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Genetic diversity of pear germplasm in Bosnia and Herzegovina, as revealed by SSR markers

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Abstract

Bosnia and Herzegovina (BIH) pear germplasm in the *ex situ* field collection at the University of Banja Luka was characterised with simple sequence repeat (SSR) markers in order to reveal redundancies, determine genetic diversity and confirm uniqueness. European pear (*Pyrus communis* L.) 67 accessions of the BIH collection and 7 reference cultivars of the *ex situ* collection at SLU in Balsgård, Sweden were analysed using a set of 10 SSR markers. All markers resulted in good amplification and easy scoring of marker alleles One-third of accessions appeared to be triploid. A total of 112 alleles were amplified in unique diploid genotypes with on average 11.2 alleles per marker. Mean observed heterozygosity (Ho) was 0.72, mean expected heterozygosity (He) – 0.80 and mean Shannon index (I) – 1.96. Inbreeding coefficient (F_{IT}) for diploid accessions ranged from −0.06 to 0.28 (mean 0.02) and Wright's inbreeding coefficient (F_{IS}) – from −0.27 to 0.17 (mean −0.03). In the BIH pear collection, mean value of genetic differentiation (F_{ST}) between the reference cultivars and pear accessions was 0.05. Principal coordinate analysis (PCoA) divided the 74 pear accessions into three groups. The first group consisted of diploid, reference and possible triploid pear accessions, while the second and third groups contained only BIH possible triploid accessions. Overall, the results revealed high levels of polymorphism and uniqueness, indicating that BIH pear germplasm represents very diverse and valuable material for future breeding programmes.

Key words: *Pyrus communis*, microsatellites, molecular characterisation, field collection, gene bank.

Introduction

European pear (*Pyrus communis* L.) cultivation has a long history in the Balkan Peninsula. Traditional pear production was based mainly on old autochthonous cultivars, which are now considered important fruit genetic resources (FGR). For centuries, the Balkan Peninsula has been known for its richness in FGR in general and in pear genetic resources in particular. Pears are grown for consumption fresh as fruit but are also very important as dried or processed products, e.g., jam and alcohol (Beširević, 2009; Đurić et al., 2009 a). The diverse uses of pears enabled the spread of old traditional cultivars across the whole Balkan Peninsula region. However, the introduction of new cultivars and migration of rural people to cities in the past 70 years have negatively affected traditional fruit production in this region. The traditional cultivars have been replaced by new varieties that are of economic importance in commercial fruit production, while the abandoned orchards have been neglected and lost, which has resulted in a high degree of genetic erosion (Beširević, 2009).

The old autochthonous cultivars are very important FGR, since they represent a diverse genetic base and can serve as a source of genes for future breeding programmes. Pear germplasm is still present in the area of Bosnia and Herzegovina (BIH), mostly in-home gardens and on old farms (Beširević, 2009; Đurić et al., 2009 b). Due to the importance of local fruit germplasm in breeding to meet future challenges and

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because of the high risk of genetic erosion, FGR must be adequately collected and described and then preserved and characterised in germplasm collections. The gene bank at the Institute of Genetic Resources, University of Banja Luka, has taken a leading role in the preservation of genetic resources in BIH in the past 10 years. It performs a wide range of activities, ranging from inventory and collection campaigns to multiplication, *ex situ*/*in situ* and on farm conservation, characterisation, documentation and information, public promotion and initial evaluation. In 2013, the Institute established an *ex situ* fruit field collection containing 87 pear accessions collected from old orchards and gardens throughout BIH. The frequent migrations of the Balkan population throughout history have led to movement of germplasm and often to changes in the names of individual genotypes. Moreover, FGR have been used in spontaneous breeding efforts by individual fruit growers in some cases. Thus, it is known that fruit collections in BIH usually contain duplicate accessions either under the same or different names (Đurić et al., 2009 a; b; Kajkut Zeljković et al., 2019).

In the past, morphological characterisation was used to reveal redundancies in fruit gene banks (Đurić, 2009 a). Over recent decades, molecular markers have become an indispensable and superior tool in the management of germplasm collections, due to their ability to reveal greater numbers of redundancies in collections, than it was possible with traditional morphological characterisation (De Andres et al., 2007). Furthermore, molecular markers are very important for evaluating the extent and structure of genetic diversity and for disclosing genetic relatedness patterns. Thus, characterisation using molecular markers provides valuable and clear information about the germplasm preserved in collections (Nybom, Weising, 2010). In BIH, the old and new genotypes preserved in the gene bank at the Institute of Genetic Resources, University of Banja Luka have to be characterised with molecular markers as the first step towards their efficient utilisation (Đurić et al., 2009 b).

