Genetic characterization of an apple gene bank (part of the Austrian Fruit Collection) using microsatellite analysis reveals rare cultivars

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Genetic characterization is an important tool and a well established method for the maintenance of fruit collections. In this study apple trees from the gene bank "Kierling" (Klosterneuburg, Austria) were characterized by molecular markers. This orchard was planted in 1997. The varieties are grafted on seedling rootstocks and raised as half-standard trees. A total of 208 trees were analyzed and 95 different varieties could be identified. The use of 28 different microsatellites in seven multiplex reactions made it possible to compare the profiles obtained from various international databases. At the same time, a reference database with over 10.000 records from international publications and cooperations with other institutions was created by harmonization of the available data for this purpose. True-to-type analysis confirmed that most of the planted cultivars were correctly recorded, as expected. However, it was also possible to identify varieties, which were previously not known to be part of this gene bank, e.g. 'Orbai alma' or 'Fameuse'. Furthermore, it was discovered by parentage analysis that 'Rumer Gravensteiner', a local variety in Tyrol, is presumably a seedling of 'Landsberger Renette' and 'Schmidberger Renette'. Four cultivars could not be verified and identified, respectively, yet as no reference profile was found in any international data set a further pomological examination has to be done. However, one of these was determined to be related to 'Bittenfelder Sämling'.
Keywords: *Malus domestica* Borkh., SSR marker, genotyping, differentiation

Die genetische Charakterisierung einer Apfelgenbank, Teil der Österreichischen Obstsortensammlung, mittels Mikrosatellitenanalyse zeigt interessante Sorten auf. Die genetische Charakterisierung ist ein wichtiges Werkzeug und eine gut etablierte Methode bei der Erhaltung von Obstsortensammlungen. In dieser Studie wurden Apfelbäume der Genbank "Kierling" (Klosterneuburg, Österreich) mittels molekularer Marker charakterisiert. Diese Streuobstwiese wurde 1997 gepflanzt. Die Sorten wurden auf Sämlingsunterlagen veredelt und als Halbstamm erzogen. Insgesamt wurden 208 Bäume analysiert und 95 verschiedene Sorten identifiziert. Die Verwendung von 28 verschiedenen Mikrosatelliten in sieben Multiplex-Ansätzen ermöglichte es, die erhaltenen Profile mit verschiedenen internationalen Datenbanken zu vergleichen. Gleichzeitig wurde zu diesem Zweck eine Referenzdatenbank mit über 10.000 Datensätzen aus internationalen Publikationen und Kooperationen mit anderen Institutionen durch die Harmonisierung der verfügbaren Daten aufgebaut. Die Überprüfung der Sortenechtheit bestätigte, dass die meisten angebauten Sorten erwartungsgemäß korrekt erfasst worden waren. Es konnten aber auch Sorten identifiziert werden, die bisher nicht als Teil dieser Genbank bekannt waren, z. B. 'Orbai Alma' oder 'Fameuse'. Weiters konnte durch

Verwandtschaftsanalysen festgestellt werden, dass 'Rumer Gravensteiner', eine lokale Sorte in Tirol, vermutlich ein Sämling von 'Landsberger Renette' und 'Schmidberger Renette' ist. Vier Sorten konnten bis jetzt noch nicht verifiziert bzw. identifiziert werden, da entweder in keinem internationalen Datensatz ein Referenzprofil gefunden wurde oder noch weitere pomologische Untersuchungen durchgeführt werden müssen. Jedoch konnte bei einer davon eine Verwandtschaft mit 'Bittenfelder Sämling' festgestellt werden.

Schlagwörter: *Malus domestica* Borkh., SSR-Marker, Genotypisierung, Differenzierung

Gene banks are used to preserve historical and traditional fruit varieties as genetic resources for the future. In times of climate change, it is desirable to preserve the largest possible gene pool, as this is the only way to ensure that varieties suitable for future growing conditions can be selected. Furthermore it provides the opportunity for adaption of our fruit crops to a changing environment. Apple (*Malus domestica* Borkh.) belongs to the fruit crops with a long life span. This and the fact that it is mostly clonally propagated ensures a very stable gene pool. Historic cultivars, especially those, which have been cultivated over centuries represent therefore a broad genetic base. This makes them very useful and necessary in the breeding of new varieties which can face the new challenges, as currently the worldwide apple production is dominated by few cultivars and their pedigrees which are genetically linked (Bannier, 2010; Veteto and Carlson, 2014; Urrestarazu et al., 2016).

