HVM44	114	32	1.00	
11.	HVM49	99 105 117	2 27 3	0.0625 0.84375 0.09375	0.27
12.	HVM 54	149 159 161	9 4 19	0.28125 0.125 0.59375	0.55
13.	HVM62	229 243 251	8 1 23	0.25 0.03125 0.71875	0.42
14.	HVM65	129 132	28 4	0.875 0.125	0.22
15.	HVM67	116 118 120	2 13 17	0.0625 0.40625 0.5375	0.54
16.	HVM68	190 204 210 214	6 6 15 5	0.1875 0.1875 0.46875 0.15625	0.68
17.	HVM74	188 190 192	15 10 7	0.46875 0.3125 0.21875	0.63
18.	HVM77	199	32	1.00	
19.	HVBKASI	197 185	30 2	0.9375 0.0625	0.12
20.	HVCSG	192 196 198 203	4 7 16 5	0.125 0.21875 0.5 0.15625	0.66

Table 4: Genetic Formulas Barley Varieties From South Ukraine

Ros	$A_{208;}$ $B_{198;}$ $C_{230;}$ $D_{251;}$ $E_{155;}$ $F_{152;}$ $G_{157;}$ $H_{108;}$ $I_{152;}$ $J_{114;}$ $K_{105;}$ $L_{159;}$ $M_{251;}$ $N_{129;}$ $O_{118;}$ $P_{210;}$ $Q_{188;}$ $R_{199;}$ $S_{197;}$ T_{192}
Pallidum 107	A 190; B 200; C 230; D 249; E 153; F 152; G 163; H 110; I 146; J 114; K 105; L 161; M 251; N 129; O 116; P 204; Q 190; R 199; S 185; T 192
Odessky 131	A 190; B 198; C 230; D 251; E 151; F 152; G 157; H 106; I 146; J 114; K 105; L 161; M 251; N 129; O 118; P 210; Q 192; R 199; S 197; T 196
Odessky 151	A 188; B 202; C 230; D 251; E 157; F 152; G 157; H 106; I 146; J 114; K 117; L 159; M 229; N 129; O 120; P 210; Q 190; R 199; S 197; T 196
Prestizh	A 186; B 202; C 230; D 251; E 155; F 150; G 157; H 106; I 146; J 114; K 105; L 149; M 251; N 129; O 120; P 204; Q 190; R 199; S 197; T 198
Deribas	A 190; B 202; C 230; D 251; E 155; F 152; G 163; H 108; I 146; J 114; K 117; L 159; M 229; N 129; O 120; P 210; Q 188; R 199; S 197; T 198
Peremozhniy	A 190; B 200; C 230; D 251; E 155; F 152; G 157; H 108; I 146; J 114; K 105; L 149; M 251; N 129; O 120; P 210; Q 188; R 199; S 197; T 203
Gambrinus	A 208; B 202; C 230; D 251; E 155; F 152; G 163; H 106; I 146; J 114; K 105; L 161; M 243; N 129; O 120; P 210; Q 188; R 199; S 197; T 196
Edem	A 208; B 200; C 230; D 251; E 155; F 150; G 157; H 106; I 152; J 114; K 105; L 149; M 251; N 129; O 120; P 210; Q 188; R 199; S 197; T 198
Stalker	A 190; B 202; C 230; D 251; E 155; F 150; G 157; H 108; I 164; J 114; K 105; L 159; M 229; N 129; O 118; P 214; Q 188; R 199; S 197; T 198
Nezalezhny	A 190; B 202; C 230; D 251; E 155; F 152; G 163; H 108; I 164; J 114; K 105; L 161; M 251; N 129; O 120; P 210; Q 188; R 199; S 197; T 196
Adapt	A 190; B 202; C 230; D 251; E 155; F 152; G 157; H 108; I 152; J 114; K 99; L 161; M 251; N 129; O 118; P 214; Q 188; R 199; S 197; T 196
Galateya	A 186; B 200; C 230; D 249; E 155; F 152; G 157; H 108; I 152; J 114; K 105 L 161; M 229; N 132; O 118; P 190; Q 190; R 199; S 197; T 192
Galaktik	A 190; B 202; C 230; D 249; E 151; F 152; G 157; H 108; I 146; J 114; K 105; L 149; M 251; N 129; O 120; P 204; Q 188; R 199; S 197; T 198
Zoryaniy	A 190; B 202; C 230; D 251; E 155; F 152; G 157; H 108; I 146; J 114; K 105; L 149; M 251; N 129; O 120; P 204; Q 190; R 199; S 197; T 198
Pivdenniy	A 190; B 200; C 230; D 251; E 155; F 152; G 157; H 108; I 146; J 114; K 105; L 161; M 229; N 129; O 120; P 210; Q 192; R 199; S 197; T 196
Getman	A 186; B 202; C 230; D 251; E 155; F 152; G 157; H 108; I 164; J 114; K 105; L 161; M 251; N 129; O 120; P 190; Q 192; R 199; S 197; T 203
Obolon	A 208; B 200; C 230; D 253; E 157; F 152; G 157; H 108; I 144; J 114; K 105; L 161; M 251; N 129; O 120; P 210; Q 192; R 199; S 197; T 198
Druzhba	A 208; B 200; C 230; D 253; E 157; F 152; G 157; H 108; I 146; J 114; K 105; L 161; M 251; N 129; O 120; P 210; Q 190; R 199; S 197; T 198
Odessky 100	A 190; B 202; C 230; D 251; E 157; F 152; G 157; H 108; I 164; J 114; K 105; L 149; M 229; N 129; O 120; P 210; Q 188; R 199; S 197; T 203
Odessky 115	A 190; B 202; C 230; D 251; E 157; F 152; G 157; H 108; I 164; J 114; K 105; L 149; M 251; N 129; O 120; P 210; Q 188; R 199; S 197; T 203
Preriya	A 190; B 202; C 230; D 251; E 155; F 152; G 157; H 108; I 164; J 114; K 99; L 161; M 229; N 129; O 118; P 214; Q 188; R 199; S 197; T 198
Taifun	A 190; B 202; C 230; D 251; E 157; F 154; G 157; H 110; I 146; J 114; K 105 ;L 161; M 251; N 129; O 118; P 214; Q 188; R 199; S 185; T 203
Eney	A 188; B 202; C 230; D 249; E 155; F 154; G 163; H 108; I 146; J 114; K 105; L 161; M 251; N 129; O 118; P 214; Q 188; R 199; S 197; T 198
Romantik	A 188; B 200; C 230; D 249; E 153; F 150; G 157; H 106; I 146; J 114; K 105; L 149; M 251; N 129; O 118; P 210; Q 188; R 199; S 197; T 198
Itil	A 186; B 202; C 230; D 249; E 151; F 152; G 157; H 108; I 152; J 114; K 105; L 161; M 251; N 129; O 118; P 204; Q 190; R 199; S 197; T 198
Pervenets	A 188; B 200; C 230; D 249; E 153; F 150; G 157; H 108; I 164; J 114; K 105; L 161; M 251; N 129; O 118; P 190; Q 190; R 199; S 197; T 198
Vestnik	A 188; B 200; C 230; D 249; E 153; F 152; G 157; H 108; I 164; J 114; K 105; L 161; M 251; N 129; O 118; P 190; Q 192; R 199; S 197; T 198
Nutans 778	A 188; B 202; C 230; D 251; E 153; F 152; G 163; H106; I 146; J 114; K 117; L 149; M 251; N 129; O 120; P 210; Q 192; R 199; S 197; T 198
Odessky 111	A 186; B 200; C 230; D 251; E 133; F 150; G 157; H 106; I 152; J 114; K 105; L 161; M 229; N 132; O 120; P 190; Q 190; R 199; S 197; T 192
Odessky 82	A 186; B 198; C 230; D 251; E 151 F 150;G 163; H 106; I 146; J 114 ; K 105; L 161; M 251; N 129; O 118; P 190; Q 192; R 199; S 197; T 196
Gelios	A 188; B 200; C 230; D253;E 155; F152; G 157; H 110; I 146; J 114; K 105; L 161; M 251; N 129; O 118; P 204; Q 190; R 199; S 197; T 198