Among the variety of molecular markers available (Nybom et al., 2014), the effectiveness of SSR markers for molecular characterisation of FGR has been demonstrated by previous studies on, e.g., the diversity of pear (Brini et al., 2008; Sehić et al., 2012; Gasi et al., 2013; Ouni et al., 2020), apple (Garkava-Gustavsson et al., 2008; 2013; Urrestarazu et al., 2016; Mažeikienė et al., 2019; Skytte af Sätra et al., 2020), plum (Gaši et al., 2020) and sweet cherry (Stanys et al., 2012). Part of the BIH pear germplasm has previously been studied with different types of molecular markers, such as RAPD markers (Kajkut et al., 2015), AFLP markers (Vučković, 2017) and SSR markers (Gasi et al., 2013).

The objective of the present study was to characterise the remaining BIH pear germplasm with SSR markers in the *ex situ* field collection at the Institute of Genetic Resources, University of Banja Luka in order to reveal redundancies, determine the genetic diversity and confirm the uniqueness of the preserved pear germplasm.

Materials and methods

Plant material and simple sequence repeat (SSR) analysis. European pear (*Pyrus communis* L.) 67 accessions of the Bosnia and Herzegovina (BIH) pear collection and 7 reference cultivars of the *ex situ* collection at SLU in Balsgård, Sweden were analysed during 2017 (Table 1).

Table 1. Bosnia and Herzegovina (BIH) pear accessions (n = 67) and reference cultivars (n = 7) analysed in this study

Accession number	Cultivar	Accession number	Cultivar
PKB-K-1	Izmirska	PKB-K-40	Nepoznato ime 2
PKB-K-2	Karamut bijeli	PKB-K-41	Duplagica
PKB-K-3	Litrenjača	PKB-K-42	Kantaruša
PKB-K-4	Urumenka	PKB-K-98	Kantaruša
PKB-K-5	Duga bostanka	PKB-K-137	Medenka
PKB-K-6	Ilinjača	PKB-K-138	Stambolka
PKB-K-7	Karamut crni	PKB-K-139	Urumenka
PKB-K-8	Batvača	PKB-K-140	Avraška
PKB-K-9	Jeribasma	PKB-K-141	Izmirska
PKB-K-10	Arapka crna	PKB-K-142	Batva
PKB-K-11	Zrnka	PKB-K-143	Duplagica
PKB-K-12	Pšeničarka	PKB-K-145	Karamut
PKB-K-13	Avraška	PKB-K-146	Lubeničarka
PKB-K-14	Ječmenka	PKB-K-219	Crnica
PKB-K-15	Nepoznato stablo 3	PKB-K-220	Mednjaka
PKB-K-16	Hošija	PKB-K-221	Sijerak
PKB-K-17	Čađanka	PKB-K-222	Šipača
PKB-K-18	Jagodnjača	PKB-K-223	Ljetnja kolačuša
PKB-K-19	Kantaruša	PKB-K-224	Karamut
PKB-K-20	Citronka	PKB-K-225	Šećernjača
PKB-K-21	Kongresovka	PKB-K-226	Crna miholjka
PKB-K-22	Čavka	PKB-K-228	Takiša
PKB-K-23	Mednica	PKB-K-233	Miholjača
PKB-K-24	Ljetna kolačuša	PKB-K-234	Divlja kruška
PKB-K-25	Nepoznato ime 2	PKB-K-236	Kaurka
PKB-K-26	Mirisavka	PKB-K-240	Sijerak
PKB-K-27	Lubeničarka	PKB-K-241	Zimnjaka
PKB-K-28	Gospoinjača	PKB-K-243	Slavkova slatka
PKB-K-29	Sarajka	PKB-K-245	Lisica
PKB-K-30	Karamut	PKB-K-246	Begarmuta
PKB-K-31	Sarevka	reference	Carola
PKB-K-32	Okrugla bostanka	reference	Clapp's Favourite
PKB-K-34	Jesenja kolačuša	reference	Clara Frijs
PKB-K-35	Glibanjka	reference	Conference
PKB-K-36	Žutica	reference	Esperens Herre
PKB-K-37	Bijela takiša	reference	Herzogin Elsa
PKB-K-38	Miholjača	reference	Pierre Corneille

In previous studies (Sehic et al., 2012), the pear genotypes from SLU in Balsgård used in this study were shown to be true-to-type. Therefore, they were used as reference cultivars in Ouni et al. (2020) and in our study, since the use of well-characterized genotypes as reference is helpful for comparing results obtained in different studies, in different laboratories and finally accessions hold in different collections.

The analysis was performed using a set of 10 SSR markers recommended by the European Cooperative Programme for Plant Genetic Resources (ECP/GR) (Evans et al., 2009) and previously used by Sehic et al. (2012) and Ouni et al. (2020) (Table 3).

Fresh leaves were sampled and the DNA was extracted using a modified CTAB (cetyl trimethylammonium bromide) extraction protocol (Doyle, Doyle, 1990). The quality and quantity of each of the 74 DNA samples were measured in 1 µL of sample using a spectrophotometer ND-1000 (Thermo Fisher Scientific, USA) following the standard procedure.

