DNA polymorphism in genetic resources of red pepper using microsatellite markers

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ABSTRACT: Genetic variability among 41 accessions of red pepper (*Capsicum annuum* L.) was assessed using eight microsatellite markers. Three of the microsatellite markers (*Hpms 1-1*, *Hpms 1-168*, and *Hpms 1-274*) had uniform spectra in all the analyzed plants. Two to eight alleles were detected for the remaining loci. In total, 28 alleles were detected, i.e. 3.5 alleles per one microsatellite locus on average. The highest number of different alleles was detected with *Hpms 1-5* (8 alleles) and *Hpms 2-21* primers (7 alleles). Molecular data were complemented with morphological measurements according to the descriptor list for the genus *Capsicum*. A dendrogram based on our genetic analysis suggests a high level of similarity between some of the accessions presumed to be distant and, at the same time, genetic variability between accessions of the same or similar name. These results show the possibility of duplicities in the current Czech collection of red pepper genetic resources.

Keywords: pepper; genetic resources; microsatellites; SSRs; variability

The often excessively large extent of the genetic resources collections prevents a detail characterization of the individual accessions that is a prerequisite of their future use and, at the same time, it hampers the process of efficient regeneration necessary to preserve the seed viability. The concept of "core" collections was introduced in the 1980's to counter the trend of extensive and unmanageable resources with a focus on preserving the broadest possible genetic spectrum of the original collection based on detailed genetic, morphological and agronomic description (Brown 1989). In order to preserve the integrity and potency of seed samples it is required that the whole spectrum of genetic diversity is preserved on a long term basis while, at the same time, sufficient amounts of seeds for the potential use is preserved. It is very likely that the gene banks contain a number of identical samples kept under various numbers of the EVIGEZ (Czech Information System of Genetic Resources). Such duplicated accessions often originate from foreign resources, where they are kept under different identification. As a result, duplications increase the costs of preserving the collection and they cause undesirable genetic erosion resulting in unfavourable ratio of collection size and overall genetic variability. All these inefficient genetic resources impede the breeding progress and considerably reduce the opportunities to respond to the requirements of farmers and consumers.

At present, a number of methods are used to evaluate the genetic diversity and variability in the collections of genetic resources; e.g. morphological characteristics, analysis of the genealogy, biochemical markers (in particular proteins and their various iso-enzyme variants) and the dynamically developing molecular (DNA) markers (Zhang et al. 2007). Within the DNA markers, the microsatellite markers (SSRs – Simple Sequence Repeats), are especially useful due to their high degree of polymorphism and co-dominant character of heredity. The use of microsatellite polymorphisms to study the genetic diversity and variability was described for a number of plant species, e.g. in pea (Haghnazari et al. 2005), tomato (Wang

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Table 1. Survey of the analyzed pepper accessions

Order	Number EVIGEZ	Name	Order	Number EVIGEZ	Name
1	09H3100071	Tetenyi	22	09H3100139	Aufrechte Cayenne
2	09H3100243	Kalocsai Furzer (Edes)	23	09H3100140	Aufrechte Cayenne
3	09H3100244	Kalocsai Furzer (Edes)	24	09H3100111	Bogyisloi
4	09H3100245	Kalocsai Furzer (Edes)	25	09H3100112	Bogyiszloi
5	09H3100290	Vinedale	26	09H3100113	Bogyiszloi
6	09H3100291	Vinedale	27	09H3100114	Bogyiszloi Vastaghusu
7	09H3100055	Astrachanskij	28	09H3100351	Japan Madarszen
8	09H3100056	Astrachanskij	29	09H3100503	Japan Madarszen
9	09H3100057	Astrachanskij	30	09H3100504	Japan Madarszen
10	09H3100058	Astrachanskij	31	09H3100505	Japan Madarszen
11	09H3100541	Astrachanskij	32	09H3100067	Tetenyi
12	09H3100059	Astrachanskij 147	33	09H3100068	Tetenyi
13	09H3100416	Hatvani	34	09H3100069	Tetenyi
14	09H3100350	Japan Madarszen	35	09H3100070	Tetenyi
15	09H3100419	Hatvani Csemege	36	09H3100288	Vinedale
16	09H3100418	Hatvani	37	09H3100349	Japan Hontakka
17	09H3100417	Hatvani	38	09H3100501	Japan Hontakka
18	09H3100354	Konservnyj Belyj 289	39	09H3100502	Japan Hontakka
19	09H3100292	Vinedale	40	09H3100352	Konservnyj Belyj 289
20	09H3100137	Aufrechte Cayenne	41	09H3100353	Konservnyj Belyj 289
21	09H3100138	Aufrechte Cayenne			

et al. 2006) and rape (Li et al. 2007). In the present study we evaluated the variability of SSR markers in selected accessions of pepper in the collection of the Crop Research Institute, Prague-Ruzyně, Department of Vegetables and Special Crops in Olomouc.