For a distinct identification and to guarantee that the desired varieties are preserved in fruit collections, a genetic characterization using microsatellite analysis is carried out in addition to classical pomological description (Monschein et al., 2004; Xuan, 2007; Storti et al., 2012). Genetic fingerprinting based on SSR markers has been used for decades in the molecular distance analysis of plants (Weising et al., 1995), animals (Blouin et al., 1996), humans (Butler, 2007), and microorganisms (Field and Wills, 1998) as well as to elucidate presumed relationships between fruit varieties and wine (Kickenweiz and Regner 2002; Holler et al., 2012). It is an extremely robust method with high discriminatory power. Moreover, this method is independent of environmental influences, in contrast to pomological analyses where the outcome can potentially be influenced by climatic and growth factors. Furthermore, genetic fingerprinting is less time-consuming than classical pomological verification.

Microsatellites are simple, in most cases non-coding sequence repetitions, so-called Simple Sequence Repeats (SSR). These usually consist of 2 to 7 base pairs repeated up to fifty times. The number of repetitions is variable for different individuals so that a good distinction is possible. In fingerprint analysis, it is common to use a larger number of markers (at least 6) to be able to compare several loci, since the presence of the same alleles in different cultivars at one locus is possible (Guilford et al., 1997; Gianfranceschi et al., 1998). In apple, several microsatellite markers have been developed and published (Guilford et al., 1997; Gianfranceschi et al., 1998; Hokanson et al., 1998; Liebhard et al., 2002). Earlier studies are not, or only in parts, comparable due to the use of different markers (Kickenweiz and Regner, 2002; Galli et al., 2005; Pereira-Lorenzo et al., 2007). Based on this fact, the ECPGR *Malus/Pyrus* working group decided to publish a recommendation for a standard set of markers to achieve comparability of different European collections (Evans et al., 2007).

In this project, the gene bank "Kierling" of the HBLA and BA Klosterneuburg was genetically examined. It holds international dessert, cider and local Austrian apple cultivars. Most of these varieties are historic to old ones, except 'Gloster', which is a recent cultivar according to the classification of Baric et al. (2020). This orchard was planted in 1997 as part of the germplasm collection of the Federal College and Research Center of Viticulture and Pomology in Klosterneuburg (Austria), with the aim to save the most relevant cultivars for Austria at that time. Some of the cultivars were taken over from an older orchard located at the same place, which holds trees that were planted in the years 1897 to 1974.

The aim of the current study was to confirm the varietal identity of the maintained trees and to identify potentially mislabelled varieties. Moreover, the work was carried out to ensure a correct allocation of each tree in the planting plan because this is the only way to maintain the desired varieties. Trees which are mislabelled

and therefore possibly multiple and not only twice planted can then be discarded and replaced with other relevant local cultivars without increasing the cost and personal resources for gene bank management. To achieve this aim a reference database had to be created for comparison with SSR profiles from different international institutions.

Material and Methods

Plant material

A total of 208 mostly old and historic apple cultivars were analyzed. The young leaves were collected in the apple orchard "Kierling", which is situated in Klosterneuburg Kierling at the edge of the Wienerwald (48°18'17.2"N, 16°15'09.2"E), 253 m above sea level. The varieties are grafted on seedling rootstocks and raised as half-standard. Per tree three young leaves were collected and leaf discs of each accession were stored at -80 °C until DNA isolation.
The reference leaf samples ('Michelin', *Malus floribunda* #821, 'Delicious', *Malus robusta* 5, M9 (Pajam2), 'Prima', 'Fiesta', 'Boiken', 'Durello di Forlì', 'Kaiser Alexander', 'Kronprinz Rudolf', 'Red Jonathan' and 'Goldparmäne') were obtained from INRAE (France) and the Laimburg Research Centre (Italy).