IDENTIFICATION AND REGISTRATION OF UKRAINIAN COMMON WHEAT VARIETIES ON THE BASIS OF STMS-ANALYSIS

Yuri M. Sivolap, Sabina V. Chebotar

Variety genotype identification is important on final stages of breeding process and is necessary for registration of varieties and protection of the author rights. The seed certification also includes identification of varieties and definition of seeds consignments genetic homogeneity.

Usage of DNA-profiling enables to operate with a unlimited amount of molecular markers permitting to test genetic stuff in various loci of genome and to reveal polymorphism. The molecular markers are not subject to influence of environment, and the developed equipment enables fast to estimate a genetic stuff.

Now in leading plant breeding corporations and research institutes which are working on by improving of agricultural plants major attention is given to second generation. DNA profiling markers. To them concerns STMS (Sequence Tagged Microsatellite Site) or SSR (Simple Sequence Repeat) analysis, in which basis testing of microsatellite (MS) of loci polymorphism.

The MS-markers are high-polymorphic, stable, have co-dominant character of inheriting, mainly genotype-specific and the possibility of usage of series of MS-markers in multiplex PCR increases efficiency analysis of microsatellite loci.

The introduction of DNA-profiling techniques to state standard of registration of varieties guesses building the database, in which the molecular-genetic characteristic of registrated varieties will be reflected.

The purpose of the given work is development of identification principles and construction database mirroring the molecular-genetic characteristic of some varieties T. aestivum of breeding from Plant Breeding and Genetics Institute and other breeding stations of Ukraine on the basis of STMS-analysis, learning of allelic structure of microsatellite loci of researched bunch wheat.