MATERIAL AND METHODS

Genetic variability was assessed in 41 accessions of pepper (*Capsicum annuum* L.) (Table 1). The plants were grown in isolation cages on plots of the Research Institute Gene Bank Olomouc and the genotypes were described according to two classifiers: IPGRI – 27 characters (IPGRI 1995) and UPOV – 44 characters (UPOV 2006). The applied classifiers are complementary since they provide different type of description. The IPGRI classifier is intended for morphological description of genetic resources and the UPOV classifier serves for morphological characterization of certified varieties. In total, 54 characteristics were evaluated for pepper: one in seedlings, eight in plants, 10 in leaves, 10 in flowers and 25 descriptors in fruits. DNA polymorphism, detected by the SSR method, was used as genetic marker.

The genomic DNA was isolated using the Invisorb Spin Plant Mini Kit (INVITEK, Germany) from leaves collected from plants at the beginning of flowering. Three plants of each accession were sampled by collecting four leaf discs from each plant (approximately 80 mg). The DNA concentration was measured fluorimetrically. Eight SSR markers described previously for pepper were used (Lee et al. 2004; Minamiyama et al. 2006). The 25 µl-reaction mixture for PCR contained: 30 ng template DNA, 1 U *Taq* polymerase (PROMEGA, USA), 1× concentrated reaction buffer, 0.2µM of fluorescence-labelled forward primer, 0.2µM of reverse primer, and 0.1mM dNTPs.

The PCR program consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 50–55°C (subject to the used pair of primers), 2 min at 72°C, and 1 cycle 10 min at 72°C. The PCR amplification was verified by agar-

Table 2. Characteristics of the SSR markers

SSR marker	Linkage group	Number of alleles	Range (bp)
Hpms 1-1	1	1	270
Hpms 1-5	6	8	269–322
Hpms 1-168	16	1	172
Hpms 1-172	11	2	338–340
Hpms 1-274	7	1	175
Hpms 2-21	10	7	266–296
Cams 163	5	2	248–250
Cams 647	3	6	188–224
Average number of alleles per locus		3.5	

Table 3. Statistical evaluation of the analyzed SSR markers

SSR marker	DI	PI	PIC
Hpms 1-1	0.00	1.00	0.00
Hpms 1-5	0.73	0.04	0.72
Hpms 1-168	0.00	1.00	0.00
Hpms 1-172	0.18	0.69	0.16
Hpms 1-274	0.00	1.00	0.00
Hpms 2-21	0.68	0.09	0.67
Cams 163	0.31	0.52	0.26
Cams 647	0.75	0.03	0.74
Average	0.33	0.55	0.32

DI – diversity index, PI – probabilities of identity, PIC – polymorphic information content

ose electrophoresis before loading of the samples on capillary electrophoresis ABI Prism 3100 (APPLIED BIOSYSTEMS, USA). The number and size of the amplicons were evaluated by the Gene Marker 1.3 software. The amplicons at polymorphic loci were scored as presence (1) or absence (0) of an allele and used to construct a binary matrix. These values were statistically evaluated using UPGMA (Jaccard coefficient) by the FreeTree programme (Hampl et al. 2001) and a dendrogram was constructed by the TreeView programme (Page 1996). Following values were assessed for each SSR marker: diversity index (DI), probability of identity (PI) and polymorphous information content (PIC) (Russell et al. 1997).

RESULTS AND DISCUSSION

We tested the variability of microsatellite markers within the collection of pepper genetic resources. Out of eight analyzed SSR markers three had a uniform spectrum (*Hpms 1-1, Hpms 1-168,* and *Hpms 1-274*) in all the analyzed pepper plants. In the other microsatellites two to eight alleles were detected (total 28), i.e. average 3.5 alleles per locus (Table 2). The size of amplicons ranged between 172 and 340 bp (Table 3). The maximal difference from the average size (Lee et al. 2004; Minamiyama et al. 2006) was determined in the SSR marker *Hpms 1-168* (+36 bp) and *Cams 163* (+46 bp). The highest number of alleles was detected in microsatellites *Hpms 1-5* (8 alleles) and *Hpms 2-21* (7 alleles). Minamiyama et al. (2006) detected a high number of alleles in the SSR markers *Cams 163* (9 alleles) and *Cams 647* (10 alleles) which, in our case, had a lower number of alleles, i.e. *Cams 647* (6 alleles) and *Cams 163* (2 alleles). The obtained number of alleles per locus is comparable with other authors who found average values of 2.9 (Minamiyama et al. 2006) and 3.0 (Kwon et al. 2007) (Table 2).

