ASSESSMENT THE GENETIC DIVERSITY OF BULGARIAN RASPBERRY GERMPLASM COLLECTION BY MICROSATELLITE AND RAPD MARKERS

Ilian Badjakov[1], Elena Todorovska[1], Violeta Kondakova[1], Raina Boicheva[2] and Atanas Atanassov[1]

[1]AgroBioInstitute, Sofia, BULGARIA
[2]Institute of Agriculture, Kjustendil, BULGARIA

(Received October 12, 2005/Accepted October 30, 2005)

A B S T R A C T

As an important producer of soft fruits Bulgaria has a rich germplasm collection of both Bulgarian and foreign varieties with valuable agronomic characters of commercial value. In addition to morphological descriptions used for classification of species and cultivars the molecular characterization of raspberry germplasm collection should enable breeders to expand genetic diversity in breeding material and can facilitate the accurate selection of genotypes with valuable agronomic characters in breeding programmes.

In this article we present our initial study on the assessment of genetic diversity among Bulgarian and foreign raspberry accessions using Simple Sequence Repeat (SSRs) and Random Amplified Polymorphic DNA (RAPDs) markers. Twenty eight raspberry genotypes (nineteen Bulgarian, eight foreign and two wild accessions) were screened for their polymorphism at four microsatellite loci. The value determined for genetic variation demonstrated a high genetic diversity in the Bulgarian raspberry germplasm collection. Genetic diversity (GD) calculated from SSR data ranged from 0.816 to 0.925 with a mean GD – 0.863. RAPD assay using 4 arbitrary 10-base primers was applied as complementary marker system for gene diversity studies in 14 varieties and elite raspberry lines. A total of 56 fragments were recorded, of which 87.5% were polymorphic. The Genetic Similarity (GS) among genotypes evaluated from RAPD data ranged from 0.480 to 0.952 thus indicating that a high level of gene diversity is present in the selected genotypes. RAPD analysis proved to be efficient for discrimination of elite raspberry lines with common pedigree.

Key words: raspberry, molecular markers, SSR, RAPD, genetic diversity

INTRODUCTION

The genus *Rubus* is one of the most diverse in the plant kingdom with over 500 heterozygous species within 12 subgenera (Jenings, 1988). The most commercially important of the domesticated subgenera is *Ideobatus* (raspberries). The subgenera contain some 200 species showing considerable differentiation, of which the most commercially important are the European red raspberry (*R. ideus* ssp. *vulgatus* Arrhen.), the North American red raspberry (*R. ideus* ssp. *Strigosus* Michx) and the black raspberry (*R. occidentalis* L.) Roach (1985) and Jennings (1988) gave account of the early domestication of red raspberry (*R. ideus*). Five parent cultivars dominate the ancestry of red raspberry: Lloyd George and Pynes Royal entirely derived from the European subspecies and Preussen, Cuthbert and Newburgh derived from both European and North American subspecies. Domestication has resulted in a reduction of both morphological and genetic diversity in red raspberry (Haskell, 1960 and Jenings, 1988) with modern cultivars being genetically similar (Dale et al., 1993; Graham and McNicol, 1995). The accurate classification of species and cultivars based on morphological characters can be difficult. The development of molecular biology has resulted in DNA based marker procedures that should lead to a greater understanding of relationships between species and more accurate taxonomic classification. These techniques should also allowed more effective understanding of crop plant's genetic architecture and utilization of genetic diversity by breeders and identification of species and cultivars by means other than morphological characteristics (Graham et al., 2002). Chloroplast DNA probes has been unable to detect variation between raspberry cultivars (Waugh et al., 1990) and minisatellite DNA and other oligonucleotides used as probes in RFLP analyses (Nybom and Schad, 1990; Parent and Page, 1992) have proved to be time consuming and require the use of radioisotopes.

In the past decade, the polymerase chain reaction (PCR) has emerged as a promising technique in molecular genetic studies (Williams et al., 1990). Different marker systems based on PCR such as RAPDs, AFLPs and microsatellites, have been extensively used in detection of DNA differences in *Rubus* and its wild progenitor *Rubus idaeus.* RAPD markers have been used in studies related to genetic variation among cultivars from Tayside area (Graham and McNicol, 1995) and spatially separated populations of wild *Rubus idaeus* (Graham and McNicol, 1995). RAPD analysis has been also applied by Gidoni et al. (1994) for fingerprinting and obtaining of markers for variety protection of commercial strawberries (*Fragaria ananassa* Dutch). Genetic maps based on RAPD markers have been constructed from Davis and Yu (1997), Levi et al. (1994), for diploid strawberry (*Fragaria vesca*) and diploid blueberry (*Vaccinium* spp.).

