Original article

Apple varieties from Kazakhstan and their relation to foreign cultivars assessed with RosBREED 10K SNP array

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Summary

Apples are one of the most important fruit tree crops in Kazakhstan. However, the local varieties remain poorly studied relative to foreign cultivars. Here we compared 24 well-characterized of *Malus domestica* specimens and 21 *Malus sieversii* specimens from Kazakhstan in comparison with foreign collection. A total of 3,119 SNP markers from the RosBREED Apple 10k SNP array were selected for data comparison after quality control. The cultivars from Kazakhstan were found to be closely related to foreign domestic apple varieties and could be classified into two distinguishable clusters. 'Saltanat', 'Zailiyskoe', 'Kazakh Jubilee', 'Talgat', 'Rubin' (KZ), 'Aport' ('Aport Alexander') together with the German cultivar 'Geheimrat Dr. Oldenburg' form the most distinct group in both admixture and genetic distance analyses. Another distinct group included five cultivars related to 'Golden Delicious'. *Malus sieversii* samples from Kazakhstan and the New Zealand collection formed a separate mixed cluster. *Malus sieversii* from Kazakhstan form a distinct compact sub-cluster comprising mostly samples from the Institute of Plant Biology and Biotechnology. *Malus sieversii* samples from the New Zealand collection formed a more heterogeneous group compared to wild and domestic apple from Kazakhstan.

Keywords

Malus domestica, *Malus sieversii*, SNP genotyping, genetic diversity, apple breeding, local varieties

Significance of this study

What is already known on this subject?

- Selections of worldwide cultivars were used extensively in apple breeding in KazSSR, but details of their involvement are mostly lost nowadays.

What are the new findings?

- Studied varieties mostly retain their relation to their genetic predecessors – 'Golden Delicious' and, presumably, 'Landsberg Reinette'.

What is the expected impact on horticulture?

- Local cultivars are introduced as a potential intermediate pool of genetic resources from *Malus sieversii*.

"Almaty" itself and its variant "Alma-Ata" used in KazSSR are derived from the Kazakh word for apple: "alma". Since the late 19th and early 20th century, the Almaty region was known for unique apple varieties, especially 'Aport' and related cultivars. Unfortunately, due to the significant isolation of the Kazakhstan agriculture industry during the Soviet period, many advances made by apple breeders in Kazakhstan were lost. Most publications describing breeding results in Kazakhstan appeared in local journals and books that now have limited accessibility. Economic crises and shocks in the 1990s caused extensive damage to agriculture and agrarian science in Kazakhstan, as well as loss of previous advances in breeding and selection. For example, the most famous cultivar from Kazakhstan, 'Aport of Almaty', has essentially ceased to exist in its unique form; its current descendants have significantly lower quality and poor expression of characteristic traits of the cultivar (Isin, 2016).

Although molecular genetic and genomic approaches in addition to classical selection are prominent in modern agricultural research, there are no genetic descriptions available for apple varieties developed in Kazakhstan. Meanwhile, *M. sieversii* has attracted substantial attention worldwide as a wild ancestor of domestic apple and a promising source of potentially important traits such as resistance to pathogens and other stresses (Pereira-Lorenzo et al., 2018). Mutual influences between some wild populations of *M. sieversii* in Kazakhstan and local domestic varieties have been shown (Omasheva et al., 2017). Negative impacts of close proximity between wild populations and apple gardens on the genetic architecture of *M. sieversii* and its resistance to pathogens

Introduction

Apple (*Malus domestica* Borkh.) is among the most important fruit crops in the world. In addition to fresh fruit consumption, apples are widely used in the food industry and as a source of biologically active compounds and other secondary metabolites. Apple plays a prominent role in the agriculture in Kazakhstan. The Tian-Shan region is the natural habitat of wild Sievers apple (*Malus sieversii* (Ledeb.) M.Roem.) which is widely recognized as the ancestor of *M. domestica* and European wild apple species (Dzhangaliev, 2010; Richards et al., 2009). Conditions found in Zhungar Alatau, Trans-Ile Alatau (part of Tian-Shan mountain system) in the Almaty region of Kazakhstan are very favorable for growth of both wild apple populations and apple orchards. The toponym

TABLE 1. Sources of genotyping data on *Malus* species.

Species	Amount	Source	Reference
<i>M. domestica</i>	27	Kazakhstan	Vanderzande et al., 2019
	122	U.S.A.	
	3	New Zealand	
<i>M. sieversii</i>	21	Kazakhstan	Kumar et al., 2014
	15	New Zealand	

were discussed (Feurtey et al., 2020). On the other hand, domestic apple cultivars with genetic material introduced from *M. sieversii* could potentially be a representative of a partial genetic pool of wild apple, for that can be used in breeding efforts. In this respect, the genetic composition of local apple varieties in Kazakhstan is of interest not only on a regional scale, but also as a novel genetic source for international breeding practice.

