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Original scientific paper

RELIABILITY OF MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF LIGHTSPROUTS FOR DIFFERENTIATION OF POTATO ACCESSIONS

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The study of reliability of morphological characterization of lightsprouts for differentiation of potato varieties was performed at the Agricultural Institute of Slovenia in cooperation with Biotechnical Faculty Podgorica in order to introduce simple method for further characterization of potato accessions in Montenegrin gene bank. Seven selected, potentially different, potato accessions preserved in the Montenegrin gene bank were used for morphological characterization of lightsprouts. Using UPOV guidelines 11 lightsprout traits were estimated. Molecular assessment was carried out in parallel with morphological characterization by six microsatellite (SSR) markers. The latter successfully distinguished all accessions but two, while four different lightsprout phenotypes were identified in morphological characterization. Though molecular markers showed more strength in resolving relationships between genotypes, characterization of lightsprouts still demonstrated its usefulness due to cheap, simple and rapid procedure.

Keywords: morphological characterization of lightsprouts, molecular differentiation, potato accessions, SSR markers

INTRODUCTION

Potato is a crop that has one of the greatest genetic diversity among all cultivated species. Its genetic resources include wild relatives, native populations, farmers' varieties and hybrids obtained by crosses between cultivated and wild species. They contain an invaluable treasure of genes responsible for various important traits such as resistance to plant diseases and pests, tolerance to different types of stress, nutritional value, flavour, high yield potential and many other quality traits. However, very often there is a little data available on potato accessions maintained in plant gene banks, and even less about their utilisation value. This is the reason why

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despite the easy accessibility of large collections of germplasm, breeders rarely request for deposited plant material (JOVOVIĆ *et al.,* 2013).

Characterization is an important element of the conservation of genetic resources. It gives a clear assessment of the value of the collection, which further leads to a significant reduction of conservation costs. Taxonomic misidentification of gene bank accessions is rather common, and misleading for the users. Very often gene bank collections consist of multiple accessions of the same or similar genotype, and their identification can often be difficult. The most applied methods for assessing the genetic diversity among living organisms are morphological, biochemical and molecular markers (DENČIĆ *et al.,* 2015). Morphological characterization methods for differentiation among different plant varieties were used for many years using UPOV (The International Union for the Protection of New Varieties of Plants) descriptors and test guidelines, among others also for DUS testing (distinctness, uniformity and stability). The UPOV guidelines for potato variety differentiation consist of 42 morphological traits on lightsprouts (11 traits), tuber, foliage and flower traits and some agronomic traits, such as earliness. For determination of new or identification of old varieties extensive database and variety collection is needed, as used also in potato breeding programmes (DOLNIČAR *et al,*. 2011, DOLNIČAR *et al.,* 2012). On the other hand, it is possible to use the same descriptors to differentiate among gene bank accessions in order to find putative duplicates (JOVOVIĆ *et al.,* 2016).

Characterization of genetic material significantly improved over the last 15 years by implementation of molecular markers (IGNJATOVIĆ-MIĆIĆ *et al*., 2015). Today, molecular markers are used extensively in molecular identification because they exclude all possible subjective influences. At the same time, combinations of different criteria are increasingly used to obtain characterization results as accurate as possible (SCHULMAN, 2007; MARIĆ *et al*., 2004). Molecular markers provide tools to test the taxonomic validity of species and can provide species specific diagnostic markers where they will become even more important in prioritization of accessions (SPOONER *et al*., 2015; BRADEEN and COLE, 2011; PIPAN *et al*., 2013; MARAS *et al.,* 2015; MARAS *et al.,* 2013). Molecular data generated by sequencing or DNA markers has led to fewer unique species or taxonomic groups, compared to the number of species determined using morphological data (SPOONER and SALAS, 2006; RODRIGUEZ and SPOONER, 2009). Molecular markers have been utilized as well to help unravel the mystery of the origin of the modern cultivated potato (SPOONER *et al.,* 2005; GHISLAIN *et al.,* 2009).