SSR markers have become quite useful in various aspects of molecular genetic studies, including assessment of genetic diversity (Amsellem et al., 2001, Ashley et al., 2003), fingerprinting (Rongwen et al., 1995), ecological-genetic studies (Li et al., 2000), marker-assisted selection (Fazio et al., 2003), and genetic linkage mapping (Akkaya et al., 1995; Broun and Tankley, 1996; Graham et al., 2004). The proven advantages of SSR markers is due to their high information content (PIC), co-dominant inheritance, locus specificity, extensive genome coverage and simple detection using labelled primers that flank the microsatellite and hence define the microsatellite locus (Graham et al., 2002; 2004). PCR-based DNA markers (RAPDs, AFLPs, genomic SSRs and EST-SSRs) and genetic linkage map in *Rubus* has been developed in Scottish Crop Research Institute by Graham et al. (2004). Such genetic linkage maps can facilitate the application of diagnostic DNA markers for polygenic traits and the identification of genes controlling complex phenotypes.

As a traditionally important producer of soft fruits Bulgaria has a rich germplasm collection of both Bulgarian and foreign varieties with valuable agronomic characters of commercial value. In addition to morphological descriptions used for classification of species and cultivars and characterization of the Bulgarian raspberry germplasm collection a programme for assessment the genetic diversity by both microsatellite and RAPD markers was recently initiated. The application of molecular markers should allowed more efficiently understanding of the crop plant genetic architecture, the identification of species and cultivars by means other than morphological characters and should also give an unable of breeders to expand genetic diversity in breeding material and can facilitate the accurate selection of genotypes with valuable agronomic characters in breeding programmes.

The objective of the present study was to use SSR and RAPD markers for assessment the genetic diversity among raspberries accessions from Bulgarian germplasm collection.

MATERIAL AND METHODS

Plant material

28 raspberry accessions of Bulgarian collection including 18 Bulgarian varieties and lines, 8 international varieties and 2 wild raspberry species: *R. occidentalis* and *R. adiene* were chosen for microsatellite analysis (Tab. 1). Twelve varieties and 14 lines obtained by intraspecific cross were subjected for RAPD analysis.

T a b l e 1 . Origin and geographical distribution of 28 raspberry accessions

Geographical region/Country					
Europe				Canada	USA
Bulgaria	Switzer-land	Germany	Norway		
1. Shopska Alena	1. Zeva-3	1. Schonemone	1. Vetten	1. Tulameen	1. Meeker
2. Lulin					2. Heritigae
3. Bulgarski Rubin					3. Fairview
4. Iskra					
5. Samodiva					
6. Elit – 1					
7. Elit – 3					
8. 27337 (Samodiva x 7667)					
9. 27555 (Samodiva x Gradina)					
10. 26666 (Samodiva x Gradina)					
11. 26664 (Samodiva x Gradina)					
12. 25927 (Samodiva x Podgorina)					
13. 27562 (Samodiva x Veten)					
14. 23006 (Samodiva 8000Ro)					
15. 23005 (Samodiva 8000Ro)					
16. 21893 (Willamette x Shopska Alena)					
17. 21840 (Willamette x Shopska Alena)					
18. 23501 (Iskra x Novost Kuzmina)					
19. 26101 (Lulin x 1193)					
20. *Rubus occidentalis*					
21. *Rubus adiene*					

DNA isolation and fragment analysis

DNA was isolated according to Murray and Thompson (1980). Four microsatellite markers (26, 108, 118 and 126) were selected for genotyping (Graham et al., 2002; 2004). PCR reactions were performed as described by Graham et al. (2002). Microsatellite alleles were detected on an automated laser fluorescence (ALF expressed II) sequencer and analyzed using the software Allele Locator 1.03 (Amersham Biosciences) by comparison to internal size standards with length of 100, 150, 200, 250, 300, 350 bp amplified from pUC19 vector.

Four 10-base primers (Operon Technologies Inc. – USA, Amersham Biosciences – USA) were selected for amplification of gDNA of 14 Bulgarian varieties and lines. Amplification reaction was performed in conformity with the requirement of the kit "Ready-To-Go RAPD Beads" – Amersham Biosciences (USA). For RAPD analyses samples were pre-denatured for 5 min at 95°C, followed by 45 cycles of polymerization reaction, each consisting of a denaturation step for 1 min, an annealing step for 1 min at 36°C and an extension step for 2 min at 72°C. The last cycle was followed by 5 min at 72°C. The amplification program was performed on a thermocycler model MiniCycler™, MJ Research (USA). Amplified DNA fragments were separated in 2% agarose gels (1xTAE) stained with ethidium bromide, visualized under UV light and photographed.

Data analysis

Fragments amplified with microsatellite and RAPD primers were scored as presence (1) and absence (0). Genetic diversity was calculated using formula [GD=1-Σ Pij2]. Genetic similarity (GS) between varieties and lines was estimated according to formula of Nei and Li (1979). The fraction of bands common between 2 genotypes was estimated using the following formula: GSij = 2Nij / (Ni+Nj).

Hierarchical cluster analysis under unweighted pair-group method with an arithmetic average (UPGMA) methodology was applied through XLSTAT 7.5 to build the dendrogram of genetic similarities.