Material and methods

Researched series of varieties Ukrainian wheat represented in table 1. With the purpose of definition of resolving power and the installations of the inferior limit of sensibility of the marker system used as check bunch: two seed samples of a variety Yubileinaya 75 and three lines of a variety Odessa krasnokolosaya, discharged by Dr. A.Khohlov.

Used 15 steams of primers to microsatelite loci (Table 2)
Xgwm 3, Xgwm18, Xgwm 155, Xgwm 165, Xgwm 169, Xgwm 190, Xgwm 261, Xgwm 325, Xgwm 357, Xgwm 408, Xgwm 437, Xgwm 577, Xgwm 631, Xgwm 680 developed in Institute for Plant Genetics and Crop Plant Research, Gatersleben. The primers to a microsatelite locus Taglgap, posed in g-gliadin a pseudo-gene on 1 B chromosome are built in John Innes Center, Norwich [5]. Eight of these a steam of primers (Xgwm 3, Xgwm 261, Xgwm 357, Xgwm 408, Xgwm 437, Xgwm 577, Xgwm 631) were used by us earlier for differentiation and identification of wheat varieties various ecology-geographical zones[1].

Intra-varieties polymorphism of varieties Albatros Odesskiy, Besostaya I, Ukrainka Odesskaya, Mironovskaya of 808 and three lines Odesskaya krasnokolosaya was estimated on sampling with 20 individual plants on microsatellite loci: Xgwm 3, Xgwm18, Xgwm 261, Xgwm 437, Xgwm 619, Taglgap.

DNA amplification conducted on the instrument Perkin-Elmer (Norwalk, CT). A reaction mixture of volume 20 мкl contain:50 mm KCl, 20 mm tris-HCl pH8.4 (25 0C), 1.5mM MgCl2, 0,01 % Tween 20, and 0,2mM each dATP, dCTP, dGTP, dTTP, 250 nM of the each primers, 100 ng DNA and 1 ut. Taq-I polymerase.

DNA amplification was in the mode 35 cycles: 94 ^{0}C - 1 min, annealing at 55 ^{0}C, 60 ^{0}C, 65 ^{0}C (in dependence on primers) - in during1 min, 72 ^{0}C - 2 min, final elongation - 10 mines at 72 ^{0}C.

Analysis of amplification fragments conducted on automatic laser sequinator (ALF, Pharmacia), using short cartridges for a gel. 6 % denaturing polyacrilamide gel by depth of - 0,35 mm prepared, using chemical agents SequaGelXR (Biozym). Electrophoresis realized in 1 xTBE buffer (0,09 M tris-borate pH8.3 and 2mM ЕДТА) at 600 V, 50mA, 50W with force of the laser 2 mW. In each track as the intrinsic standard of a molecular weight added fragments with known molecular masses. Dimension of amplification fragments calculated with the help of the program Fragment Manager Version 1.2 (Pharmacia).

For installation of genetic distances on the data of STMS-analysis have constituted a template, where presence of a amplification fragment designated 1, absence - 0. The genetic distances calculated according to algorithm

$$PD = 1-2Nij / (Ni + Nj),$$

Where Nij - number of common fragments for varieties i and j, and (Ni +Nj) - total number of fragments, detected for both varieties, using the program NCLAS from the software package SYN-TAX IV. For dendrogram construction used a computer program DENDPLOT from the same package of computer programs.

Results and Discussion

The analysis of a polymorphism of researched wheat varieties on microsatellite loci (Table 2) has revealed on the average 3.7 alleles for varieties of PBGI breeding and 4.2 alleles on all sampling of researched varieties. The index of polymorphism PIC among varieties of PBFI breeding has constituted 0.49, on common sampling of varieties this parameter was 0.51.

The minimum value PIC - 0.08 (among varieties PBGI) and 0.19 among 29 researched varieties and lines, was supervised for a locus Xgwm 261. The locus Xgwm 190 found out two alleles both among varieties of PBGI breeding and on common sampling of varieties, the index compounded it polymorphism 0.28 and 0.22 accordingly. Thus, 86 % of tested microsattelite loci were high-polymorphic, them PIC on the average compounded 0.54 on sampling of varieties PBGI and 0.56 on sampling 29 varieties. At research with the help of STMS-analysis 12 wheat varieties [2] revealed on the average 3.2 alleles, in work [3] at research 10 wheat genotypes 3.5 alleles were tested. At account PIC according to RFLP-aanalysis fulfilled Chao et al. [4] average value is compounded 0.06].

The total number of alleles, detected on microsatellite loci in our researches for PBGI varieties has constituted 56, for 29 varieties - 63. In Table 3 the alleles tested in our researches on loci, select for analysis represented.

The analysis of intra-varieties polymorphism on series of microsatellite loci for 7 varieties has shown average allele frequency reference 0,95 (Table 4).

