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THE MORPHOLOGICAL DESCRIPTION AND DNA TOOLS ANALYSIS: FOR DETECTION OF DUPLICATIONS IN THE CZECH GERMPLASM COLLECTION OF PEPPER (*Capsicum annuum* L.)

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Abstract

STAVĚLÍKOVÁ, H., HANÁČEK, P., VYHNÁNEK, T.: *The morphological description and DNA tools analysis: for detection of duplicitions in the Czech germplasm collection of pepper (Capsicum annuum L.).* Acta univ. agric. et silvic. Mendel. Brun., 2010, LVIII, No. 1, pp. 191–198

The pepper (*Capsicum annuum* L.) is very popular annual vegetable either for fresh consume either as spice after drying and grinding. The fruit contains high amounts of vitamin C, provitamin A, E, P (citrin), B_1 (thiamine), B_2 (riboflavin) and B_3 (niacin). Crop Research Institute (CRI), Department of Vegetable and Special Crops, Olomouc, the Czech Republic is the holder of the collection of pepper genetic resources. The collection of pepper consists of 504 accessions, currently. It is necessary to find duplications within collection for effective work with genetics resources. For analyses totally 41 accessions were chosen. These were divided into ten groups according name: 1. Astrachanskij, 2. Aufrechte Cayenne, 3. Bogyisloi, 4. Hatvani, 5. Japan Hontakka, 6. Japan Madarszen, 7. Kalocsai Fuszer (Edes), 8. Konservnyj Belyj 289, 9. Tetenyi and 10. Vinedale. Two approaches were used for the detection of duplications – morphological description and polymorphism of DNA. The accessions were characterized for 54 morphological traits: 1 character in seedlings, 8 characters in the plants, 10 characters in leaves, 10 characters in flowers and 25 characters in fruits. The polymorphism of DNA was analysed using the SSR (*Simple Sequence Repeats*) method with 8 SSR markers (*Hpms 1-1*, *Hpms 1-5*, *Hpms 1-168*, *Hpms 1-172*, *Hpms 1-274*, *Hpms 2-21*, *Cams 163* and *Cams 647*) which are localised on different chromosomes. The results from DNA analysis were complemented with the morphological characterization. Possible duplications were in 4 groups: 1. Astrachanskij, 4. Hatvani, 5. Japan Hontakka and 7. Kalocsai Fuszer (Edes). This work is the first step for the determination of duplications in the Czech germplasm collection of pepper.

pepper, genetic resources, microsatellites, SSRs, variability, morphological descriptors

The pepper is very popular, widespread in the world, annual vegetable, to produce high amounts of vitamin C, provitamin A, E, P (citrin), B_1 (thiamine), B_2 (riboflavin) and B_3 (niacin) (Valšíková, 1987; Bosland & Votava, 2000). This files in class: *Magnoliopsida*, order: *Solanales*, tribe: *Solanaceae,* genus: *Capsicum.* Various authors describe 25 species to the genus *Capsicum.* The major species of this genus are *Capsicum annuum* L., *Capsicum fructescens* L., *Capsicum chinense* Jacq., *Capsicum minimum* Roxb., *Capsicum pubescenns* Ruiz & Pav. and *Capsicum baccatum* var. *pendulum* (Basu & De, 2003).

Pepper has been grown very long time. The oldest known records of pepper come from the desert valley of Tehuacán, in Southern Mexico. It is known that the indigenes were eating peppers as early 7000 B.C. Now we do know that peppers were among the first plants to be domesticated in the Americas (Smith, 1984). Christopher Columbus brought the pepper to the Europe (Bosland & Votava, 2000). At the beginning the pepper was planted as the ornamental and medicinal plant in the Spain and Portugal and later in Italy. In the 16th century the pepper was brought by Turks to Bulgaria. The Bulgarian gardeners expanded the pepper to other Europe coun-

tries (Valšíková, 1987). Now the pepper is grown on 521 681 ha on the world (Tab. I), (FAO). The pepper was known as spice plant in 16th century in Bohemia (Müller, 1959). In the Czechoslovakia the intense growing of pepper started after the First World War (Valšíková, 1987). Now the pepper is grown on 243 ha in the Czech Republic (Tab. II) (Buchtová, 2006; 2008).