Potato is a species with many morphologically similar varieties. In a series of publications cultivated traditional accessions and modern varieties of potato have been characterized using molecular markers. Already in 1996 and in 1997 MANDLINO *et al.* and SCHNEIDER and DOUCHES, respectively, were able to distinguish between potato varieties using SSR, RFLP and RAPD markers combined with morphological traits. Rapid method for the differentiation and identification of potato cultivars has become increasingly more important also as an additional support to DUS testing based on morphological traits. Simple sequence repeat (SSR) markers have proved to be the most robust tool for the rapid differentiation of potato cultivars (JOVOVIĆ *et al.,* 2013).

The aim of this study was to examine the reliability of the morphological characterization of lightsprouts for quick assessment of the genetic diversity of Montenegrin potato accessions. The results of morphological characterization and additional evaluation using

SSR markers are expected to result in identification of duplicates, and conservation of representative samples of the total genetic diversity.

MATERIALS AND METHODS

During eight expeditions throughout Montenegro in the period from 2008 to 2010 52 traditional potato varieties were collected. The tubers are kept in the Montenegrin Plant Gene Bank. For the purpose of this study seven most valuable potato accessions were chosen: MNE 00030, MNE 00125, MNE 00127, MNE 00139, MNE 00142, MNE 00173 and MNE 00264. Selection of genotypes was based on the history of their cultivation, planted area and the economic importance for the local community.

Morphological characterization of lightsprouts and molecular differentiation were performed at Agricultural Institute in Ljubljana in 2012. For morphological characterization four tubers per accession were put into growing chamber with diffuse light and temperature approx. 20 ^{o}C for three months. Afterwards tubers were brought to daylight and assessed for morphological characteristics of lightsprouts according to UPOV descriptor list for potato:

A – Lightsprout size (3-small, 5-medium, 7-large)
B – Lightsprout shape (1-spherical, 2-ovoid, 3-conical, 4-broad cylindrical, 5-narrow cylindrical)
C – Intensity of anthocyanin coloration of base (1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong)
D – Proportion of blue in anthocyanin coloration of base (1-absent or low, 2-medium, 3-high)
E – Pubescence of base (1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong)
F – Size of tip in relation of base (3-small, 5-medium, 7-large)
G – Habit of tip (1-closed, 3-intermediate, 5-open)
H – Anthocyanin coloration of tip (1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong)
I – Pubescence of tip (1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong)
J – Number of root tips (3-few, 5-medium, 7-many)
K – Length of lateral shots (3-short, 5-medium, 7-long).

In molecular analysis of seven potato accessions maintained at the Montenegro Gene Bank, six microsatellite markers were used: STM1024, STM2022, STM3012, STM2028, STM5136 and STM5148. Light sprouted tubers were planted in greenhouse and genomic DNA was isolated from leaves using GenElute Plant Genomic DNA Miniprep Kit (Sigma). Amplification reactions were performed with a Gene Amp PCR thermocycler (Applied Biosystems) in a 15 µL reaction mixture. Fluorescently labeled PCR products were mixed with formamide and internal size standard Rox 500 and genotyped on the ABI Prism 3130xl Genetic Analyzer using the GeneScan Analysis Software.

Every morphotype and SSR allele was scored for the presence (1) and absence (0) for each accession. The binary data matrix was used to calculate Jaccard's similarity coefficients (Jaccard, 1908); GSij = a/(a+b+c), where GSij is the similarity between two individuals i and j, a is the number of bands present in both i and j, b is the number of bands present in i and absent in j and c is the number of bands present in j and absent in i. Associations among the seven accessions

were displayed by cluster analysis using the UPGMA algorithm in the NTSYS-pc package (ROHLF, 1998).

RESULTS AND DISCUSSION

Results of morphological evaluation of lightsprouts are shown in Table 1. Four different phenotypes were identified among the seven Montenegrian accessions. Accessions MNE 00125, MNE 00127 and MNE 00264 classified in the UPGMA dendrogram into one group (Figure 1). Another two accessions, MNE 00030 and MNE 00173, showed identical phenotype and belonged to the second group. MNE 00139 and MNE 00142 showed distinct phenotypes to the rest of the accessions and could not be assigned into neither of the first two groups (Figure 1). Accessions that classified into the same group represent putative duplicates.