SSRAnalysis

Genomic DNA was isolated using a rapid extraction method for small leaf samples according to the protocol developed by Bertsch et al. (2006). Fragment length analysis was performed with 28 primers in seven multiplex reactions (Table 1). The markers CH01f02, CH01f03b, CH01h01, CH01h10, CH02c06, CH02c09, CH02c11, CH02d08, CH03d07, CH04c07, CH04e05, CH05f06 (Liebhard et al., 2002), CH-Vf1 (Vinatzer et al., 2004), GD12, GD147 (Hokanson et al., 1998) and Hi02c07 (Silfverberg-Dilworth et al., 2006) are those which are recommended by the ECPGR *Malus/Pyrus* working group (Urrestarazu et al., 2016). For better comparison with the database from the Laimburg Research Centre the following markers were applied: CH01c06, CH01d08, CH01f07a, Ch2b10, CH02c02a, Ch02d12, CH02h11a, CH03a04 (Liebhard et al., 2002) and COL (Gianfranceschi et al., 1998) according to the

publication of Baric et al. (2009). Additionally, markers CH01b07, Ch01b11 and CH04c06 (Liebhard et al., 2002) were selected to complete the seventh multiplex approach. As the markers were combined for multiplex PCR reactions, the forward primers were labelled with four fluorescent dyes (Yakima Yellow, Atto550, Atto565 und FAM). The 11 µl PCR reactions consisted of 3 µl template DNA, 2x KAPA2G Fast Multiplex Master Mix (KAPABIOSYSTEMS, Cape Town, South Africa) and between 0,045 µM and 0,18 µM of each primer; exact concentrations are listed in Table 1. For multiplex (MP) 1, MP2, MP3, MP4 and MP7 PCR reactions were run under the following conditions: 5min at 95°C, 4 cycles with 30s at 94°C, 45s at 60°C with -1°C per cycle, 45s at 72°C, 34 cycles 15s at 94°C, 45s at 57°C, 45s at 72°C and 15min final extension at 72°C; for MP5 and MP6 annealing temperature was changed to 57.5°C. The resulting SSR amplification products were analyzed on an ABI 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by Eurofins (Ebersberg, Germany). Fragment length analysis was carried out with the Peak Scanner™ Software (Applied Biosystems, Foster City, CA, USA). At least six of the 14 reference cultivars were used as control profiles.

Table 1: List of microsatellite loci used in this study with the corresponding multiplex and concentration; genetic description of the 64 unique diploid cultivars (Na = number of alleles; Ne = number of effective alleles; I = information index; Ho = observed heterozygosity; He and uHe = expected and unbiased expected heterozygosity; F = fixation index; PIC = polymorphic information content; $F_{(Null)}$ = estimated frequency of null alleles)