Polymerase chain reaction (PCR) was performed using 10 fluorescence labelled SSR marker pairs. The total volume of the PCR reaction was 18 µL, to which

10 × PCR buffer containing 20 mM $MgCl_2$, 10 mM dNTP, 0.25 U Taq polymerase and 10 ng DNA; 0.8 µL of each primer (10 µM) were added. The amplification was conducted using an initial denaturation at 94°C for 5 min followed by 10 cycles with 94°C for 30 s, 55–50°C (−0.5°C/cycle) for 45 s and 72°C for 60 s. A further 25 cycles with 94°C for 30 s, 50°C for 45 s and 72°C for 60 s were then performed. The amplification was stopped with a final extension at 72°C for 15 min. The PCR reaction was carried out using a thermal cycler Mastercycler EP Gradient S (Eppendorf, Germany). Successful amplification was verified by separating the PCR products on 2% agarose gel electrophoresis in 1 × TBE buffer. Staining and visualisation were performed using GelRed™ (Biotium, USA) and a UV-light transilluminator (Saveen Werner AB, Sweden). For fragment analysis, the PCR products were multiplexed in the following combinations: CH01d08 (NED), CH01d09 (VIC) and CH03d12 (6-FAM); CH05c06 (6-FAM), EMPc117 (VIC), GD147 (PET) and EMPc11 (NED); CH04e03 (PET), CH03g07 (6-FAM) and CH01f07a (VIC). Multiplexed products were separated using a 3500 Series Genetic Analyzer (Thermo Scientific, USA). Fragment analysis was carried out using the software *Gene-Marker*, version 1.85 (SoftGenetics, LLC).

To check for the reproducibility of the results, three samples of 'Karamut bijeli', 'Kantaruša' and 'Nepoznato ime 2' were repeated twice in each run. Furthermore, three non-template controls, where no amplification was expected, were included in each run. Finally, SSR profiles of the reference cultivars were compared to those obtained in the previous studies (Sehic et al., 2012; Ouni et al., 2020).

Statistical analysis. A genetic distance matrix based on presence (1) or absence (0) of scored alleles was calculated for all 74 accessions by simple matching coefficient (SMC) (Sokal, Sneath, 1963; Gower, 1971). A dendrogram was produced using the unweighted pair group method with arithmetic mean (UPGMA) and bootstrapping based on 100 iterations with the software *R*, version 3.4.4 (R Core Team, 2018). Marker polymorphism and diversity were calculated for the 44 diploid accessions (duplicates excluded). The polymorphic information content (PIC) for each marker was calculated using the software *MolKin*, version 3.0 (Gutiérrez, Goyache, 2009). Genetic diversity estimators such as number of different alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and Shannon index (I) were calculated using the software packages *GeneALEx*, version 6.5 (Peakall, Smouse, 2006) and *R*, version 3.4.4. Allele frequencies and *F* statistics (inbreeding coefficient (F_{IT}), Wright's inbreeding coefficient (F_{IS}) and mean value of genetic differentiation (F_{ST}) (Weir, Cockerham, 1984) were also analysed using the software *GeneALEx*, version 6.5. Probability of identity (PI) was calculated for all diploid accessions using the same software. The grouping and dispersion patterns for molecular data of the 74 accessions (diploid and triploid accessions in the BIH pear collection and references) were analysed in accordance with the data structure by cluster analysis and principal coordinate analysis (PCoA) (Kolář et al., 2017; Meirmans et al., 2018) using the software *R*, version 3.4.4.

Results and discussion

Marker polymorphism. The 10 SSR primer pairs used for evaluation of genetic diversity within the BIH pear collection resulted in good amplification and easy scoring of scorable SSR fragments. The presence of a third marker allele in one or several loci was recorded in 27 accessions, which can be an indication of triploidy (Table 2).

Table 2. Possible triploid accessions (n = 27) identified among the Bosnia and Herzegovina (BIH) pear accessions analysed