It is known, that the majority of the Ukrainian wheat varieties as well as Easteuropean consists of several biotypes. In our researches was not is detected of a considerable level intravarieties polymorphism on loci: Xgwm 3, Xgwm 261, Xgwm 437, Xgwm 619, Taglgap

In too time, on a locus Xgwm18 for variety Albatros odesskiy are detected two alleles 182 п.н. and 188 п.н. with frequency - 0,55 and 0,45, accordingly. It is interesting to mark, that the variety Ukrainka odesskaya, built by selection from Albatros odesskiy has inherited an allele 188 b.p. Conducted by us earlier RAPD -analysis of structure of a variety Albatros odesskiy detected 4 biotypes of this variety (unpublished data).

Screening wheat varieties on 15 microsatellite loci has allowed to discriminate parsed varieties. Each variety identified as a unique genotype according to STMS-analysis.

The information on a genotype of a variety so-called passport that reflect the data on an allelic state of microsatellite loci, represented in Table 5.

In a course of conducted researches the genetical polymorphism of microsatellite loci localized on different chromosomes of a Triticum aestivum L., for some wheat varieties registrated now on Ukraine is investigated. This of research have allowed to differentiate genotypes and to identify them as unique. The date base of the researched genotypes keeping the molecular-genetical characteristic of an allelic state of microsattelite loci of genomes of wheat varieties .

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Table 1. Soft wheat analised varieties and lines.

Variety, line	Variety author	Pedigree
Albatros odesskiy	PBGI	(M-57xMayak)xPromin
Besostaya 1	Krasnodar ARI	Selection from Besostaya 4
Vimpel	PBGI	Zirka x Brigantina x Stepnyak x Odesskaya 95
Donskaya polukarlikovaya	Donskoy ARI	Rusalka x Severodonskaya
Zolotava	PBGI	Donskaya polukarlikovaya x Olvsya
Mirleben	Krasnodar ARI	IO 16208/83{/23833/75x(Geyneh Mironovskaya 808 6)]Alcedo}
Mironovskaya 808	Krasnodar ARI	*
Obriy	PBGI	Red River 68 x Odesskaya 51^2
Odesskaya 51	PBGI	Odesskaya 16 x Besostaya 1
Odesskaya 117	PBGI	Odesskaya 51 x Odesskaya 66
Odesskaya 132	PBGI	(Krasnodarsriy karlik x Odesskaya 51) x Priboy
Odesskaya krasnokolosaya (I-II-III)	PBGI	(Odesskaya 75 x Purdue 4930 x Chayka)x Zaporojskaya ostistaya
Odesskaya polukarlikovaya	PBGI	Krasnodarsriy karlik x Odesskaya 51
Odom	PBGI	(Acteka x Avtora) x Zirka
Simvol odesskiy	PBGI	GK Protein x Albatros odesskiy
Strumok	PBGI	(Red River 68 x Odesskaya 51^2) x Priboy x Yujnaya zarya
Tira	PBGI	{[(Odesskaya 75 x Vel.97) x Priboy] x Promin} x Yunat odesskiy
Ukrainka odesskaya	PBGI	IO Albatros odesskiy
Fedorovka	PBGI	Eritrospermum 1022-79 x Brigantina
Kharkovskaya 50	URPBGI	Donskaya polukarlikovaya x Kharkovskaya 20
Kharkovskaya 93	URPBGI	(Saratovskaya 29 x Milturum 215)
Khersonskaya 86	URMI	Obriy x Odesskaya polukarlikovaya
Eritrospermum 127	AI	Vigodyanskaya 2 x Besostaya 1
Eritrospermum 1072	PBGI	Mutant from a Priboya
Eritrospermum 272-87	PBGI	Obriy x B-16
Eritrospermum 949-38	PBGI	Obriy x B-16
Yubileinaya 75	PBGI	(Tr114/65 x Priboya) x Odesskaya polukarlikovaya x(Lerma Roho x Kavkas)

*- The winter forms are received at threetime seed in the late autumn (1950-1952). spring wheat Artemovka . By repeated group selection of morphology homogeneous plants and mass selection on efficiency and others agricultural important properties from specified materials after severe winter 1955 -1956 the family is allocated, on the basis of which the variety Mironovskaya 808 has created. (on S.V. Rabinovich, 1972).

Table 2. Analysed microsatellite loci.

N	Loci	Chromosome location	Fragment sizes on example Chienese spring	Alleles number on sample of varieties PBGI	Alleles number on sample of general sample	PIC on sample of varieties PBGI	PIC on sample of general sample
1	*Xgwm* 3	3D	84	5	5	0.47	0.52
2	*Xgwm* 18	1B	186	4	5	0.63	0.71
3	*Xgwm* 155	3A	140-150*	4	4	0.60	0.58
4	*Xgwm* 165	4A	191	4	4	0.47	0.39
5	*Xgwm* 169	6A	185-215*	5	5	0.49	0.45
6	*Xgwm* 190	5D	>201	2	2	0.28	0.23
7	*Xgwm* 261	2D	192	2	3	0.08	0.19
8	*Xgwm* 325	6D	138	5	5	0.69	0.66
9	*Xgwm* 357	1A	123	4	4	0.65	0.61
10	*Xgwm* 408	5B	176	5	5	0.42	0.51
11	*Xgwm* 437	7D	109	3	4	0.61	0.66
12	*Xgwm* 577	7B	133	3	5	0.59	0.69
13	*Xgwm* 631	7A	197	3	4	0.44	0.50
14	*Xgwm* 680	6B	123	3	3	0.45	0.38
15	*Taglgap*	1B	255-337*	4	5	0.48	0.58

Fragment sizes tested on varieties sample.