I: *The area harvest in the world*

Year	Area harvest (ha)
2003	456 141
2004	502 401
2005	503 038
2006	524 008
2007	521 681

II: *The area harvest in the Czech Republic (source Czech and Moravian Vegetable Union (CMVU))*

Year	Area harvest (ha)
2003	211
2004	315
2005	300
2006	276
2007	270
2008	243

It is necessary to find duplications within collection for effective, efficient and rational work with genetics resources on the national and international level (Dotlačil, 2007; ECPGR, 2008a). Now many methods for studying the genetic diversity and variability in the collections of genetic resources are; 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).

MATERIAL AND METHODS

The objective of the present study was to detect the duplications in selected genetic resources of pepper. The collection of pepper held by CRI consists of 504 accessions (acc.), currently (Stavělíková *et al*. 2009). All accessions of pepper have been described for 27 characters taken from Descriptors for Capsicum (*Capsisum* spp.) [IPGRI, (1995)]. Documentation photos of all accessions have been taken. The passport data of the collection are fully recorded, computerized and entered in EVIGEZ (Plant Genetic Resources Documentation in the Czech Republic),http://genbank.vurv.cz/genetic/resources/) and in the ECPGR (The European Cooperative Programme for Plant Genetic Resources) Pepper Database http://www.ecpgr.cgiar.org/Databases/Crops/Pepper.htm.

We chose 41 acc. pepper from the collection of pepper genetic resources to DNA analysis. The material for analysis was prepared as follows: Pepper seeds were drilled to the small pots with pearlite on 28th March. These were put on Jacobsen's germination apparatus for ten days. (Tree days the temperature was 35 °C and seven days the temperature was 25 °C. System (period) of light was 12 hour light and 12 hours dark.). The seedlings were transplanted to plastic pots, diameter 8 cm, two plants per pot with growing substrate in 8th April. The pepper plantings were planted out to the isolation cages in16th May. 20 plants from accessions were pricked in. Planting distance was 25 × 30 cm. During all growing period the pepper growth was watered twice per week. The fertilizer was not used. The insecticides with effectual substance lambda-cyhalothrin (Karate 2,5 WG) and pirimicarb (Pirimor 2,5 WG) were used protection against *Aphis* ssp. The samples for DNA analysis were taken in 17th July.

The accessions were split into ten groups according name (Tab. III). These acc. were described according Descriptors for Capsicum (*Capsisum* spp.) [IPGRI, (1995)](Descriptor) – 27 characters and descriptor list by International union for the protection of new varieties of plants (UPOV) (UPOV, 2006) – 44 characters. Some characters are both in Descriptor and in UPOV (plant habit, pedicule attitude, fruit colour etc.). Finally 54 characters were used for pepper description – 1 character in seedlings – anthocyanin coloration of hypocotyl; 8 characters in the plants – stem pubescence, height, habitus, length of stem, shortened internode (in upper part), anthocyanin coloration of nodes, intensity of anthocyanin coloration of nodes, hairiness of nodes; 10 characters in leaves – length, pubescence, length of blade, width of blade, intensity of green color, shape undulation of margin, blistering, glossiness, profile in cross section; 10 characters in flowers – number of flowers per axil, flower position, corolla colour, corolla spot colour, anther colour, filament colour, calyx annular constriction, calyx margin, stigma exsertion to anthesis, anthocyanin coloration in anther and 25 characters in fruits – anthocyanin spots or stripes, colour at intermediate stage, intensity of color (before maturity), position, set, colour at mature stage, intensity of color (at maturity), shape, length, ratio length/diameter, width, shape at pedicel attachment, neck at base of fruit, shape at blosoom end, cross-sectional corrugation, surface, sinuation of pericarp at basal part, sinuation of pericarp excluding basal part, glossiness, depth of interloculary groove, number of locules, thickness of flesh, length and thickness of stalk, aspekt of calyx. We took photo of the acc. twice per growing season – in phase of flowering and in phase plants with the ripe fruits (Fig. 1). Photo – documentation of fruits contains fruit sideways/sidelong look, top point of view, cross section, too. The characters were assessed by scale from 1 to 9. Number 1 presents none or very weak expression of monitored character, number 3 presents weak intensity of expression, number 5 presents middle inten-