No.	Cultivar	CH05c06	CH03d12	GD147	EMPc117	EMPc11	CH01d08	CH01d09	CH01f07a	CH03g07	CH04e03
1.	Izmirska	91:107	89:91	121:127:129	103:113:117	141:147	277:277	149:169:171	195:207	233:241	179:179
2.	Urumenka	83:107:109	107:113:115	121:121	115:119	141:147	279:301	123:141	183:183	241:243	179:195
3.	Ilinjača	87:89:97	89:89	121:125	93:111	147:155	275:277	137:141	191:207	233:241	179:179
4.	Karamut crni	91:107	89:91	121:127:129	103:113:117	141:147	275:277	149:169:171	195:207	231:233:241	179:179
5.	Batvača	89:97:107	89:111	121:129	117:135	137:141:143	277:277	133:147:149	189:195:211	199:237:265	179:199
6	Arapka crna	87:91:111	89:123	121:147	91:117	147:153	281:285	129:135:141	181:187:207	225:227:255	179:179
7.	Ječmenka	87:101	119:119	121:127:147	117:123	153:153	275:281	129:151	183:195	213:243	179:199
8.	Čađanka	87:89:101	107:107	121:125	117:123	135:153	277:281	129:151	183:189	201:241	179:179
9.	Jagodnjača	87:91:95	123:123	121:147	109:117	147:153	275:277	129:153	175:185	241:247	179:205
10.	Kantaruša	87:91:95	123:123	121:147	109:117	147:153	275:277	129:153	175:185	241:247	179:205
11.	Ljetnja kolačuša	91:107	89:91	121:127:129	103:113:117	141:147	277:277	149:169:171	195:207	233:241	179:179
12.	Nepoznato ime	89:89	89:111	129:147	117:123	135:153	275:281:301	129:129	183:189	201:241	179:195
13.	Mirisavka	87:99	107:111:123	127:147	99:113	113:135	275:281:301	129:141:149	183:207	207:223:231	179:179
14.	Gospoinjača	87:101	119:119	121:127:129	117:123	153:153	275:281	129:153	183:195	213:243	179:199
15.	Sarajka	91:107	89:91	121:127:129	103:113:117	141:147	277:277	149:169:171	195:207	231:233:241	179:179
16.	Karamut	91:107	89:91	121:127:129	103:113:117	141:147	277:277	149:169:171	195:207	231:233:241	179:179
17.	Sarevka	91:107	89:91	121:127:129	103:113:117	141:147	277:277	149:169:171	195:207	231:233:241	179:179
18.	Okrugla bostanka	87:99	107:109	121:127	87:123:125	153:155	275:285	163:165	175:183	231:241	179:179
19.	Kantaruša	87:89:101	107:107	121:125	117:123	135:153	277:281	129:151	183:189	201:241	179:179
20.	Kantaruša	87:97	109:117:123	121:123	85:87:113	135:139	275:277	129:139:151	181:183:207	231:241	179:195
21.	Medenka	89:97:107	89:111	121:129	117:135	137:141:143	279:281	133:147:149	189:195:211	199:237:265	179:199
22.	Urumenka	83:107:109	89:89	121:147	115:119	141:147	279:301	123:141	183:183	241:243	179:195
23.	Batvača	89:97:107	89:111	121:129	117:135	137:141:143	281:281	133:147:149	189:195:211	199:237:265	179:199
24.	Karamut	91:107	89:91	121:127:129	103:113:117	141:147	277:277	149:169:171	181:181	233:241	179:179
25.	Mednjaka	89:97:107	89:111	121:129	117:135	137:141:143	279:281	133:147:149	189:195:211	199:237:265	179:199
26.	Lisica	91:95:107	89:111	129:147	95:117	139:153	277:277	139:165	175:183	221:241	179:197
27.	Begarmuta	87:99	107:109	123:129	87:107:113	135:149	275:279:301	129:129	175:181:183	231:247	179:195

Similar findings of more than two alleles of individual loci have been reported in other SSR-based studies of pear (Sehic et al., 2012; Gasi et al., 2013; Ferradini et al., 2017; Ouni et al., 2020). A total of 112 alleles were amplified in the set of 44 unique diploid genotypes (37 BIH accessions and 7 reference cultivars) after all the duplicates had been removed (Table 3). The number of alleles per locus ranged from 6 alleles for markers CH01d08 and CH04e03 to 20 alleles for marker CHd01d09 with an average number of 11.20 alleles per locus.

Table 3. The 10 SSR markers used for evaluation of genetic diversity in this study