Table 1. Description of lightsprouts of seven Montenegrin accessions using UPOV technical guidelines

ACCESSION\TRAIT	A	B	C	D	E	F	G	H	I	J	K
MNE00125	5	3	3	1	3	7	5	1	3	3	3
MNE00127	5	3	3	1	3	7	5	1	3	3	3
MNE00264	5	3	3	1	3	7	5	1	3	3	3
MNE00030	7	5	3	1	7	3	3	1	3	3	3
MNE00173	7	5	3	1	7	3	3	1	3	3	3
MNE00142	7	5	3	1	3	3	1	1	1	5	5
MNE00139	7	5	5	1	5	5	1	3	5	3	3

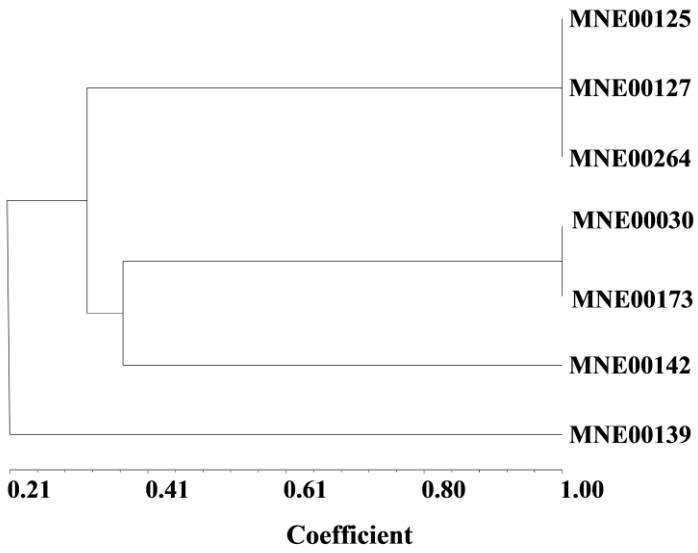

Fig. 1. UPGMA dendrogram of seven potato accessions from Montenegro based on morphological data

Fig. 2. Different groups of lightsprouts

In the analysis of six SSR loci 23 alleles were detected, of which 15 (65%) were polymorphic (Table 2). Only two alleles were detected at STM 1024 and they were both monomorphic. The most alleles (6) were scored at STM 5148, followed by STM 2028 (5) and STM 5136 (4).

Table 2. SSR alleles scored at six loci of seven potato accessions (1= present, 0 = absent)

	SSR markers																						
	STM3012			STM 5136				STM 5148						STM 1024		STM 2022			STM 2028				
ACCESSION	171	200	205	226	235	237	255	410	415	435	441	462	472	149	153	152	182	197	290	299	368	396	472
MNE 00125	0	1	1	1	1	1	0	0	1	1	0	1	0	1	1	1	0	1	0	1	1	1	1
MNE 00127	0	1	1	1	1	1	0	0	1	1	0	1	0	1	1	1	0	1	0	1	1	1	0
MNE 00264	0	1	1	1	1	1	0	0	1	1	0	1	0	1	1	1	0	1	0	1	1	0	0
MNE 00030	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	0	0
MNE 00173	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	0	1	0	0
MNE 00139	1	1	0	0	1	1	1	0	1	1	0	0	1	1	1	1	0	1	1	0	1	0	0
MNE 00142	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0

Accessions MNE 00125, MNE 00127 and MNE 00135 that had identical phenotypes differed by just one or two SSR alleles between each other. In the UPGMA dendrogram they alone constituted the first group (Figure 2). Another two accessions with identical phenotype, MNE 0030 and MNE 00173, were distinguished by just one SSR allele and formed group two in the dendrogram. MNE 00142 and MNE 00139 differed from the rest of accessions by four or more SSR alleles and clustered near the second group.

The two dendrograms based on morphological and SSR dendrograms showed very similar clustering patterns of the seven accessions. Due to higher polymorphic rate SSR markers succeeded to distinguish all accessions between each other. The second difference was positioning of accession MNE 00139 in the dendrograms. In the SSR dendrogram it clustered close to a set of three accessions while it differed from the rest of accessions by the same degree in morphological dendrogram.