III: *Analysed pepper accessions*

Order	Accession number	Name Country of origin
1. group Astrachanskij – former Soviet Union		
7	09H3100055	Astrachanskij
8	09H3100056	Astrachanskij
9	09H3100057	Astrachanskij
10	09H3100058	Astrachanskij
12	09H3100059	Astrachanskij 147
11	09H3100541	Astrachanskij
2. group Aufrechte Cayenne – France		
20	09H3100137	Aufrechte Cayenne
21	09H3100138	Aufrechte Cayenne
22	09H3100139	Aufrechte Cayenne
23	09H3100140	Aufrechte Cayenne
3. group Bogyisloi – Hungary		
24	09H3100111	Bogyisloi
25	09H3100112	Bogyiszloi
26	09H3100113	Bogyiszloi
27	09H3100114	Bogyiszloi Vastaghusu
4. group Hatvani – Hungary		
13	09H3100416	Hatvani
17	09H3100417	Hatvani
16	09H3100418	Hatvani
15	09H3100419	Hatvani Csemege
5. group Japan Hontakka – Hungary		
37	09H3100349	Japan Hontakka
38	09H3400501	Japan hontakka
39	09H3100502	Japan hontakka
6. group Japan Madarszem – Hungary		
14	09H3100350	Japan Madarszem
28	09H3100351	Japan Madarszem
29	09H3100503	Japan Madarszen
30	09H3100504	Japan madarszen
31	09H3100505	Japan madarszen
7. group Kalocsai Fuszer (Edes) – Hungary		
2	09H3100243	Kalocsai Fuszer (Edes)
3	09H3100244	Kalocsai Fuszer (Edes)
4	09H3100245	Kalocsai Fuszer (Edes)
8. group Konservnyj Belyj 289 – former Soviet Union		
18	09H3100354	Konservnyj Belyj 289
40	09H3100352	Konservnyj Belyj 289
41	09H3100353	Konservnyj Belyj 289
9. group Tetenyi – Hungary		
32	09H3100067	Tetenyi
33	09H3100068	Tetenyi
34	09H3100069	Tetenyi
35	09H3100070	Tetenyi
1	09H3100071	Tetenyi
10. group Vinedale – Canada		
5	09H3100290	Vinedale
6	09H3100291	Vinedale
19	09H3100292	Vinedale
36	09H3100288	Vinedale

1: *Photo of the acc. 09H3100069 (Tetenyi) over the growing season*
(a) in phase of flowering, (b) in phase plants with the ripe fruits

sity of expression, 7 presents strong intensity of expression and level 9 presents very strong intensity of expression. Only two possibilities 1 and 9 were used for characters – seedling: anthocyanin coloration of hypocotyl, plant: shortened internode (in upper part), plant: anthocyanin coloration of nodes, inflorescence: calyx annular constriction, fruit: anthocyanin coloration, fruit: neck at base of fruit, fruit: stalk cavity and calyx: aspect. Characters – plant: height, leaf: length, fruit: length and width were measured on ten plants. The wide scope of characters for morphological description was used. We chose important characters which are in Minimum descriptors (ECPGR, 2008b) for comparison accessions (Tab. IV). The polymorphism of DNA in pepper was analysed using the SSR (*Simple Sequence Repeats*) method (Rohrer et al., 2009). Three plants of each accession were sampled. We analysed 8 SSR markers chosen in accordance with literature (Lee et al. 2004; Minamiyama et al. 2006). SSR markers (*Hpms 1-1*, *Hpms 1-5*, *Hpms 1-168*, *Hpms 1-172*, *Hpms 1-274*, *Hpms 2-21*, *Cams 163* and *Cams 647)* are localised on different chromosomes. The PCR amplification was verified by agarose electrophoresis before loading of the sampleas on capillary electrophoresis ABI Prism 3000 (Applied Biosystems, USA). The number and size of the amplicons were evaluated by the Gene Marker 1.3 software.

RESULTS AND DISCUSSION

Morphological characteristics can detect only a degree of polymorphism, and may be sensitive to environmental conditions. These characters suffer from limitations of number interaction with the environment in which the plant variety grows and the subjectivity in decisionmaking (Sing et al., 2004). Kwon et al. (2007) recommended using of the phenotypic and molecular (SSRs) markers for analyzing the duplicities in collection of plant genetic resources. A dendrogram (Rohrer et al., 2009) based on our genetic analysis suggests a high level of similarity between some of the accessions presumed to be