Table 3. Microsatellite loci alleles, revealed on general sample of varieties.

Loci	Number alleles	Molecular weight tested alleles in b.p.				
Xgwm 3	5	80	82	84	86	88
Xgwm 18	5 (4 PBGI)	178	180	182	184	186
Xgwm 155	4	136	140	142	144	
Xgwm 165/I	4	180	184	186	188	
Xgwm 169	5	156	160	164	172	184
Xgwm 190	2	203	207			
Xgwm 261	3 (2 PBGI)	174	192	196		
Xgwm 325	5	129	134	136	138	142
Xgwm 357	4	114	116	118	120	
Xgwm 408	5	166	176	184	189	0
Xgwm 437	4 (3 PBGI)	90	98	112	124	
Xgwm 577	5 (3 PBGI)	120	134	144	152	154
Xgwm 631	4 (3 PBGI)	180	184	192	200	
Xgwm 680	3	124	126	130		
Taglgap	5	0	254	286	334	338

Table 4. Intravariety polymorphism analysis of microsatellite loci on 20 individual plants sample .

variety (line)	Xgwm3		Xgwm18		Xgwm261		Xgwm619		Xgwm437		Taglgap	
	Allele	Frequency allele	Allele	Frequency allele	Allele	Frequency allele	Allele	Frequency allele	Allele	Frequency allele	Allele	Frequency allele
Albatros odesskiy	80 p.n.	1	182 188	0.55 0.45	192	1	134	1	98 -	0.95 0.05	214	1
Besostaya 1	78 80 -	0.55 0.05 0.4	182 188	0.95 0.05	192	1	136 134 130 136+144	0.8 0.05 0.15 0.05			214 -	0.9 0.1
Ukrainka odesskaya	80 79 78 -	0.8 0.05 0.05 0.05	188	1	192	1	134 130	0.95 0.05	98	1	211	1
Mironovskaya 808	78 -	0.95 0.05	184	1	174	1	144 -	0.95 0.05	98	1	211	1
Odesskaya krasnokolosaya I			184	1			134	1			236	1
Odesskaya krasnokolosaya 2			184 -	0.95 0.05			134	1			236	1
Odesskaya krasnokolosaya 3			184	1			134	1			236	1

Maize genotypes differentiation, identification and registration by SSR-markers

N. Kozhukhova, Yuri Sivolap

The differentiation and identification of maize breeding materials and commercial genotypes (lines and hybrids) is important element of breeding and seed production. Phenotype traits expressions are depended on environment conditions and its registration demands plant growing up to complete ripening. Molecular markers usage allows to avoid the most difficulties during solution such tasks as discrimination of nearly relative genotypes, pedigree comparison, genetic purity evaluation, hybridity level determination, genotype characterization on any stage of plant development and etc.

The purpose of our work consisted in molecular-genetic characterization of maize lines and simple hybrids zoned in Southern region of Ukraine and elaboration of maize genotypes registration system by data of SSR-analysis. Researches were realized on such directions: 1) system creation of maize lines and hybrids differentiation and identification, 2) analyzed genotypes registration as formula reflects its heterozygosis, 3) creation on its basis the catalogue of maize genotypes, 4) inbred lines homogeneity determination and hybrid typicalness estimation, 5) paternity testing of maize simple hybrids.

Material and methods

Plant material: 23 inbred lines and 17 simple hybrids were analyzed. The analyzed genotypes zone in Southern region of Ukraine and are brought in «The state list of plant varieties».

DNA extraction: DNA were extract from mix of ten 7-days shoots according to the CTAB-Protocol. DNA concentration was determined on DNA Fluorometer, model TKO 100 ("Hoefer Scientific Instruments", USA).

PCR-amplification: PCR realized on termocycler "Tertsik" ("DNA-Technology", Russia) in the following temperature conditions: an initial denaturation – 93 ^{o}C, 1 min; 30 cycles – 93 ^{o}C, 20 sec., 60 ^{o}C; 20 sec., 70 ^{o}C, 20 sec.; final elongation - 70 ^{o}C, 2 min. The reaction mixture of volume 20 mkl kept: 1 x buffer, included 50 mM KCl; 20 mM tris-HCl pH 8,4; 4 mM $MgCl_2$; 0,01 % twin-20; 0,2 mM each dNTP; till 0,2 mkM of the direct and reverse SSR-primers; 20 ng DNA; 1 unit DNA-Polymerase Taq. Ten SSR-loci were analyzed by PCR. The information about SSR-loci and used primers is shown in Table 1.