Locus	PCR Multiplex	Primer concentration (μM)	Na	Ne	I	Ho	He	uHe	F	PIC	$F_{(Null)}$
CH02c06	MP1	0.18	16	8.23	2.35	0.95	0.88	0.89	-0.08	0.87	-0.042
CH01h10	MP1	0.18	13	2.89	1.56	0.77	0.65	0.66	-0.17	0.63	-0.098
CH01f03b	MP1	0.045	12	5.34	1.89	0.80	0.81	0.82	0.02	0.79	0.008
CH01h01	MP1	0.045	16	7.85	2.27	0.91	0.87	0.88	-0.04	0.86	-0.020
CH-Vf1	MP2	0.09	13	3.58	1.68	0.77	0.72	0.73	-0.06	0.69	-0.033
CH05f06	MP2	0.09	9	5.62	1.90	0.92	0.82	0.83	-0.12	0.80	-0.062
CH04e05	MP4	0.09	13	4.62	1.90	0.84	0.78	0.79	-0.08	0.76	-0.044
CH02d08	MP3	0.09	18	8.84	2.42	0.86	0.89	0.90	0.03	0.88	0.017
Hi02c07	MP3	0.09	11	4.89	1.80	0.88	0.80	0.80	-0.10	0.77	-0.049
CH02c11	MP2	0.125	12	9.86	2.36	0.91	0.90	0.91	-0.01	0.89	-0.006
CH01f02	MP3	0.09	15	9.1	2.37	0.92	0.89	0.90	-0.04	0.88	-0.019
GD147	MP3	0.18	14	6.25	2.18	0.86	0.84	0.85	-0.02	0.83	-0.010
GD12	MP4	0.18	12	3.38	1.66	0.70	0.70	0.71	0.00	0.68	0.013
Ch03d07	MP2	0.125	15	6.82	2.19	0.90	0.85	0.86	-0.06	0.84	-0.035
CH04c07	MP4	0.18	17	6.45	2.28	0.88	0.84	0.85	-0.04	0.83	-0.019
CH02c09	MP4	0.09	10	7.03	2.05	0.92	0.86	0.87	-0.07	0.84	-0.037
CH01c06	MP5	0.045	12	5.15	1.90	0.81	0.81	0.81	-0.01	0.78	-0.004
CH01d08	MP7	0.18	13	4.67	1.86	0.78	0.79	0.79	0.01	0.76	0.001
CH02h11a	MP7	0.09	10	5.44	1.90	0.91	0.82	0.82	-0.11	0.80	-0.059
CH02b10	MP5	0.09	17	10.1	2.57	0.94	0.90	0.91	-0.04	0.89	-0.023
CH01f07a	MP5	0.09	12	6.64	2.11	0.86	0.85	0.86	-0.01	0.83	-0.008
CH02c02a	MP6	0.09	26	17.03	3.02	0.89	0.94	0.95	0.05	0.94	0.028
CH02d12	MP6	0.09	16	5.48	2.13	0.89	0.82	0.82	-0.09	0.80	-0.056
CH03a04	MP6	0.18	16	8.81	2.40	0.78	0.89	0.89	0.12	0.88	0.064
COL	MP6	0.09	10	4.25	1.65	0.78	0.76	0.77	-0.02	0.73	-0.009
CH01b07	MP5	0.125	11	4.51	1.78	0.61	0.78	0.78	0.22	0.75	0.121
Ch01b11	MP7	0.09	9	3.16	1.46	0.67	0.68	0.69	0.02	0.63	0.001
CH04c06	MP7	0.09	13	6.94	2.14	0.84	0.86	0.86	0.01	0.84	0.005
Mean			13.61	6.53	2.06	0.84	0.82	0.83	-0.02	0.80	
Total			381	183							

Statistical description of the genetic diversity

To assess the genetic diversity of the gene bank the data set was reduced to 64 unique diploid cultivars, triploids were excluded. The calculation was done with the software GenAlEx version 6.503 (Peakall and Smouse, 2012) for the following parameters: number of alleles (Na), number of effective alleles (Ne), Shannon`s information index (I), observed (Ho), expected (He) and unbiased expected (uHe) heterozygosity and fixation index (F). Additionally, the polymorphic information content (PIC) of markers and the esti-

mated frequency of null alleles (F_{null}) was calculated with the software CERVUS version 3.0.7 (Kalinowsky et al., 2007).

True-to-type investigation

It is a known fact that SSR data achieved by different laboratories need harmonization, as there are often allelic shifts due to different laboratory methods and different automatic sequencers used (Baric et al., 2008; Evans et al., 2007; Sutton et al., 2011; Testolin et al., 2019). That is the reason why for comparison and verification of the SSR profiles with other international databases correspondence tables were established. These correspondence tables were done for each

marker and each allele as developed by Caroline Denancé from INRAE and implemented in Urrestarazu et al. (2016). The same approach has been developed further for assigning the MUNQ (Malus UNiQue genotype) codes as described in Muranty et al. (2020) and the INRAE dataset (https://data.inrae.fr/dataset.xhtml?persistentId=doi:10.15454/HKGMAS; September, 13th, 2021). The MUNQ code data from these sources were used for evaluation of our data.