RESULTS

GENETIC DIVERSITY STUDIES USING SSR MARKERS

Microsatellite polymorphism

A set of 4 raspberry microsatellite markers was used for assessment of genetic diversity in 26 raspberry accessions (*R. idaeus*) and 2 wild raspberry species: *R. occidentalis* and *R. adiene* from Bulgarian germplasm collection. A total of 58 alleles were detected at 4 studied microsatellite loci. The number of alleles per locus varied between 6 for locus **108** to 21 for locus **118** with an average number of 14.5 alleles per locus (Tab. 2).

A total number of 33 alleles with a mean 8.25 alleles/locus were obtained for 19 Bulgarian raspberry genotypes. For European, Canadian and American genotypes were detected a total 28 alleles with a mean 7 alleles per locus.

A comparison of the used SSR markers (Tab. 2) with regard to their information content (PI) (number of alleles and Probability of Identity value)

was done. The probability of identity was quite variable, as were the diversity index reflecting the range and frequencies of alleles detected by each microsatellite marker (Tab. 3). PI showed that the most informative loci for the studied varieties were **118** and **26** with the highest number of alleles and PI values 0.0182 and 0.060 respectively.

Table 2. Description of 4 microsatellite markers, allele size, major allele and number of alleles across 28 raspberry accessions

Microsatellite	Motif	Allele size [bp]	Major allele	Number of alleles
108	$(CT)_9$ $(AT)_5$	150-159	157	7
118	$(CT)_{25}$	60-157	107	21
126	$(CT)_{31}$ $(C)_{22}$	88-196	88	17
26	$(CT)_{11}(C)_{29}$	87-196	136, 182	13
Total				**58**
Mean				**14.5**

SSR marker was for distinguishing of two mutant lines (No 23006 and No 23005) obtained by γ irradiation of "Samodiva" was obtained. Both lines

Genetic diversity

Table 3. Genetic diversity (GD) in 27 raspberry varieties and 2 wild species, observed (Ho) and expected (He) heterozigosity at 4 microsatellite loci

Micro-satellite	Genetic diversity				Heterozigosity		Probability of identity [PI]
	Bulgarian varieties	European varieties	American varieties	total GD	Ho	He	
108	0.7731	0.7222	0.8000	0.816	0.5357	0.8157	0.113
118	0.8843	0.6111	0.7400	0.925	0.5357	0.9279	0.018
126	0.7577	0.6666	0.7400	0.858	0.7143	0.8546	0.060
26	0.6929	0.7777	0.7600	0.854	0.5714	0.8584	0.063
Mean	0.7770	0.6944	0.7600	0.863	0.5892	0.8642	$7.878x10^{-6}$

Genetic diversity (GD) was studied in a set of 28 raspberries from Bulgarian collection and separately in the groups representing different geographical regions: Europe, Canada and United States. Mean Genetic Diversity (MGD) in all studied loci was **0.863** (Tab. 3). The lowest GD was calculated for locus 108 with **0.816** and highest for locus 118 with **0.925** respectively. Genetic diversity (GD) in the group of Bulgarian varieties was **0.777** compared to European with **0.694** and American – **0.760** respectively. The higher GD observed in the set of Bulgarian raspberry varieties is probably due to more genotypes included in the trial.

Estimate heterozigosity

The application of co-dominant SSR markers has allowed the evaluation of heterozigosity. The estimated values of the expected heterozigosity of the studied loci ranged from 0.8156 at locus 108 to 0.9279 at locus 118 with a mean value of 0.8641.

Correspondingly the estimated value of the observed heterozigosity of the studied loci (the percentage of heterozygous individuals among all tested ones) varied between 0.535 at loci 108 and 118 to 0.714 at locus 26 with a mean value of 0.5892. The observed heterozigosity is lower than the expected one in all studied microsatellite loci. Conversely, the study of the set of 50 *Rubus* genotypes, including *Rubus* species material, European and North American red raspberry cultivars (*R. idaeus*), black raspberry (*R. occidentalis*) and purple raspberry (*R. idaeus* x *R. occidentalis*), blackberries (*R. fructosus* L. agg) and hybrids with 10 SSR markers showed that the observed heterozigosity (*H*o) is higher than the expected ones in most loci (Graham et al., 2002). Different level of mean heterozigosity has also been observed among parental lines (40% for Glen Moy and 84% for Latham, respectively) including in creation of mapping population for construction of genetic map with AFLPs, genomic- and EST- SSR markers (Graham et al., 2004). In our study different levels of mean heterozigosity was observed for the parental lines of cv. Samodiva (25% for B. Rubin and 75% for Shopska Alena). The level of heterozigosity in elite Bulgarian raspberry lines varied between 50 and 100%. Lines No27337, No26101, No26666 and No21840 are characterized by 50% heterozigosity. 75% heterozigosity was found for lines No 21839, No 25927, No 26664, No 27555, No 23006 and No 23005. 100% heterozigosity at four SSR loci was found in lines No 23501 (Iskra x Novost Kuzmina) and No 27562 (Samodiva x Veten).