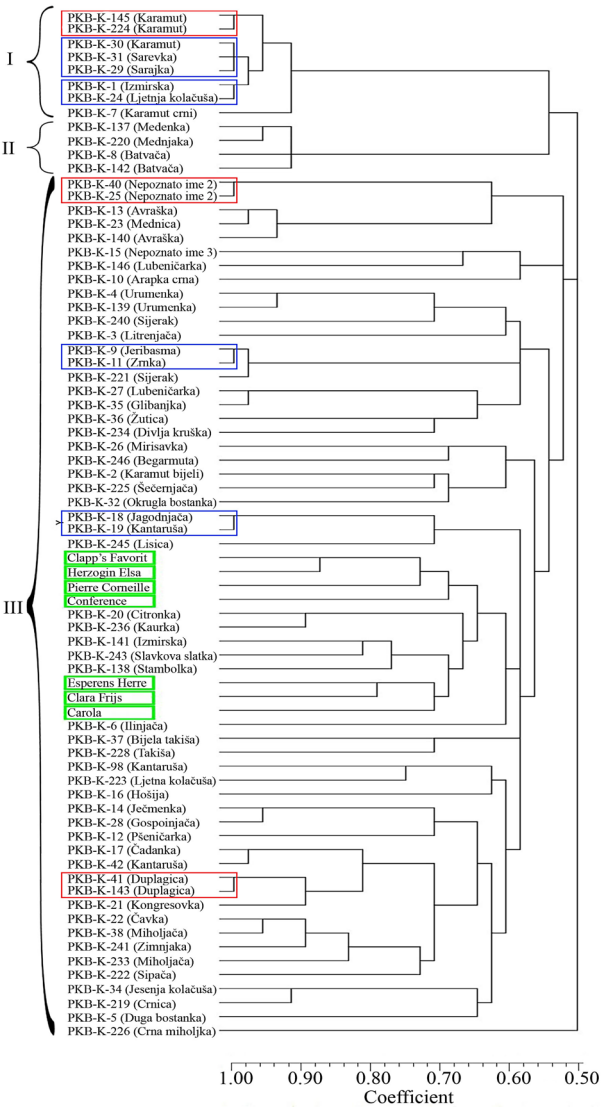
Marker	Size range bp	Reference	Number of alleles (Na)	Number of effective alleles (Ne)	Polymorphic information content (PIC) value
GD147	121–147	Hokanson et al., 1998	7	2.93	0.65
CH01d08	277–301	Liebhard et al., 2002	6	4.15	0.76
CH01d09	123–175	Liebhard et al., 2002	20	11.56	0.90
CH01f07a	175–211	Liebhard et al., 2002	13	6.85	0.85
CH03d12	89–123	Liebhard et al., 2002	8	5.66	0.82
CH03g07	195–265	Liebhard et al., 2002	15	8.38	0.87
CH04e03	179–221	Liebhard et al., 2002	6	2.17	0.55
CH05c06	83–111	Liebhard et al., 2002	11	6.06	0.83
EMPc11	135–155	Fernández-Fernández et al., 2006	10	6.60	0.85
EMPc117	85–135	Fernández-Fernández et al., 2006	16	9.33	0.89

This is comparable to the average number of 11.30 alleles per locus found by Queiroz et al. (2015). Slightly lower values, 5.45 and 9.50 alleles per locus, have been reported by Liu et al. (2015) and Rana et al. (2015), respectively. The number of effective alleles per locus varied from 2.17 (CH04e03) to 11.56 (CH01d09) with an average number of 6.40 effective alleles per locus, which is higher than the value of 3.30 found by Ouni et al. (2020). The PIC value ranged between 0.55 for marker CH04e03 and 0.90 for marker CH01d09 with an average of 0.80. All the markers were very informative in the current study (Table 3).

Identification of duplicates, synonyms and homonyms. Using simple matching coefficient (SMC) analysis, different duplicates, synonyms and homonyms were identified (Fig. 1).

Upon bootstrapping, three stable groups of genotypes were identified, which grouped together with the following high stability coefficients: I – 0.95, II – 0.98 and III – 0.62. In total, three pairs of duplicates: PKB-K-25 and PKB-K-40 (‘Nepoznato ime 2’), PKB-K-41 and PKB-K-143 (‘Duplagica’) and PKB-K-145 and PKB-K-224 (‘Karamut’), were identified. Furthermore, four groups of synonymous accessions were identified with group 1 containing the accessions PKB-K-9 (‘Jeribasma’) and PKB-K-11 (‘Zrnka’), group 2 containing the accessions PKB-K-18 (‘Jagodnjača’) and PKB-K-19 (‘Kantaruša’), group 3 containing the accessions PKB-K-1 (‘Izmirska’) and PKB-K-24 (‘Ljetnja kolačuša’), and group 4 containing the accessions PKB-K-29 (‘Sarajka’), PKB-K-30 (‘Karamut’) and PKB-K-31 (‘Sarevka’). Beside the groups of duplicates and synonyms, a total of seven groups of homonyms were identified. Group 1 contained the two accessions PKB-K-4 and PKB-K-139 (‘Urumenka’). Group 2 contained two accessions PKB-K-13 and PKB-K-140 known as ‘Avraška’. Group 3 contained two genetically different accessions PKB-K-27 and PKB-K-146, which are designated ‘Lubeničarka’. Group 4 consisted of two ‘Ljetnja kolačuša’ accessions PKB-K-24 and PKB-K-223, group 5 of the ‘Miholjača’ accessions PKB-K-38 and PKB-233, group 6 of the ‘Sijerak’ accessions PKB-K-221 and PKB-K-240, and group 7 of the ‘Kantaruša’ accessions PKB-K-42 and PKB-K-98.

Seven SSR loci and some accessions: ‘Jeribasma’, ‘Ječmenka’, ‘Karamut’, ‘Lubeničarka’, ‘Miholjača’ and ‘Takiša’, were common for our study and a study of Gasi et al. (2013) also describing BIH pear germplasm. One of the reference cultivars ‘Conference’



Note. Three groups of duplicates (marked in red boxes) and four groups of synonymous accessions (marked in blue boxes) were identified.

Figure 1. Grouping of the Bosnia and Herzegovina (BIH) pear 67 accessions and the 7 reference cultivars (marked in green boxes) based on simple matching coefficient (SMC) analysis and grouping based on bootstrapping with 100 iterations

was analysed by Sehic et al. (2012), Gasi et al. (2013) and Ouni et al. (2020), and this cultivar had identical SSR profile in all the analyses after correction for allelic size differences among the studies.