For the verification of the apple cultivars, the resulting SSR profiles were compared with the published data from the following studies,: Urrestarazu et al. (2016), Larsen et al. (2017) and Baric et al. (2020). Furthermore, data from the following institutions were used for comparison because of cooperation agreements: Federal Office for Agriculture FOAG (Switzerland), Julius Kühn Institut JKI Dresden (Germany) and FruitID, which includes the data of the National Fruit Collection (UK).

In addition, a dendrogram was generated with the software Past 3 (Hammer et al., 2001) for the identification and verification of duplicates and possible mislabelled accessions (data not shown). The distances were calculated by Euclidean distance and the phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA).

Results

Twenty-eight microsatellite markers were used for the genetic fingerprinting of the apple cultivars planted in the gene bank "Kierling". They all generated clear and consistent DNA profiles as expected. All loci showed three alleles for some genotypes. Most of these genotypes are known triploid cultivars, e. g. 'Galloway Pippin'. Cultivars with unknown ploidy levels, which showed more than three different alleles at four or more loci were assumed to be triploids. The complete fingerprinting data are presented in

Supplement S1

(https://www.weinobst.at/service/klosterneuburger-mitteilungen/archiv/jahresverzeichnis-2022.html)

Genetic diversity analysis of the 64 unique diploid genotypes (shown in table 1) showed that the number of alleles varied from 9 (CH01b11 and CH05f06) to 26 (CH02c02a) with a mean number of 13.6 alleles per locus. Ho values ranged from 0.61 to 0.95 with a mean value of 0.84 which was quite similar to the mean He value of 0.82 (range from 0.65 to 0.94). Marker CH01b07 yielded the greatest difference between Ho and He value, which is also reflected in the F-indexes with the greatest deviation from zero with 0.22. This marker exhibited also an estimated frequency of null allele $F_{Null}>0.1$, which implies that this locus is carrying null alleles. All used markers can be considered as highly informative and highly polymorphic as PIC values were greater than 0.5.

Table 2: Parentage analysis of 'Rumer Gravensteiner',
cultivar '7-007 unknown' and 'Rolling'

Table block 1

Cultivar	CH01c06	CH01d08	CH01f02	CH01f03b	CH01h01	CH01h10	CH02b10	CH02c06	CH02c09	CH02c11
Rumer Gravensteiner	154 / 162	240 / 254	173 / 193	162 / 174 / 0	122 / 137	100 / 104	143 / 155	220 / 256 / 0	235 / 241 / 0	227 / 231 / 0
Schmidberger Renette	156 / 162	240 / 254	173 / 191	141 / 174	122 / 0	0 / 104	127 / 155	220 / 236 / 0	235 / 257 / 0	227 / 237 / 0
Landsberger Renette	154 / 156	240 / 0	187 / 193	162 / 182	120 / 137	94 / 104	119 / 143	242 / 256 / 0	241 / 247 / 0	231 / 233 / 0
7-007 unknown 1	154 / 170	240 / 272	177 / 208	141 / 162 / 172	120 / 133	100 / 108	0 / 133	220 / 238 / 254	247 / 251 / 0	219 / 227 / 0
Bittenfelder Sämling	158 / 170	240 / 254	177 / 187	141 / 182	120 / 133	100 / 118	125 / 133	232 / 254 / 0	245 / 251 / 0	227 / 235 / 0
Rolling	156 / 162	254 / 272	187 / 208	141 / 174	122 / 124	94 / 100	131 / 155	220 / 246 / 0	235 / 247 / 0	223 / 233 / 0
Farum Æble	n.a. / n.a.	n.a. / n.a.	187 / 208 / 225	141 / 174	122 / 124	94 / 100	131 / n.a.	220 / 232 / 246	235 / 247 / 253	223 / 233 / 237