In order to characterize further the structure and grouping of 29 raspberry accessions from Bulgarian raspberry germplasm collection a dendrogram deriving from UPGMA cluster analysis based on the genetic similarity (GS) coefficient matrix was designed (Fig. 1). Generally all accessions could be distinguished. The dendrogram clearly shows the presence of 2 main clusters. Grouping of accessions originating from different geographical regions was observed. All Bulgarian raspberries were grouped in one cluster together with 2 European (Schonemone and Veten), Canadian (Tulameen) and one American (Willamette) varieties. The hybrid – cv. Samodiva (B. Rubin x Shopska Alena) and one of its parents (B. Rubin) are grouping in one of the subclusters obtained. We suggest that a specific flow of the studied chromosomal regions (alleles) from one of the parents into the genome of cv. Samodiva occurred. Mutant lines No23006 and No23005 (Samodiva 8000 Ro) which are characterizing with highest level of genetic similarity are grouped close to each other in one of subclusters.

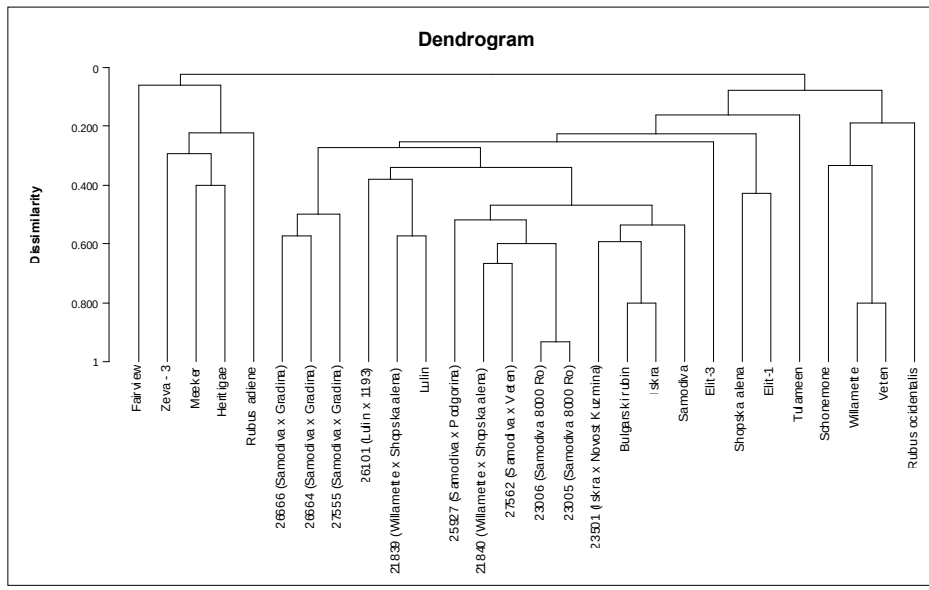


Figure 1. Genetic relationship between 28 raspberry accessions based on SSR's data using UPGMA cluster analysis

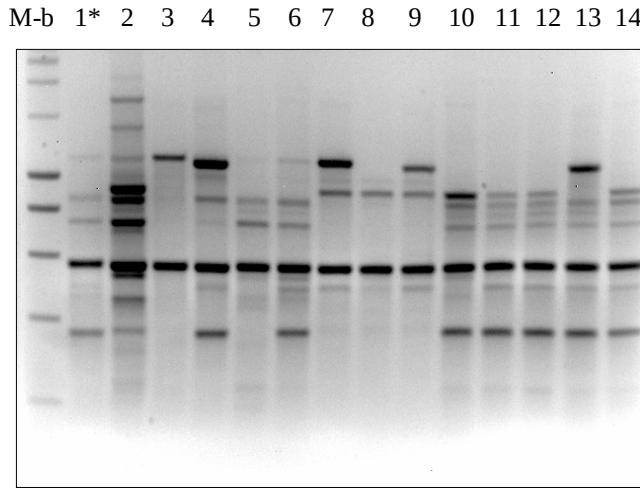
RAPD ANALYSIS AS A TOOL FOR EVALUATION OF GENETIC DIVERSITY AMONG BULGARIAN VARIETIES AND ELITE LINES

RAPD analysis of 14 raspberry varieties and elite lines was performed using a set of four 10-base oligonucleotides with arbitrary sequence and 60-70% (G+C) content. RAPD patterns obtained are characterized with high reproducibility under the established PCR conditions. A total of 56 fragments, ranging from 200 to 1800 bp were recorded, 87.5% of which were polymorphic.

The four primers (OPA07, OPA17, OPB19 and OPC08) used in RAPD analysis generated high number of polymorphic bands and could be successfully used for discrimination of lines No 26666, No 26664, No27555 (Samodiva x Gradina), and lines No21839 and No21840 (Willamette x Shopska Alena) with common pedigree (Tab. 1) but characterizing with different morphological characters (data not shown).