The situation with other cultivars was somewhat different. Thus, SSR profiles of 'Miholjača' (PKB-K-233) and 'Takiša' (PKB-K-228) corresponded to those of 'Miholjača' (SR35) and 'Takiša' (SA12) in Gasi et al. (2013) in all loci, except CH03d12. Furthermore, indication of triploidy was obtained for 'Karamut', designated PKB-K-30 in our study and SR23 in Gasi et al. (2013). However, Gasi et al. (2013) scored three alleles only in one locus CH03g07, while in our study third allele was scored also in loci EMPc117, CH01d09 and GD147 (the latest was not included in Gasi et al., 2013) in addition to CH03g07 (Table 2). This emphasizes need for careful standardization of procedures, while analysing and comparing different germplasm collections including sufficient number of relevant reference cultivars and overlapping accessions represents maximum of available alleles, which allows correction for allele size differences among the studies and collections (Garkava-Gustavsson et al., 2013; Sehic et al., 2013). Harmonization among different collections and phenotypic evaluation of the material are the next very important steps towards efficient germplasm management in BIH.

Efficient germplasm management includes preservation of maximum genetic diversity and minimum redundant genotypes. The probability for the pairs of fingerprints to be identical by chance was very low (Fig. 2). By that, accessions with identical SSR profiles are expected to derive from the same genotype or arise as sport mutations.

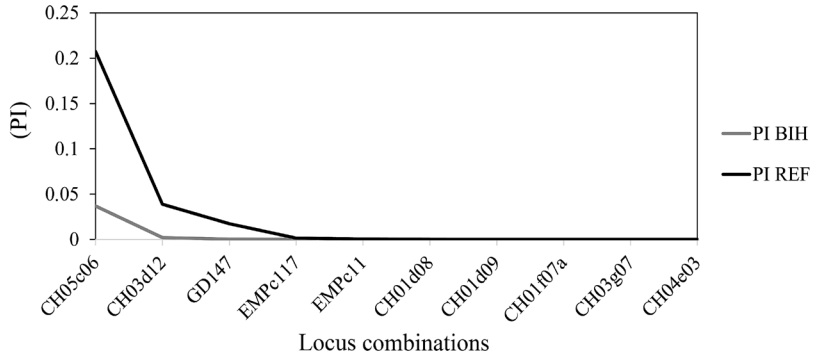


Figure 2. Probability of identity (PI) values for diploid Bosnia and Herzegovina (BIH) pear accessions and references (REF) cultivars identified with the set of 10 SSR markers

Genetic diversity. The mean values of the three genetic diversity estimators (Ho, He and I) calculated for the 44 (37 BIH accessions and 7 reference cultivars) unique diploid genotypes are shown in Table 4.

Table 4. Value of genetic diversity estimators for the diploid Bosnia and Herzegovina (BIH) pear accessions (n = 37) and reference cultivars (n = 7)

Marker	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Shannon index (I)
GD147	0.59	0.66	1.43
CH01d08	0.77	0.76	1.56
CH01d09	0.77	0.91	2.70
CH01f07a	0.91	0.85	2.17
CH03d12	0.48	0.82	1.85
CH03g07	0.96	0.88	2.36
CH04e03	0.61	0.54	1.16
CH05c06	0.73	0.84	2.07
EMPc11	0.75	0.85	2.02
EMPc117	0.68	0.89	2.43

The highest value of observed heterozygosity was found for locus CH03g07 (0.96), while the lowest one was detected for locus CH03d12 (0.48). The highest value of expected heterozygosity was found for locus CH01d09 (0.91) and the lowest one for locus CH04e03 (0.54). The highest value of Shannon index was detected for locus CH01d09 (2.67) and the lowest one for locus CH04e03 (1.16). The mean value of observed heterozygosity was 0.73, which is similar to previously reported values of 0.74 (Sehic et al., 2013) and 0.71 (Brini et al., 2008) for old Swedish and Tunisian cultivars, respectively. Higher than 0.64 value was reported by Rugienius et al. (2013) for wild pear (*Pyrus pyraster* (L.) Burgst.), but lower than the 0.85 one was reported by Queiroz et al. (2015) for Portuguese pear landraces. The mean value of expected heterozygosity was 0.80, which is similar to the values of 0.78 and 0.74, previously reported by Sehic et al. (2013) and Ferradini et al. (2017), respectively, but lower than that of *P. pyraster* reported by Rugienius et al. (2013). The mean value of Shannon index for all 10 SSR markers was 1.96, which is high and comparable to the value of 1.95 reported for *P. pyraster* by Wolko et al. (2015). Erfani-Moghadam and Zarei (2018) found a mean Shannon index value of 1.04, which was lower than that in this study. The values of genetic diversity estimators calculated for the 37 unique diploid BIH pear accessions were 0.72, 0.80 and 1.97, respectively. Thus, the BIH pear germplasm accurately represents the diversity of the *Pyrus communis* species and is well in line with the outcrossing and self-incompatible nature of pear. The level of diversity harboured by the pear collection in BIH is high and comparable to that in other collections.