Table block 2

Cultivar	CH02d08	CH02h11a	CH03d07	CH04c07	CH04e05	CH05f06	GD12	GD 147	CH-Vf1	Hi02c07	CH01f07a
Rumer Gravensteiner	229 / 256 / 0	100 / 122	188 / 230	112 / 124 / 0	178 / 201 / 0	179 / 189 / 0	170 / 192	142 / 155	146 / 171 / 0	112 / 131	177 / 0
Schmidberger Renette	229 / 262 / 0	100 / 116	220 / 230	110 / 124 / 136	178 / 0 / 0	179 / 189	160 / 192	136 / 155	146 / 171 / 179	114 / 131	177 / 197
Landsberger Renette	221 / 258 / 0	114 / 122	188 / 228	112 / 124 / 0	178 / 201	179 / 181	160 / 170	142 / 144	146 / 0 / 0	112 / 114	177 / 0
7-007 unknown 1	213 / 217 / 0	116 / 122	190 / 210	108 / 110 / 0	205 / 213 / 219	179 / 185	164 / 192	142 / 149	146 / 179	106 / 114	193 / 195
Bittenfelder Sämling	217 / 227 / 0	98 / 116	190 / 208	108 / 136	178 / 213	177 / 179	172 / 192	129 / 142	148 / 179	114 / 116	193 / 0
Rolling	215 / 258 / 0	122 / 0	194 / 228	98 / 0	178 / 205 / 0	187 / 189 / 0	160 / 0	153 / 0	148 / 179 / 0	112 / 114	0 / 0
Farum Æble	215 / 229	n.a. / n.a.	194 / 228	98 / 124 / 138	178 / 205	185 / 187 / 189	160 / 0	136 / 153	n.a. / n.a. / n.a.	108 / 114	0 / 0

Table block 3

Cultivar	CH02c02a	CH02d12	CH03a04	CH04c06	COL	CH01b07	CH01b11
Rumer Gravensteiner	177 / 183	181 / 191	92 / 108	182 / 191	219 / 0	85 / 91	197 / 199
Schmidberger Renette	177 / 201	181 / 199	92 / 112	182 / 0	219 / 231	85 / 94	197 / 199
Landsberger Renette	177 / 183	177 / 191	108 / 120	186 / 191	219 / 0	91 / 0	197 / 199
7-007 unknown 1	155 / 165	181 / 185	98 / 106	178 / 182	231 / 239	85 / 91	197 / 199
Bittenfelder Sämling	165 / 167	181 / 0	92 / 98	178 / 0	219 / 231	85 / 91	197 / 199

True-to-type investigation led to the identification and verification of 91 different cultivars of the 208 examined accessions. This was done by comparison of the gained and harmonized SSR profiles (Supplemental Electronic Material S1; https://www.weinobst.at/service/klosterneuburger-mitteilungen/archiv/jahresverzeichnis-2022.html) with the international data sets in a newly established Microsoft Excel database (unpublished). Furthermore, the UPGMA clustering method was used to identify possible mislabelled or duplicate accessions. The combination of these two approaches was very useful, only three cultivars could not be matched with any genetic profile and one still has to be pomologically examined as it matches two different profiles.

For the unknown, unclear and some local varieties, a parentage analysis was done with the help of the Software DNA Explorer-P2P Malus v7.06 developed by Peter Laws from FruitID.com and with hierarchical cluster analysis performed with the Software Past 3 (data not shown). It was found that the Austrian cultivar 'Rumer Gravensteiner' is presumably a seedling of 'Landsberger Renette' and 'Schmidberger Renette'. For the cultivar 'Rolling' a possible relationship to the Danish cultivar 'Farum Æble' was found. The cultivar labelled 'unknown 1' seems to be related to 'Bittenfelder Sämling'. (Data shown in table 2.)

Discussion

The use of 28 genetic markers resulted in the creation of distinct fingerprinting profiles for each examined accession, except for the cultivars 'Belle de Boskoop' and 'Red Boskoop'. The reason is that the discrimination of clones or sports, these are somatic mutants, of a cultivar is a limit of microsatellite analysis (Mhelembe, 2015; Nybom and Lācis, 2021). Moreover, it was possible to compare these profiles with different international data sets. These data sets were primarily harmonized with the establishment of correspondence tables to enable direct comparison, as it is known that there are deviations between various laboratories due to different chemicals and sequencing equipment used (Frey et al., 2007; Sehic et al., 2013).