IV: *Morphological description in group number 7**

Accession number	Name accession	Plant growth habit	Plant height cm	Number of flowers per axil	Corolla colour	Fruit colour		Fruit			
						intermediate stage	mature stage	shape	length (cm)	width (cm)	surface
09H3100243	Kalocsai Fuszer (Edes)	erect	46–65	two	white	green	red	triangual	11–15	3–5	smooth
09H3100244	Kalocsai Fuszer (Edes)	intermediate	66–85	one	white	green	red	elongate	11–15	< 3	smooth
09H3100245	Kalocsai Fuszer (Edes)	intermediate	67–85	one	white	green	red	elongate	6–10	< 3	smooth

* – minimum descriptors (ECPGR, 2008b)

V: *The size of amplicons (bp) in the possible duplications*

Group	Number	SSR markers							
	EVIGEZ	*Hpms 1-1*	*Hpms 1-5*	*Hpms 1-168*	*Hpms 1-172*	*Hpms 1-274*	*Hpms 2-21*	*Cams 163*	*Cams 647*
1.	09H3100055	270	318-320*	172	340	175	290	250	218–224*
	09H3100056	270	320	172	340	175	290	250	218
	09H3100057	270	312	172	340	175	296	250	218
	09H3100058	270	308	172	340	175	292–294*	250	212
	09H3100059	270	308	172	340	175	294	250	212
	09H3100541	270	318–322*	172	340	175	294	250	224
4.	09H3100416	270	296	172	340	175	294	250	224
	09H3100417	270	318	172	340	175	294	250	212
	09H3100418	270	318	172	340	175	294	250	212
	09H3100419	270	318	172	340	175	294	250	188
5.	09H3100349	270	308	172	340	175	266	250	218
	09H3400501	270	308	172	340	175	290	250	218
	09H3100502	270	306–308*	172	340	175	294	250	218
7.	09H3100243	270	320	172	340	175	292	250	224
	09H3100244	270	308	172	340	175	294	248	218
	09H3100245	270	308	172	340	175	294	248	218

* – interval from 3 individual plants

distant and, at the same time, genetic variability between accessions of the same or similar name. Our results in pepper show the possibility of duplicities in group number 1 (group Astrachanskij), 4 (group Hatvani), 5 (group Japan Hontakka) and 7 (group Kalocsai Fuszer (Edes)) (Tab. V).

1. **group Astrachanskij** –according morphological characterization 09H3100059 Astrachanskij 147 was different from others acc. in height plant and colour fruit at intermediate stage. The acc. 09H3100059 and 09H3100057 were different from others acc., according DNA analysis.
2. **group Aufrechte Cayenne** – within group the genotype 09H3100140 was different in length of blade, width of blade and shape of fruit according morphological characterization. DNA analysis presented the small differences in all accessions but big differences were between acc. 09H3100137, 09H3100138, 09H3100139 and 09H3100140.
3. **group Bogyisloi** – the fundamental morphological differences were not found within group. According DNA analysis acc. 09H3100113 and 09H3100114 were very similar. The small differences were between acc. 09H3100111 and acc. 09H3100112. All these acc. were in one subgroup.
4. **group Hatvani** – the morphological differences were not among acc. 09H3100417, 09H3100418 and 09H3100419. The plants of acc. 09H3100416 had heterogeneous phenotype expression. 09H3100416 was dissimilar to the rest group, according DNA analysis.
5. **group Japan Hontakka** – the acc. 09H3100502 was different from 09H3100349 and 09H3400501 in the position, shape and size of fruits. The results of DNA analysis were the identical.
6. **group Japan Madarszem** – the individual accessions were differed in the size of leafs, size and shape of fruits. The biggest differences were between acc. 09H3100350 and 09H3100351 and among the acc. 09H3100503, 09H3100504 and 09H3100505 according DNA analysis. These groups were put in different cluster.
7. **group Kalocsai Fuszer (Edes)** – the genotype 09H3100243 was different from 09H3100244 and 09H3100245 in the shape and position of fruits and in vegetation. The result of DNA analysis is identical with morphological description.
8. **group Konservnyj Belyj 289** – the morphological differences were not found within group. According DNA analysis acc. 09H3100354 and 09H3100352 were the same. The small differences were found between acc. 09H3100353 and acc. 09H3100354 and 09H3100352.
9. **group Tetenyi** – according morphological description it is possible to split up two parts this group. 09H3100068 and 09H3100071 form the first subgroup. These acc. have low plants, erect and triangular fruits, the fruit colour at intermediate stage is yellowish and light red at mature stage. The acc. 09H3100067, 09H3100069 and 09H3100070 form the second subgroup have elongace and drooping (declining) fruits. The fruits of this group are green at intermediate stage and red at mature stage. The result of DNA analysis is the same. Within the second subgroup small variability was found.
10. **group Vinedale** –identical accessions were not found according both morphological description and DNA analysis. Within this group the accessions were different in all important morphological characters.