Inbreeding coefficient (F_{IT}) ranged between −0.06 and 0.28 with a mean value of 0.02 (Fig. 3). In a similar study on BIH pear accessions by Gasi et al. (2013), a F_{IT} value of 0.07 was found indicating no loss of heterozygosity. Wright's inbreeding coefficient (F_{IS}) was within the range −0.27 to 0.17 with a mean value of −0.03. A negative value of this coefficient indicates a very slight excess of heterozygotes in the pear germplasm collection. Giovannini et al. (2011) also reported a negative F_{IS} value (−0.09) for pear germplasm from southern Italy. Ferreira dos Santos et al. (2011) reported a mean F_{IS} value of 0.10 for Spanish pear germplasm, while Gasi et al. (2013) reported a value of 0.05 for BIH

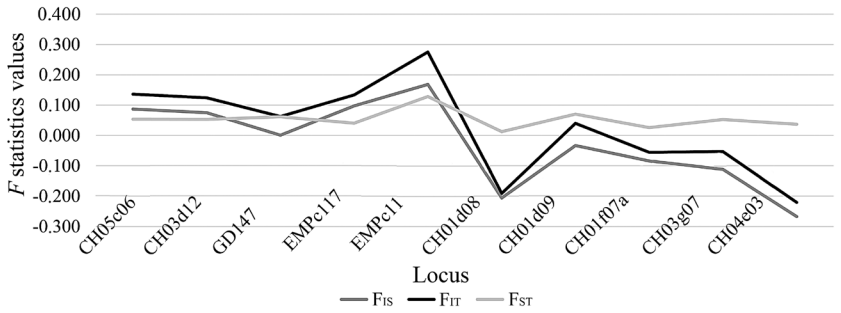


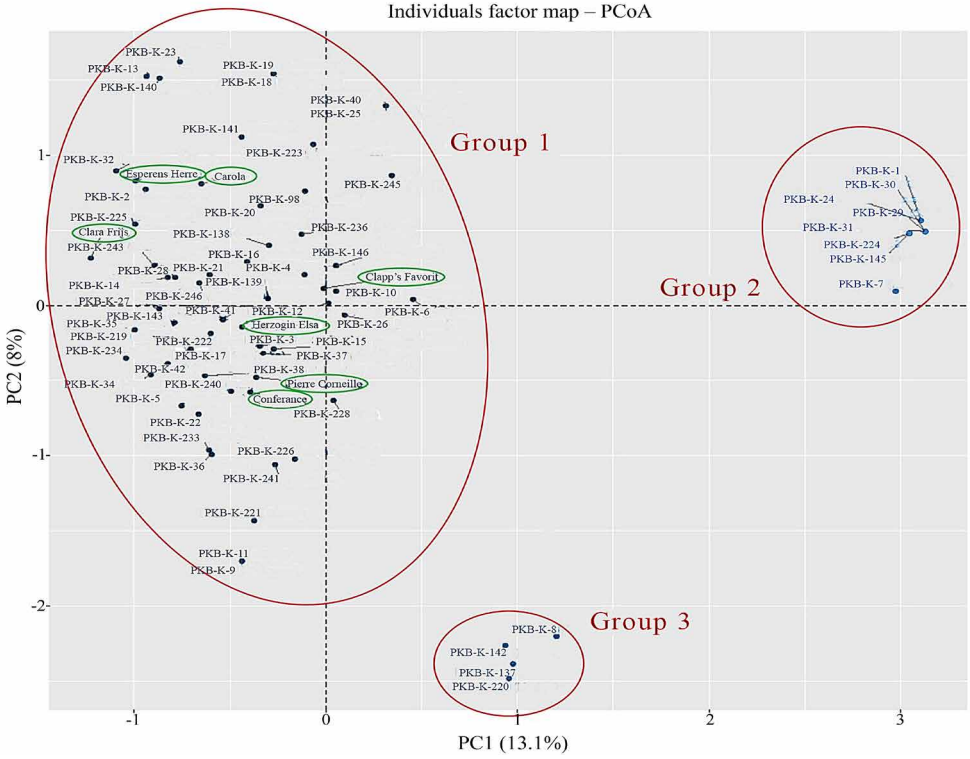
Figure 3. Wright's inbreeding coefficient (F_{IS}), inbreeding coefficient (F_{IT}) and mean value of genetic differentiation (F_{ST}) for the diploid Bosnia and Herzegovina (BIH) pear accessions (n = 37) and reference cultivars (n = 7)

pear germplasm. Mean value of genetic differentiation (F_{ST}) between the reference cultivars and the BIH pear accessions was 0.05, which is higher than the 0.02 reported by Gasi et al. (2013) and the 0.01 reported by Ferradini et al. (2017). Thus, the BIH pear germplasm analysed in this study was quite different from the set of reference cultivars obtained from a Swedish collection.