RAPD polymorphism generated with primer OPA07 in elite lines with common pedigree

Primer OPA07 produced an additional band of 700 bp in lines No26666 and No27555 in comparison to No26664. An additional band with length of 300 bp was detected in No26666 in comparison to No26664 and No27555. An additional band of 400 bp and absence of 650 bp band was detected in No27555 in comparison to other 2 lines (No26666 and No26664) deriving from the same cross (Samodiva x Gradina) (Fig. 2).



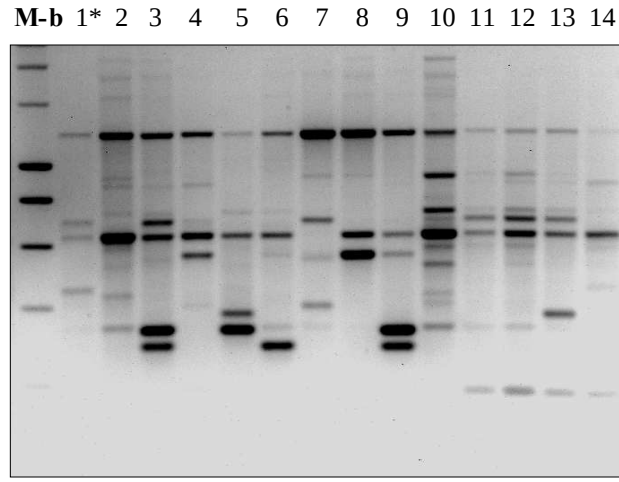
*Line 1 – 27337 (Samodiva x 7667)
Line 2 – 26101 (Lulin x 1193)
Line 3 – 26666 (Samodiva x Gradina)
Line 4 – 21839 (Willamette x Shopska Alena)
Line 5 – 25927 (Samodiva x Podgorina)
Line 6 – 26664 (Samodiva x Gradina)
Line 7 – 27555 (Samodiva x Gradina)
Line 8 – 21840 (Willamette x Shopska Alena
Line 9 – 23501 (Iskra x Novost Kuzmina)
Line 10 – 27562 (Samodiva x Veten)
Line 11 – 23006 (Samodiva 8000 Ro)
Line 12 – 23005 (Samodiva 8000 Ro)
Line 13 – Shopska Alena
Line 14 – Lulin

Figure 2. RAPD banding patterns of 14 Bulgarian raspberry accessions generated by primer OPA17; M-b – Marker SmartLadder

RAPD polymorphism generated with primer OPA17 in elite lines with common pedigree

A. *OPA17 RAPD polymorphism in elite lines of the cross* (*Willamette* x *Shopska Alena*): OPA17 RAPD pattern of line No21840 is characterizing with absence of 2 bands with length 380 bp and 1100 bp respectively in comparison to No21839.
B. *OPA17 RAPD polymorphism in elite lines of the cross* (*Samodiva* x *Gradina*): Absence of 2 fragments (390 bp and 900 bp) was observed in No26666 and No26664 in comparison to No27555. Two additional

bands (750 bp and 850 bp) were detected in OPA17 RAPD pattern of line No26664 in comparison to lines No26666 and No27555 (Fig. 3).



***For explanation, see Figure 2**

Figure 3. RAPD RAPD banding patterns of 14 Bulgarian raspberry accessions generated by primer OPA07; M-b – Marker SmartLadder

RAPD polymorphism generated with primer OPB19 in elite lines with common pedigree

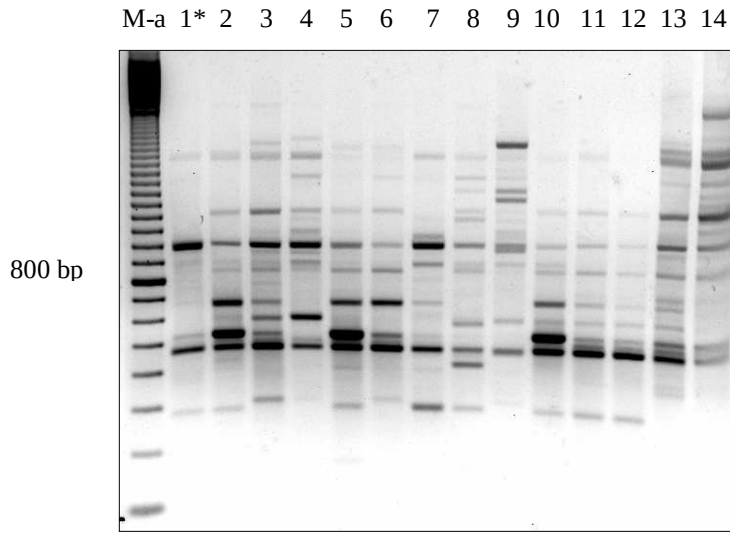
A. *OPB19 RAPD polymorphism in elite lines of the cross* (*Willamette* x *Shopska Alena*): Two additional bands (300 bp and 450 bp) were observed in OPB19 RAPD pattern of line No21840 in comparison to No21839.