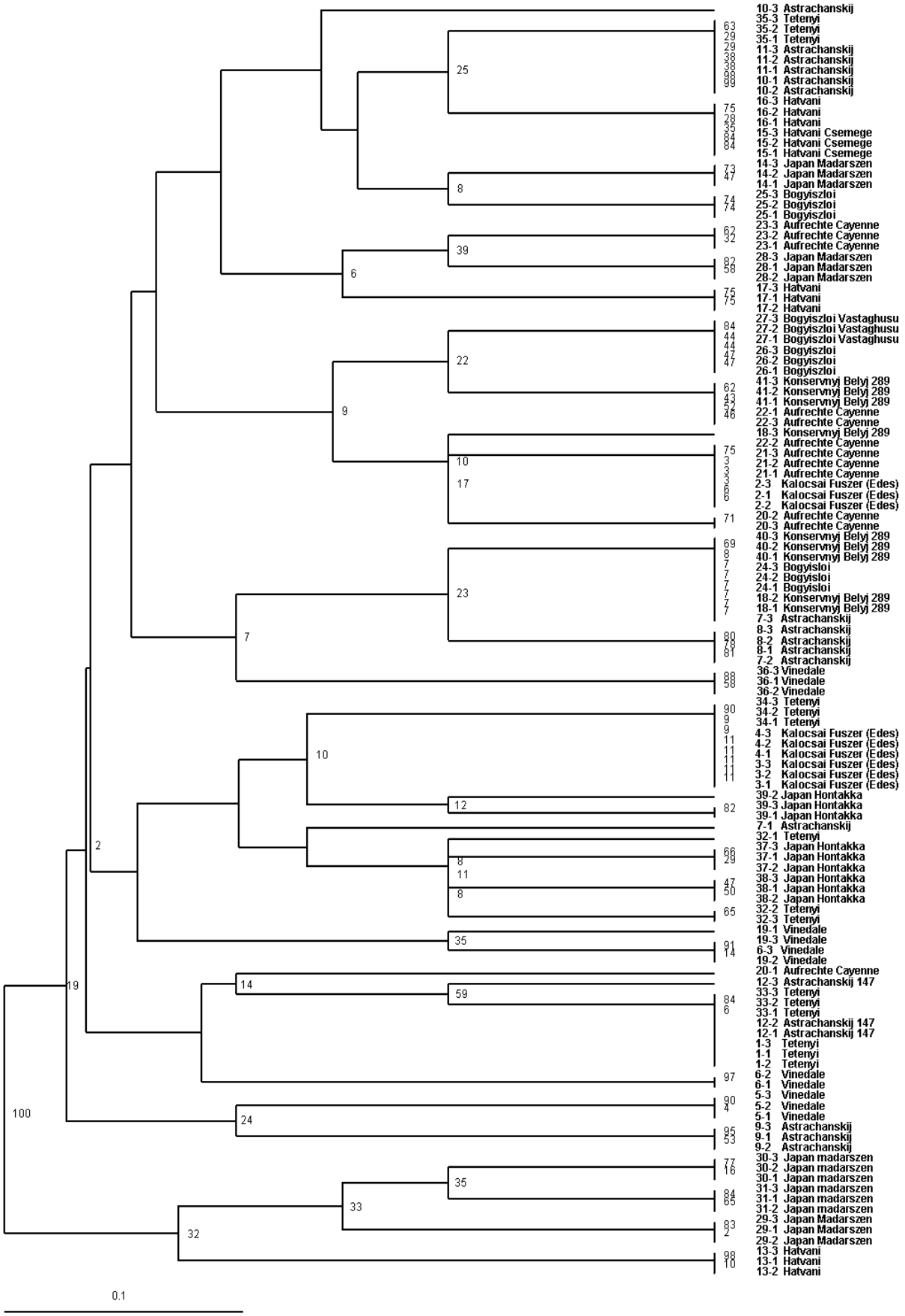

Fig. 1. Dendrogram of similarity of the analyzed pepper plants

The average DI (diversity index) value was 0.33 (0.00–0.74), average of PI (probability of identity) 0.55 (0.04–1.00) and for PIC (polymorphous information content) average value was 0.32 (0.00 to 0.73) (Table 3). The average value of PIC was lower than the value of 0.76 described by Lee et al. (2004) when studying various members of the genus *Capsicum*. Minamiyama et al. (2006) quoted a similar value of 0.46 in their studies of dihaploid pepper lines (*C. annuum*). The low value of PIC implies a higher level of genetic similarity within the analyzed pepper genotypes.