High discrimination power of microsatellite markers observed here has already been reported in potato elsewhere (GAVRILENKO *et al.*, 2010; REID *et al.*, 2011). In some species, like

common bean, microsatellite markers have been shown also to be superior to morphological characterisation (ASFAW *et al*., 2009).

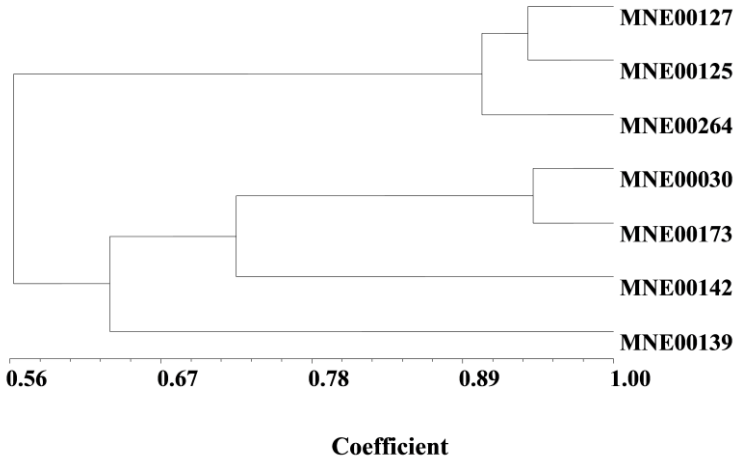

Fig. 3. UPGMA dendrogram of seven potato accessions from Montenegro based on SSR analysis

CONCLUSION

The study presented here showed that morphological characterization of the lightsprouts can be used in differentiation of potato genotypes by significant degree of confidence. We could have differentiated the accessions with higher precision if all 42 UPOV traits had been included in morphological characterization, but this would be time consuming and not reliable due to high virus infection of the preserved material. By using SSR markers in addition to morphological characterisation the results were substantially improved. We could clearly differentiate among different genotypes. The results showed that for rapid differentiation of potato genotypes with high degree of confidence both the morphological characterization of lightsprouts and SSR markers should be used. That was suggested also by other authors for potato (GAVRILENKO *et al.*, 2010; REID *et al.*, 2011), wheat (NOLI *et al.*, 2008), maize (IGNJATOVIĆ-MIĆIĆ *et al.*, 2015) and common bean (ASFAW *et al*., 2009). Although molecular identification has several advantages over the morphological characterization, phenotypic characteristics and other agronomic and morphological properties will continue to play an important and often crucial role in the characterization of genetic resources especially for rapid and robust evaluation of genetic resources and in situation when available financial resources are limited.

The results showed that this simple method can be reliably used in the further characterization of the potato gene pool, especially if it is taken into account that the Montenegrin Gene Bank is still didn't establish a system for DNA evaluation. Method of morphological characterization of lightsprouts can be of great assistance for quick identification of potato genotypes, and thus finding possible duplicates. On the other hand, could contribute to substantial reduction of conservation costs.

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POUZDANOST MORFOLOŠKE I MOLEKULARNE KARAKTERIZACIJE KLICA ZA DIFERENCIRANJE AKSEŠENA KROMPIRA

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Izvod

Studija pouzdanosti morfološke karakterizacije klice za diferenciranje sorti krompira izvedena je na Poljoprivrednom institutu Slovenije u saradnji sa Biotehničkim fakultetom iz Podgorice u cilju uvođenja jednostavnog metoda dalje karakterizacije aksešna krompira u Crnogorskoj banci gena. Za morfološku karakterizaciju klice odabrano je sedam potencijalno različitih aksešna krompira koji se čuvaju u Crnogorskoj banci gena. Ocjena 11 osobina klice izvršena je na osnovu UPOV deskriptora. Paralelno sa morfološkom karakterizacijom urađena je i molekularna procjena uz upotrebu šest mikrosatelitskih (SSR) markera. Molekularnom procjenom su uspješno izdvojeni svi aksešni osim dva, dok su morfološkom karakterizacijom identifikovana četiri različita fenotipa klice. Iako su se molekularni markeri pokazali kao efikasniji u određivanju veza između genotipova, karakterizacija klice se ipak pokazala kao korisna zbog jeftine, jednostavne i brze procedure.

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