CONCLUSION

This work was the first step for the determination of duplications in the collection of genetic resources of pepper in the Czech Republic. The collection is very large to have 504 accessions, currently. The thorough morphological description gives better information about material to user of genetic resources – plant-breeders, research workers. The detection of duplications leads to effective work with genetics resources. In future we would like to continue in the determination of duplications on the basis of increase number of SSR markers and morphological description. Better number of SSR markers gives results with higher predicative ability about variability within collection and in the scope individual accessions.

SUMMARY

The objective of the present study was to detect the duplications in selected genetic resources of pepper of the Crop Research Institute, Department of Vegetables and Special Crops in Olomouc. The collection of pepper consists of 504 accessions (acc.), currently. 41 acc. were chosen for morphological description and DNA analysis. These were divided into ten groups according name of accessions. These acc. were described according Descriptors for Capsicum (*Capsisum* spp.) [IPGRI, (1995)] (Descriptor) for 27 characters and by descriptor list by International union for the protection of new varieties of plants (UPOV) (UPOV, 2006) for 44 characters. We took photo of the acc. twice per growing season – in phase of flowering and in phase plants with the ripe fruits. Photo – documentation of fruits contains fruit sideways/ sidelong look, top point of view, cross section, too. The polymorphism of DNA in pepper was analysed using the SSR (*Simple Sequence Repeats*) method. Totally 8 SSR mar-

kers are localised on different chromosomes were chosen. The possible duplications were detected on the basis evaluation of apmlicons' size individually SSR markers from analysis of individual plants (one accession presents tree plants) (Tab. V). The possible duplications were found in 4 groups (Astrachanskij, Hatvani, Japan Hontakka and Kalocsai Fuzser (Eder)) in the collection of genetic resources. The detection of duplications leads to effective work with genetics resources. In future we would like to continue with the determinative of duplications on the basis of increase number of SSR markers and morphological description.

SOUHRN

Využití morfologických deskriptorů a DNA analýzy pro detekci duplicit v české kolekci genetických zdrojů papriky

Cílem prezentované studie byla detekce duplicit u vybraných genetických zdrojů papriky Výzkumného ústavu rostlinné výroby, oddělení zelenin a speciálních plodin v Olomouci. V současnosti obsahuje tato kolekce 504 položek. Hlavní část kolekce prezentují staré odrůdy z Maďarska (129 položek), Sovětského svazu (68 položek), Československa (52 položek), USA (46 položek), Bulharska (44 položek) a 17 položek je z České republiky. Nové položky jsou získávány ze semenářským firem a jiných genových bank. Pro morfologický popis a DNA analýzy bylo vybráno 41 položek, které byly rozděleny do deseti skupin podle názvu. Tyto položky byly popsány podle deskriptoru pro papriku (*Capsicum* spp.) [IPGRI, (1995)](Descriptor) – 27 znaků, a podle Mezinárodní úmluvy na ochranu nových odrůd rostlin (UPOV) (UPOV, 2006) – 44 znaků. V průběhu vegetace byla pořizována fotodokumentace ve dvou fázích: (a) ve fázi kvetení, (b) ve fázi rostlin se zralými plody. Polymorfizmus DNA u papriky byl analyzován pomocí SSR (*Simple Sequence Repeats*) metody. Bylo vybráno osm SSR markerů podle literárních pramenů (každý z SSR markery byl lokalizován na jiném chromozomu). Na základě vyhodnocení velikosti apmlikonů jednotlivých SSR markerů získaných z analýz jednotlivých rostlin (1 položka = 3 rostliny) byly odhaleny možné duplicity v kolekci (Tab. V). V kolekci genetických zdrojů byly nalezeny pravděpodobné duplikace u čtyř skupin (Astrachanskij, Hatvani, Japan Hontakka a Kalocsai Fuzser (Eded)). Detekce duplicit umožňuje efektivní práci s genetickými zdroji. V budoucnu bychom rádi pokračovali ve vyhledávání duplicit na základě molekulárních markerů a morfologického popisu.

paprika, genetické zdroje, mikrosatelity, SSR, variabilita, morfologické znaky

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