It was found that some of the accessions in the gene bank were not true-to-type, which is not unusual as it was shown that the use of genetic markers reveals more mislabelling than traditional pomological analysis (Nybom and Weising, 2010; Sehic et al., 2013; Mhelembe, 2015). Eight accessions were detected to be other cultivars than assumed, others were found to be incorrectly localized in the records. Also cultivars could be found that were not known to have been planted in this gene bank, for example 'Orbai alma' ('Apfel von Orba'), which is an old cultivar from Transylvania (Romania) (Votteler, 2005). This cultivar could be recognized because of accordance with a profile from the NFC. Another example is 'Fameuse' ('Amerikanischer Schneeapfel'), this accession was presumed to be probably a 'Gravensteiner' clone, but has not been pomologically examined and identified yet. The genetic profile matched profiles from the JKI, NFC, and CRA-W (Centre Wallon de Recherches Agronomiques), respectively. Two trees were assumed to be probably 'Roter Stettiner', but it could be shown that these are accessions of 'Baumann's Reinette'. The cultivar planted as 'Ilzer Rosenapfel' turned out to be 'De Grignon',or Contessa matching profiles from JKI and Laimburg Research Centre. According to the work of Bannier and Schuricht (2021) and personal correspondence is the correct name of this cultivar Beauty of Kent. A total of 19 accessions were revised. These results show the usefulness of genetic characterization as a helpful tool in gene bank management.

Overall 95 different cultivars from 208 analyzed accessions could be identified. Some of these cultivars are local ones, for example 'Remsen', which is an old cultivar for cider production. Another one is 'Rumer Gravensteiner', which is a cultivar mainly located in Tyrol. This cultivar has an uncertain history. It is presumed that it was found and named by Anton Falch (president of the regional organisation of fruit and horticultural associations Tyrol), who lived in Rum near Innsbruck. As described in the chapter results, this variety is not an offspring of 'Gravensteiner' but presumably a by-chance seedling of 'Landsberger Renette' and 'Schmidberger Renette'. This seems very plausible as both cultivars are old ones and not rarely found in Austria. Parentage analysis revealed also that the cultivar 'Rolling', which is an old Austrian cultivar, seems to be related to the Danish cultivar 'Farum Æble'. So, the use of

the database offers new insights into the relationships between Austrian and other European cultivars.

Nevertheless four of the 95 cultivars are still not identified or verified. The first (labelled 'unknown 1' in Supplement S1) seems to be related to 'Bittenfelder Sämling', but it does not match any other profile in the different data sets. The variety 'Remfield' (marked as 'unclear' in Supplement S1) is a cultivar, which could not be found in any literature yet and is not in accordance with any other genetic profile. The primary tree was planted 1890 in the orchard next to the gene bank and was 1997 propagated on a seedling rootstock in the new gene bank orchard. The third accession (labelled 'unknown 2' in Supplement S1) was planted as 'Roter Passamaner', which is a synonym for 'Danziger Kantapfel', but the profile does not match with this cultivar nor any other. The fourth cultivar was originally recorded as 'Rosset Apfel', which was found to be either 'Pomella verde brisca' (University Bozen match) or 'Carrara brusca' (NFC match). These four cultivars will have to be pomologically examined to unravel their identity. All in all, based on this work the orchard gene bank in Kierling was brought up to date and true-to-variety preservation can be ensured. The genetic profiles created in this study are stored in a database and can thus be used for future comparisons. Furthermore, a reference database with over 10 000 genetic profiles has been established. This database shall be enlarged further in the next few years through international cooperation. On the basis of this work, the second apple gene bank of the HBLA and BA Klosterneuburg at Haschhof (Klosterneuburg) will be subjected to a genetic examination in future.

Summing up the study proved that for good gene bank management it is necessary to examine the planted accessions not only by morphological criteria but also through genetic analyses. A constant application of both procedures will allow to maintain the desired cultivars for the future.

Literature

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