Within the analyzed accessions, alleles specific for certain genotypes were detected, e.g. 296 bp amplicon of *Hpms 1-5* specific for the accession Hatvani (No. 13) and in *Hpms 2-21* the following genotype-specific sizes: Astrachanskij (No. 9) – 296 bp; Japan Madarszen (No. 28) – 292 bp and Japan Hontakka (No. 37) – 266 bp. Intragenotype variability was detected for some markers; e.g. in Japan Hontakka (No. 39) – 306 bp and 309 bp alleles (*Hpms 1-5*) were determined in individual plants. In the same microsatellite marker we detected a unique 332 bp allele on one of the three plants of the accession Astrachanskij 147 (No. 12) which did not appear in any other of the remaining 122 analyzed plants.

Based on statistical evaluation we constructed a similarity dendrogram of the analyzed pepper genotypes (Jaccard coefficient) (Fig. 1). Four accessions were significantly (Hatvani (No. 13), Japan Madarszen (No. 29, No. 30 and No. 31)) different from the other 37 analyzed accessions. These four accessions differed not only in their SSR markers but also according to descriptive morphological data (No. 29 – chilli pepper; Nos. 30 and 31 – spice pepper). The distribution of the analyzed genotypes in the dendrogram indicated a high level of similarity within some items of the same or similar name, e.g. Kalocsai Furzer (Edes) (Nos. 3 and 4), Hatvani and Hatvani Csemege (Nos. 15 and 16); Bogyiszloi (No. 26) and Bogyiszloi Vastaghusu (No. 27). No differences among these accessions were detected even when evaluating morphological characters using the UPOV classifier. Minimal differences were observed when the IPGRI classifier was used for the evaluations, i.e. in characteristics which could be affected by the environment, e.g. different level of nutrients in the soil. Taken together we can consider these samples as examples of duplicity within the pepper genetic resources.

By contrast, other accessions within the studied collection can be considered as being able to encompass genetically different material since we detected variability of microsatellites in individual plants within the presumed accessions. The highest variability was detected in the accession Astrachanskij (No. 7) as is documented by the position of individual analyzed plants in the dendrogram. A lower level of variability was observed also in accessions Astrachanskij (No. 10), Konservnyj Belyj 289 (No. 18) and Tetenyi (No. 32). The differences detected at the molecular level could not be confirmed by morphological evaluation as all three plants appeared as identical.

CONCLUSION

Distribution of the analyzed accessions in the dendrogram implies a high level of similarity within some accessions and, in contrast, the presence of genetically different material within other accessions of the same or similar name. Molecular data were complemented with morphological measurements according to the descriptor list for the genus *Capsicum*. Taken together, the results show the possibility of duplicities in the current collection of genetic resources of red pepper.

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Polymorfizmus DNA genetických zdrojů papriky pomocí mikrosatelitních markerů

ABSTRAKT: Genetická variabilita mezi 41 položkami papriky (*Capsicum annuum* L.) byla hodnocena pomocí osmi mikrosatelitních markerů. Tři z těchto mikrosatelitních markerů (*Hpms 1-1*, *Hpms 1-168* a *Hpms 1-274*) měly uniformní spektrum u všech analyzovaných rostlin. Dvě až osm alel bylo detekováno ve zbývajících lokusech. Celkem bylo detekováno 28 alel, což je v průměru 3,5 alely na jeden mikrosatelitní lokus. Nejvyšší počet alel byl detekován pomocí primerů *Hmps 1-5* (8 alel) a *Hpms 2-21* (7 alel). Molekulární data byla doplněna o morfologický popis podle klasifikátoru pro rod *Capsicum*. Dendrogram na základě našich genetických analýz naznačuje vyšší stupeň podobnosti mezi některými položkami v kontrastu s jinými položkami se stejným označením. Výsledky ukazují na možnost duplicit v rámci studované kolekce genových zdrojů papriky.

Klíčová slova: paprika; genetické zdroje; mikrosatelity; SSR; variabilita

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