Electrophoresis: The PCR-products (2 mkl of reaction mixture) fragmented by electrophoresis in 10 % denaturating polyacrylamide gels. Electrophoresis was held four hours at 500 V in the 1 x TBE-buffer in vertical electrophoresis block ("Hoefer Scientific Instruments", USA). Visualization of PCR-products realized by silver staining. The electrophoretic DNA-profiles images and fragment size calculation were reaches by documentation and electrophoresis gels analysis system “ImageMaster VDS” ("Amersham Pharmacia Biotech", UK).

Mathematical analysis: For estimation of used SSR-system informativeness such parameters were calculated: expected heterozygosis H_e (corresponds to Polymorphic Index Content (PIC)); average heterozygosis H_a; sum effective allele number N_e; probability of non-parent forms exclusion PE; combined probability of exclusion CPE and used such formulas:

$H_e = 1 - \Sigma^n f_i^2$, where f_i^2 - frequency i-allele;
$H_a = \Sigma H_e / n$, where n – analyzed allele number;
$N_e = \Sigma (n_e - 1)$, where n_e - effective allele number for each loci and $n_e = 1 / \Sigma f_i^2$;
$PE = 2 f_i^2 f_j^2$;
$CPE = \prod_{i=1}^{k} (1\text{-}PE_i)$.

Results

Unique DNA-profiles of 40 analyzed genotypes were obtained by PCR-amplification of 10 SSR-loci. Number of alleles per locus varied from two to five. For each locus alleles frequencies and other indexes of informativeness were calculated and summed in Table 2.

As much as male and female forms of the majority of hybrids differed upon DNA-profiles, SSR-analysis was used to determine of the hybridity level of simple hybrids. For this purpose DNA-profile of each hybrid was compared with DNA-profiles of corresponding parent lines. Comparative analysis data given in Table 3.

The presence of the hybrid heterozygote was noted in 100 % comparisons minimum for two (for hybrid MelodyaC) and maximum for eight (for hybrid Rose) analyzed SSR-loci. For each locus the probability of exclusion of the non-parental male's forms was calculated by comparison with pairs the maternal form/offspring of known genotypes in population. Since the analyzed loci were not linked, the value of combined probability of exclusion was 0,99. This value shows, that the non-parental male's form could be incorrectly identified as paternal only in 1 % comparisons.

The unique differentiation of genotypes has allowed to compose the genotypes formulas of maize lines and simple hybrids. Each SSR-locus was encoded by the character of the latin alphabet (Table 1). As an inferior index allele size of given locus was indicated (in b.p.). If locus was homozygous, one allele was indicated. In Table 4 formulas of 40 maize genotypes that were composed by 10 SSR-loci analysis data are shown. The hybrid formula sums the data on the corresponding parent forms. Knowing the simple hybrid formula and one of lines it is possible to determine the second amounting.

Conclusions

The PCR-analysis of 10 SSR-loci has allowed to differentiate uniquely 40 maize genotypes. SSR-testing of simple hybrids DNA revealed heterozygotes for all analized loci, that has enabled to identify the parent forms of simple hybrids with high probability of exclusion of the non-parental forms. The genotypes formulas model were constructed with the purpose of DNA-profiling database creation.

Table 1. Some Characteristics of analyzed SSR-loci

N	SSR-locus	Code	Chromosome localization	Primer sequences (5'- 3')	Repeat size (b.p.)
1	MZEADH2N	A	4S027	TGCGAAGAAGCAGTAGCAAA TGGAGGTAGAAGACGCACG	4
2	phi064	B	1.11	CCGAATTGAAATAGCTGCGAGAACCT ACAATGAACGGTGGTTATCAACACGC	4
3	phi127-2	C	2.07	ATATGCATTGCCTGGAACTGGAAGGA AATTCAAACACGCCTCCCGAGTGT	4
4	phi 083	D	5.06	CGAGACCACCATCATCTGGAAG TTTGCAATCGCTTCGGGGACC	4
5	phi 015	E	8.08	GCAACGTACCGTACCTTTCCGA ACGCTGCATTCAATTACCGGGAG	4
6	phi 061	F	9.03	GACGTAAGCCTAGCTCTGCCAT AAACAAGAACGGCGGTGCTGATTC	8
7	phi 079	G	4.04	TGGTGCTCGTTGCCAAATCTACGA GCAGTGGTGGTTTCGAACAGACAA	5
8	phi 116	H	7.06	GCATACGGCCATGGATGGGA TCCCTGCCGGGGACTCCTG	7
9	phi070	I	6.06	GCTGAGCGATCAGTTCATCCAG CCATGGCAGGGTCTCTCAAG	5
10	phi113	J	5.02	GCTCCAGGTCGGAGATGTGA CACAACACATCCAGTGACCAGAGT	4