These results show that germplasm of the BIH pear collection represents valuable material that can be used directly for growing or for starting a future pear breeding programme in BIH. Based on the data obtained, BIH pear germplasm represents a diverse gene pool. This diversity could be explained by the influence of migration of people through the Balkan Peninsula and by spontaneous hybridisation (Đurić et al., 2009 a; b). Noticeable genetic variation in the pear gene bank collection of Iran was reported by Erfani-Moghadam and Zarei (2018), who attributed this high level of genetic variation to high crossability among pear germplasm.

Applying PCoA, where the first two principal coordinates explained 21.1% of the variation, the plot revealed separation of the BIH pear accessions into three groups (Fig. 4).

The Group 1 consisted of diploid and possible triploid BIH pear accessions, grouped together with the reference cultivars. The Group 2 consisted of only BIH pear accessions with synonyms, duplicates and one unique accession that were all possible triploids. The Group 3 comprised four BIH pear accessions with similar names, but all had unique genetic profiles and were possible triploids. The Groups 2 and 3 were distant from the Group 1 (Fig. 4). Separation of the BIH pear germplasm in these three groups can be explained by the influence of germplasm being introduced into the region, mainly from the East, over the centuries.



Note. Group 1 contains diploid and possible triploid BIH pear accessions grouped together with the reference cultivars (marked in boxes); Group 2 contains only possible triploid BIH pear accessions; Group 3 contains only the remaining possible triploid BIH pear accessions.

Figure 4. Two-dimensional principal coordinate analysis (PCoA) plot of the 67 Bosnia and Herzegovina (BIH) pear accessions and 7 reference cultivars analysed using 10 SSR markers

Conclusions

1. Bosnia and Herzegovina (BIH) pear germplasm has high levels of diversity. This diversity is well represented in the *ex situ* field gene bank collection at the Institute of Genetic Resources, University of Banja Luka, as shown by the SSR marker results.

2. The collection at the Institute of Genetic Resources, University of Banja Luka has quite a low level of redundancies and a high level of diversity. This is in line with the outcrossing and self-incompatible nature of pear and is comparable to the levels in other collections.

3. Following molecular characterisation of the BIH pear germplasm, in future work morpho-pomological characterisation should be conducted to fully exploit the potential of this collection to become a diverse and valuable material for utilisation in breeding programmes. Furthermore, comparisons and harmonization among different collections are important steps towards efficient germplasm management in BIH.

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Paprastosios kriaušės genetinės medžiagos įvairovė Bosnijoje ir Hercegovinoje atskleista taikant paprastųjų pasikartojančių sekų žymeklius

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Santrauka

Siekiant atskleisti dubliavimą, nustatyti genetinę įvairovę ir patvirtinti unikalumą, Banja Luka universiteto *ex situ* lauko Bosnijos ir Hercegovinos (BIH) kolekcijos paprastosios kriaušės (*Pyrus communis* L.) genetinė medžiaga apibūdinta taikant paprastųjų pasikartojančių sekų (PPS) žymeklius. Švedijos žemės ūkio mokslų universiteto BIH kolekcijos 67 kriaušės ir 7 *ex situ* kolekcijos genotipai išanalizuoti taikant 10 PPS žymeklių rinkinį. Žymekliai leido amplifikuoti lengvai įvertinamus žymeklių alelius. Trečdalis genotipų pasirodė esantys triploidai. Unikaliuose diploidiniuose genotipuose iš viso buvo amlifikuota 112 alelių, vidutiniškai 11,2 alelių vienam žymekliui. Vidutinis nustatytas heterozigotas (Ho) buvo 0,72, vidutinis tikėtinas heterozigotas (He) – 0,80, vidutinis Šanono indeksas (I) – 1,96. Diploidinių genotipų inbrydingo koeficientas (F_{IT}) svyravo nuo −0,06 iki 0,28 (vidurkis 0,02), Wrighto inbrydingo koeficientas (F_{IS}) – nuo −0,27 iki 0,17 (vidurkis −0,03). BIH kolekcijoje vidutinė genetinių diferenciacijų (F_{ST}) vertė tarp referentinių veislių ir kriaušės genotipų buvo 0,05. Taikant principinę koordinačių analizę 74 kriaušės genotipai buvo suskirstyti į tris grupes. Pirmą grupę sudarė diploidiniai, referentiniai ir numanomi triploidiniai genotipai, antroje ir trečioje grupėse buvo tik numanomi triploidiniai BIH kolekcijos kriaušės genotipai. Tyrimo rezultatai atskleidė aukštą polimorfizmo ir unikalumo lygį, rodantį, kad BIH kolekcijos kriaušės genetinė medžiaga yra labai įvairi ir vertinga būsimoms selekcinėms programoms.

Reikšminiai žodžiai: *Pyrus communis*, paprastosios pasikartojančios sekos, molekulinis apibūdinimas, lauko kolekcija, genų bankas.