B. *OPB19 RAPD polymorphism in elite lines of the cross* (*Samodiva* x *Gradina*): An additional band with 600 bp length was detected in OPB19 RAPD pattern of line No26666 in comparison to lines No 26664 and No27555. Variation in the length of low molecular weight bands was observed (320 bp in No 26666 and No 26664, and 300 bp in No27555 (Fig. 4).

RAPD polymorphism generated with primer OPC08 in elite lines with common pedigree

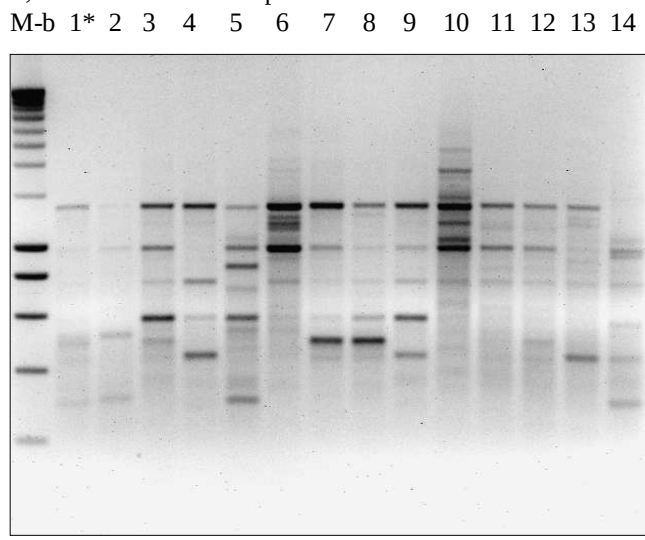
A. *OPC08 RAPD polymorphism in elite lines of the cross* (*Willamette* x *Shopska Alena*): Absence of 1000 bp fragment in OPC08 RAPD pattern of line No21839 and variation in the length of lower molecular weight fragments (450 bp in line No21839 and 500 bp in line No21840) were detected.

B. *OPC08 RAPD polymorphism in elite lines of the cross* (*Samodiva* x *Gradina*): Few additional high molecular weight bands (between 1300-1400 bp) and absence of 600 bp band was observed in OPC08 RAPD pattern of line No26664 in comparison to lines No26666 and No27555 (Fig. 5).



*For explanation, see Figure 2

Figure 4. RAPD banding patterns of 14 Bulgarian raspberry accessions generated by primer OPB19; M-a – Marker 100 bp



*For explanation, see Figure 2

Figure 5. RAPD banding patterns of 14 Bulgarian raspberry accessions generated by primer OPC08; M-b – Marker SmartLadder

Genetic variation among lines No23006 and No23005 which are experimentally induced mutants, obtaining by ionizing radiation (8000Ro) was not detected using the set of applied in RAPD analysis primers (Fig. 2, 3, 4, 5).

Polymorphic bands were obtained in RAPD patterns of varieties “Shopska Alena” and “Lulin” generated by all used primers. Additional fragments with length 350 bp and 700 bp in OPA07, 1050 bp in OPA17 and 1480 bp in OPC08 RAPD patterns were observed in Shopska Alena in comparison to Lulin.

Some unique bands observed in RAPD patterns may transfer into SCAR markers for varieties identification and breeder right protection.

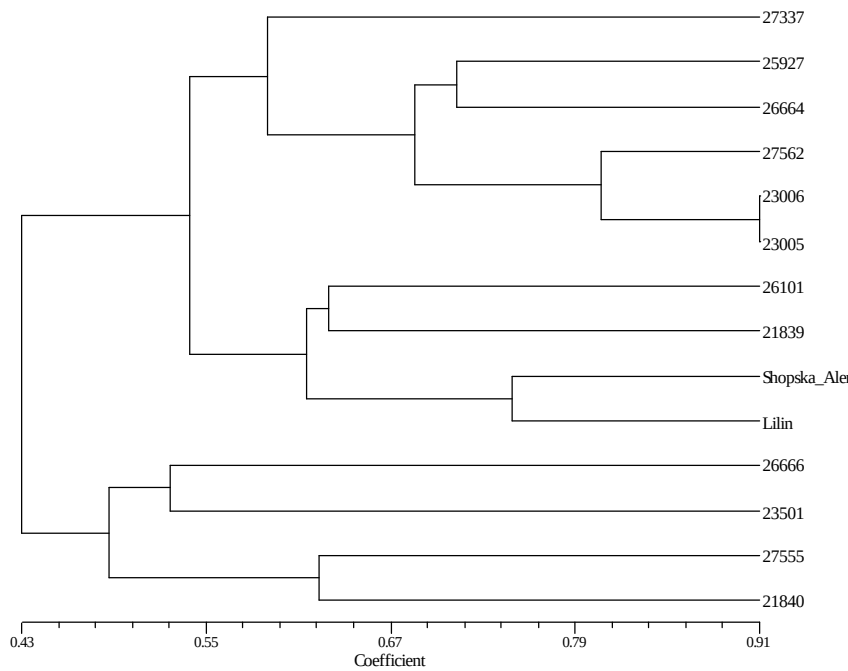


Figure 6. Genetic relationship between 14 raspberry accessions based on RAPD data using UPGMA cluster analysis