Table 2. Informativeness indexes of analyzed SSR-loci

N	SSR-loci	Number of alleles	Number of Homozygotes	Number of Heterozygotes	Number of Heterozygotes among hybrids (%)	H_e	n_e
1	MZEADH2N	5	31	9	53	0,53	2,125
2	phi064	4	30	10	59	0,70	3,353
3	phi127-2	2	34	6	35	0,40	1,663
4	phi083	2	32	8	47	0,48	1,940
5	phi015	2	28	12	71	0,50	1,995
6	phi061	2	33	7	41	0,48	1,905
7	phi079	3	32	8	47	0,50	2,015
8	phi116	3	26	14	82	0,64	2,827
9	phi070	3	28	12	71	0,67	2,994
10	phi113	2	35	5	39	0,41	1,695

Table 3. Heterozygous and homozygous DNA-profiles of hybrids

Hybrid	Heterozygotes (+) and homozygotes (-) on SSR-loci									
	MZEA DH2N	phi 064	phi 127-2	phi 083	phi 015	phi 061	phi 079	phi 116	phi 070	phi 113
MelodyaC	-	-	-	-	-	-	-	+	+	-
P3978M	+	+	-	+	+	+	-	-	-	+
Syren	+	+	-	+	+	+	-	+	+	-
W401m x OK44zm	+	-	+	-	+	-	+	-	-	-
Stozhar	+	+	+	-	+	-	+	+	+	-
SmenaM	-	+	+	-	-	-	-	-	+	-
SurpriseM	+	-	+	-	+	-	-	+	+	-
RoseM	-	+	+	+	-	+	+	+	+	+
Cross 403	+	-	-	-	-	-	+	+	-	-
Od329zm x Od871-88	+	+	-	-	+	+	-	+	+	-
Od329zm x Om150	+	+	-	-	-	+	-	+	-	-
Od329zm x Od310	-	-	+	-	+	+	-	+	+	-
Od329zm x OdB84	-	+	-	+	+	-	+	+	-	+
Od329zm x K1503v	-	+	-	+	+	-	+	+	+	+
Od329zm x B84early	-	-	-	+	+	-	+	+	+	-
Od329zm x A634	+	+	-	+	+	+	+	+	+	-
Od329zm x F564-12zs	-	-	-	+	+	-	-	+	+	+