In order to characterize further the structure and grouping of 14 raspberry accessions from Bulgarian germplasm collection the genetic similarity tree based on RAPD data was designed (Fig. 6). Generally all accessions could be distinguished excluding mutant lines No23006 and 23005 obtained by γ – irradiation of cv. Samodiva. The dendrogram clearly shows the presence of 2 main clusters. Most of genotypes were ordered as expected from their pedigree data and both RAPD and SSR trees showed similar grouping of cultivars and lines included in this study. The only difference is closely

Table 4. Genetic similarity (GS) between 14 raspberry accessions was estimated according to the formula suggested by Nei and Li (1979)

	27337	**26101**	**26666**	**21839**	**25927**	**26664**	**27555**	**21840**	**23501**	**27562**	**23006**	**23005**	**Shopska Alena**	**Lulin**
27337	1	0.692	0.583	0.560	0.727	0.636	0.608	0.608	0.545	0.782	0.800	0.761	0.720	0.695
26101	0.692	1	0.733	0.838	0.785	0.785	0.551	0.689	0.571	0.758	0.692	0.666	0.774	0.758
26666	0.583	0.733	1	0.689	0.692	0.692	0.666	0.740	0.692	0.666	0.583	0.560	0.620	0.592
21839	0.560	0.774	0.689	1	0.592	0.666	0.642	0.714	0.592	0.571	0.640	0.615	0.733	0.785
25927	0.727	0.785	0.615	0.592	1	0.833	0.560	0.640	0.500	0.880	0.818	0.782	0.740	0.640
26664	0.636	0.642	0.692	0.666	0.833	1	0.480	0.560	0.583	0.800	0.818	0.782	0.740	0.640
27555	0.608	0.551	0.592	0.642	0.560	0.480	1	0.769	0.560	0.615	0.521	0.583	0.571	0.538
21840	0.608	0.689	0.740	0.714	0.640	0.560	0.769	1	0.72	0.692	0.608	0.666	0.642	0.615
23501	0.545	0.571	0.692	0.592	0.583	0.583	0.560	0.720	1	0.560	0.545	0.521	0.592	0.480
27562	0.782	0.758	0.666	0.571	0.880	0.800	0.615	0.692	0.560	1	0.869	0.916	0.785	0.692
23006	0.800	0.692	0.583	0.560	0.818	0.818	0.521	0.608	0.545	0.869	1	0.869	0.800	0.782
23005	0.761	0.666	0.560	0.615	0.782	0.695	0.583	0.666	0.521	0.916	0.952	1	0.769	0.750
Shopska Alena	0.720	0.774	0.620	0.733	0.740	0.740	0.571	0.642	0.592	0.785	0.800	0.769	1	0.857
Lulin	0.695	0.758	0.592	0.785	0.640	0.640	0.538	0.615	0.480	0.692	0.782	0.750	0.857	1

grouping of No 26664 (Samodiva x Gradina) with lines No23337 (Samodiva x 7667), 25927 (Samodiva x Podgorina), 27562 (Samodiva x Veten), 23005 (Samodiva 8000Ro) and 23005 (Samodiva 8000Ro) in RAPD tree in comparison to the SSR tree.

We can hypothesizes that the different grouping of 26664 (Samodiva x Gradina) is due to the higher percentage of introgression of 'Samodiva' germplasm in its genome rather than 'Gradina' as in the case No 26666 (Samodiva x Gradina) and 27555 (Samodiva x Gradina) which are positioned in different cluster.

The paper reports the first results of the research on molecular characterization of Bulgarian raspberry germplasm collection. The work is a part of long-term studies on preservation and utilization of small fruit's genetic resources on a regional level.

REFERENCES

Akkaya M.S., Shoemaker R., Specht J.E., Bhagwat A.A., Cregan P.B. 1995. Integration of simple sequence repeat (SSR) DNA markers into a soybean linkage map. CROP SCI. 35: 1439-1445.

Amsellem L., Dutech C., Billote N. 2001. Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir (Rosaceae), an invasive weed in La Reunion island. MOL. ECOL. NOTES 1: 33-35.

Ashley M.V., Wilk J.A., Styan S.M.N., Craft K.J., Jones K.L., Feldheim K.A., Lewers K.S., Ashman T.L. 2003. High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill (Rosaceae). THEOR. APPL. GENET. 107: 1201-1207.

Broun P., Tanksley S.D. 1996. Characterization and genetic mapping of simple repeat sequences in the tomato genome. MOL. GEN. GENET. 250: 39-49.

Dale A., Moore P.P., McNicol R.J., Sjulin T.M., Burmistrov L.A. 1993 Genetic diversity of red raspberry varieties throughout the world. J. AM. SOC. HORTIC. SCI. 118: 119-129.

Davis T.M, Yu H. 1997. A linkage map of the diploid strawberry *Fragaria vesca*. J. HERED 88: 215-221.

Fazio G., Chung S.M, Staub J.E. 2003. Comparative analysis of response to phenotypic and marker-assisted selection for multiple lateral branching in cucumber (*Cucumis sativa* L.) THEOR. APPL. GENET. 107: 875-883.

Gidoni D., Rom M., Kunik T., Zur M., Izsak E., Izhar S., Firon N. 1994. Strawberry cultivar identification using randomly amplified polymorphic DNA (RAPD) markers. PLANT BREED. 113: 339-342.

Graham J., McNicol R.J. 1995. An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. THEOR. APPL. GENET 90: 1128-1132.

Graham J., Smith K., Woodhead M., Russel J. 2002. Development and use simple sequence repeat SSR markers in *Rubus* species. MOL. ECOL. NOTES 2: 250-252.

Graham J., Smith K., MacKenzie, Jorgenson L., Hackett C., Powell W. 2004. The construction of a genetic linkage map of red raspberry (Rubus idaeus subspp. idaeus) based on AFLPs, genomic-SSR and EST-SSR markers. THEOR. APPL. GENET 109: 740-749.

Haskell G. 1960. The raspberry wild in Britain. WATSONIA 4: 238-255

Jenings D.L. 1988. Raspberries and blackberries: their breeding, diseases and growth. Academic, London.

Levi A., Rowland L.J., Galletta G. J., Martelli G., Greco I. 1994. Identification of strawberry genotypes and evaluation of their relationships using Randomly Amplified Polymorphic DNA (RAPD) ANALYSIS. ADV. STRAWBERRY RES. 13: 36-38.

Li Y., Fahima T.,. Korol A.B,. Peng J,. Roder M.S,. Kirzhner V,. Beiles A, Nevo E. 2000. Microsatellite diversity correlated with ecological-edaphic and genetic factors in three microsatellites of wild emmer wheat in north Israel. MOL. BIOL. EVOL. 17: 851-862.

Muray M.G., Thompson W.F. 1980. Rapid isolation of high. molecular weight plant DNA. NUCLEIC ACID. RES. 8: 4321-4325.

Nei M., Li W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. PROC. NAT. ACAD. SCI. USA 76: 5267-5273

Nybom H., Schaal B.A. 1990. DNA "fingerprints" reveal genotypic distributions in natural populations of blackberry and raspberries (*Rubus*, Rosaceae) AMER. J. BOTANY 77: 883-888.

Parent J.G., Page D. 1992. Identification of raspberry cultivars by nonradioactive DNA fingerprinting. HORT. SCI. 27: 1108-1110.

Roach F.A. 1985. Cultivated fruits of Britain: their origin and history. Blackwell, Oxford.

Rongwen J., Akkaya M.S., Bhagwat A.A., Levi U., Cregan P.B. 1995. The use of microsatellite DNA markers for soybean genotypes identification. THEOR. APPL. GENET. 90: 43-48.

Waugh R., Van de Ven W.T.G., Phillips M.S., Powel W. 1990. Chloroplast DNA diversity in the genus *Rubus* (Rosaceae) revealed by Southern hybridisation. PLANT SYSTEM EVOL. 172: 65-75.

Williams J.G.K.,. Kubelik A.R. Livak K.J. Rafalski J.A., Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. NUCLEIC ACIDS RES.18: 6531-6535.

ZASTOSOWANIE TECHNIKI SSR I RAPD DO OCENY ZRÓŻNICOWANIA GENETYCZNEGO MALINY Z BUŁGARSKIEGO BANKU GENÓW

Ilian Badjakov, Elena Todorovska,
Violeta Kondakova, Raina Boicheva
i Atanas Atanassov

S T R E S Z C Z E N I E

Bułgaria dysponuje bogatą kolekcją w banku genów obejmującą zarówno odmiany rodzime, jak i odmiany zagraniczne roślin sadowniczych o dużej wartości produkcyjnej. Autorzy opisują wyniki wstępnych badań nad zróżnicowaniem genetycznym odmian maliny. Dwadzieścia osiem genotypów (18 bułgarskich, 8 zagranicznych i 2 genotypy dzikie) były testowane na obecność polimorficznego DNA z użyciem 4 starterów mikrosatelitarnych. Zróżnicowanie genetyczne (GD) określone na podstawie danych uzyskanych w reakcjach SSR wynosiło od 0,816 do 0,925 (średnio – 0.863). Technika RAPD z 4 arbitralnymi starterami była zastosowana jako system komplementarny dla badania zróżnicowanie genetycznego. Testy oparte na RAPD prowadzono dla 14 odmian i linii maliny. Łącznie uzyskano 56 fragmentów, spośród których 87.5% było polimorficzne. Pokrewieństwo genetyczne określone na podstawie wyników RAPD wynosiło od 0,480 do 0,952. Technika RAPD posłużyła także do rozróżnienia linii maliny od uprawianych odmian.

Słowa kluczowe: malina, markery molekularne, SSR, RAPD, zróżnicowanie genetyczne