Table 4. The maize genotypes formula of inbred lines and hybrids

Lines, *hybrids*	Formula
F564c	$A_{134}B_{128}C_{132}D_{133}E_{82}F_{97}G_{182}H_{168}I_{120}J_{154}$
MelodyaC	$A_{134}B_{128}C_{132}D_{133}E_{82}F_{97}G_{182}H_{161}H_{168}I_{115}I_{120}J_{154}$
F564-12	$A_{134}B_{128}C_{132}D_{133}E_{82}F_{97}G_{182}H_{161}I_{115}J_{154}$
P346m	$A_{134}B_{128}C_{132}D_{133}E_{94}F_{89}G_{182}H_{161}I_{110}J_{158}$
P3978M	$A_{134}A_{142}B_{128}B_{132}C_{132}D_{125}D_{133}E_{82}E_{94}F_{89}F_{97}G_{182}H_{161}I_{110}J_{154}J_{158}$
P502мв	$A_{142}B_{132}C_{132}D_{125}E_{82}G_{187}F_{97}G_{182}H_{161}I_{110}J_{154}$
P346м	$A_{134}B_{128}C_{132}D_{133}E_{94}F_{89}G_{182}H_{161}I_{110}J_{158}$
Syren	$A_{134}A_{170}B_{128}B_{132}C_{132}D_{125}D_{133}E_{82}E_{94}F_{89}F_{97}G_{182}H_{161}H_{168}I_{110}I_{115}J_{158}$
IK205-2zm	$A_{170}B_{132}C_{132}D_{125}E_{82}F_{97}G_{182}H_{168}I_{115}J_{158}$
W401m	$A_{174}B_{140}C_{128}D_{125}E_{82}F_{89}G_{187}H_{168}I_{120}J_{154}$
W401mxOK44zm	$A_{134}A_{174}B_{140}C_{128}C_{132}D_{125}E_{82}E_{94}F_{89}G_{182}G_{187}H_{168}I_{120}J_{154}$
OK44zm	$A_{134}B_{140}C_{132}D_{125}E_{94}F_{89}G_{182}H_{168}I_{120}J_{154}$
GK26	$A_{134}B_{144}C_{128}D_{125}E_{94}F_{97}G_{187}H_{175}I_{115}J_{154}$
Stozhar	$A_{134}A_{142}B_{132}B_{144}C_{128}C_{132}D_{125}E_{82}E_{94}F_{97}G_{182}G_{187}H_{161}H_{168}I_{110}I_{115}J_{154}$
P502mv	$A_{142}B_{132}C_{132}D_{125}E_{82}G_{187}F_{97}G_{182}H_{161}I_{110}J_{154}$
GK26	$A_{134}B_{144}C_{128}D_{125}E_{94}F_{97}G_{187}H_{175}I_{115}J_{154}$
SmenaM	$A_{134}B_{140}B_{144}C_{128}C_{132}D_{125}E_{94}F_{97}G_{187}H_{175}I_{115}I_{120}J_{154}$
P101zm	$A_{134}B_{140}C_{132}D_{125}E_{94}F_{97}G_{187}H_{175}I_{120}J_{154}$
GK11	$A_{170}B_{140}C_{128}D_{133}E_{94}F_{97}G_{182}H_{175}I_{110}J_{158}$
SurpriseM	$A_{142}A_{170}B_{140}C_{128}C_{132}D_{133}E_{82}E_{94}F_{97}G_{182}H_{168}H_{175}I_{110}I_{115}J_{158}$
Ом74zm	$A_{142}B_{140}C_{132}D_{133}E_{82}F_{97}G_{182}H_{168}I_{115}J_{158}$
P346m	$A_{134}B_{128}C_{132}D_{133}E_{94}F_{89}G_{182}H_{161}I_{110}J_{158}$
Rose	$A_{134}B_{128}B_{144}C_{128}C_{132}D_{125}D_{133}E_{94}F_{89}F_{97}G_{177}G_{182}H_{161}H_{168}I_{110}I_{115}J_{154}J_{158}$
GK26zm	$A_{134}B_{144}C_{128}D_{125}E_{94}F_{97}G_{177}H_{168}I_{115}J_{154}$
DK437	$A_{134}B_{140}C_{128}D_{125}E_{82}F_{89}G_{182}H_{175}I_{120}J_{154}$
Cross403	$A_{134}A_{142}B_{140}C_{128}D_{125}E_{82}F_{89}G_{177}G_{182}H_{168}H_{175}I_{120}J_{154}$
DK403	$A_{142}B_{140}C_{128}D_{125}E_{82}F_{89}G_{177}H_{168}I_{120}J_{154}$
Od329zm	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
*Od329zm**x**Od871-88*	$A_{134}A_{142}B_{128}B_{140}C_{132}D_{125}E_{82}E_{94}F_{89}F_{97}G_{182}H_{161}H_{168}I_{110}I_{115}J_{154}$
Od871-88	$A_{142}B_{140}C_{132}D_{125}E_{94}F_{89}G_{182}H_{168}I_{115}J_{154}$
Od329zm	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
Od329zmxОм150	$A_{134}A_{166}B_{128}B_{144}C_{132}D_{125}E_{82}F_{89}F_{97}G_{182}H_{161}H_{175}I_{110}J_{154}$
Ом150	$A_{166}B_{144}C_{132}D_{125}E_{82}F_{89}G_{182}H_{175}I_{110}J_{154}$
Од329зм	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
Од329змхОд310	$A_{134}B_{128}C_{128}C_{132}D_{125}E_{82}E_{94}F_{89}F_{97}G_{182}H_{161}H_{175}I_{110}I_{120}J_{154}$
Од310	$A_{134}B_{128}C_{128}D_{125}E_{94}F_{89}G_{182}H_{175}I_{120}J_{154}$
Од329зм	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
Од329змхОдВ84	$A_{134}B_{128}B_{132}C_{132}D_{125}D_{133}E_{82}E_{94}F_{97}G_{182}G_{187}H_{161}H_{168}I_{110}J_{154}J_{158}$
ОдВ84	$A_{134}B_{132}C_{132}D_{133}E_{94}F_{97}G_{187}H_{168}I_{110}J_{158}$
Од329зм	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
Од329змхК1503	$A_{134}B_{128}B_{140}C_{132}D_{125}D_{133}E_{82}E_{94}F_{97}G_{177}G_{182}H_{161}H_{168}I_{110}I_{120}J_{154}J_{158}$
К1503	$A_{134}B_{140}C_{132}D_{133}E_{94}F_{97}G_{177}H_{168}I_{120}J_{158}$
Од329змхВ84early	$A_{134}B_{128}C_{132}D_{125}D_{133}E_{82}E_{94}F_{97}G_{177}G_{182}H_{161}H_{168}I_{110}I_{115}J_{154}$
B84early	$A_{134}B_{128}C_{132}D_{133}E_{94}F_{97}G_{177}H_{168}I_{115}J_{154}$
Од329зм	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
Од329змхА634	$A_{134}A_{142}B_{128}B_{144}C_{132}D_{125}D_{133}E_{82}E_{94}F_{89}F_{97}G_{177}G_{182}H_{161}H_{175}I_{110}I_{120}J_{154}$
А634	$A_{142}B_{144}C_{132}D_{133}E_{94}F_{89}G_{177}H_{175}I_{120}J_{154}$
Од329зм	$A_{134}B_{128}C_{132}D_{125}E_{82}F_{97}G_{182}H_{161}I_{110}J_{154}$
Од329змхF564-12зс	$A_{134}B_{128}C_{132}D_{125}D_{133}E_{82}E_{94}F_{97}G_{182}H_{161}H_{168}I_{110}I_{115}J_{154}J_{158}$
F564-12зс	$A_{134}B_{128}C_{132}D_{133}E_{94}F_{97}G_{182}H_{168}I_{115}J_{158}$

[End of Annex III and of document]