The present study aims to provide new information about the relationship of selected apple cultivars in Kazakhstan with *M. sieversii* and clarify links with commonly known foreign varieties. SNP genotyping with the RosBREED 10K SNP array was performed on local apple varieties for the first time to provide a basis for the introduction and further development of marker-assisted selection practices in apple breeding in Kazakhstan.

Materials and methods

Material sampling and storage

A total of 17 *M. sieversii* samples were selected from the collection of apple leaf material at the Laboratory of Molecular Biology of the Institute of Plant Biology and Biotechnology, Almaty (IPBB). This collection was created in 2011–2015 in cooperation with Dr. Sergey Chekalin (Institute of Botany and Phytointroduction, Almaty, Kazakhstan). Samples were randomly selected from Chernoff river, Uryukty, Krutoe tract and Lepsy right bank populations (near Alakol lake) (Omasheva et al., 2014). Another four samples from *M. domestica* cultivars ('Saltanat', 'Ainur', 'Zarya Alatau', 'Zailiyskoe') were also selected. Samples of 'Golden Delicious' and 'Starkrimson' cultivars were used as reference samples. All samples were stored at -80°C before use.

21 samples of local *M. domestica* cultivars and four *M. sieversii* samples of unknown origin were collected from the apple collection in the garden at the Fruit and Vegetable Research Institute, Almaty (FVRI). Leaves were collected in September 2019, dehydrated using silica gel and stored in a dry place at room temperature before use.

DNA isolation and SNP genotyping

We used a classical CTAB protocol (Doyle and Doyle, 1990) with adjustments to sample volumes and research environment to extract DNA from 0.1 g and 0.05 g of frozen and dried leaves, respectively. Low temperature conditions were maintained during processing. Quality check and quantification of the resulting DNA samples were conducted using NanoDrop3000 spectrophotometer (Thermo Scientific, U.S.A.) and by electrophoresis on 1% agarose gel.

Genotyping was conducted on the Illumina iScan system using the RosBREED Apple 10k SNP array (Chagné et al., 2012)¹ according to the manufacturer's protocol. Genotype calling and quality control were performed using the Genotyping module of GenomeStudio v. 2.0.4 software (Illumina, U.S.A.). Markers with call rate lower than 0.8, 50% GeneCall score lower than 0.5 or those that significantly deviated from the expected allele clustering pattern, revealed by manual examination of clustering plots, were excluded from further analysis. Data was exported in PLINK format (.ped + .map) using PLINK Input Report Plug-in v. 2.1.4 for GenomeStudio.

Data analysis

Data processing was conducted using PLINK1.9 (Purcell et al., 2007) and R 3.6.3 (R Core Team, 2019). Additional SNP data related to *M. domestica* and *M. sieversii* diversity that were obtained using RosBREED Apple 8K SNP panel (Kumar et al., 2014; Vanderzande et al., 2019) were kindly provided by Dr. David Chagné (New Zealand). These external datasets were loaded into R and transformed to fit the PLINK data input format before selected individuals were merged with our data. The merged dataset included *M. sieversii* samples from the New Zealand collection and *M. domestica* samples from the U.S.A. collection having known cultivar identity (Table 1). Only SNPs that were consistent across all datasets with overall call rate 0.95 or higher were retained for analysis.

Principal component analysis (PCA) was performed using the 'ade4' package (Jombart and Ahmed, 2011) and visualized using 'ggplot2' (Wickham, 2016). A matrix of genetic distances was calculated used to create a neighbor-joining tree using the 'ape' package (Paradis et al., 2004) visualization using FigTree software (Rambaut, 2018). Genetic structure analysis was performed with ADMIXTURE software (Alexander and Lange, 2011) and visualized by CLUMPAK (Kopelman et al., 2015).

Results

After filtering of genotyping data, 4,432 SNPs were selected to describe apple genotypes from Kazakhstan. Two samples, one *M. domestica* and one *M. sieversii*, had shown no signal and were excluded, leaving a total 46 samples of apples from Kazakhstan. After merging with two external datasets, 3,119 markers that were consistent across all samples with a missing value rate ≤ 0.05 were chosen for analysis. The post-merge analysis revealed that no missing values were present in the final dataset, indicating that all loci having incomplete total genotypes had call rate < 0.95 and thus were discarded by the selected filtering threshold.

¹ Since the original development, the RosBREED Apple SNP array was being provided in three different editions (8K, 9K, 10K), which share original SNP set with minor extensions. As confirmed by Dr. Chagné in a personal communication, the cited paper on development of 8K SNP array for apple is also a valid reference for 10K array used in the present study, and the data is mostly inter-compatible between editions.

FIGURE 1. Principal component analysis (PCA) of apple genotypes from Kazakhstan in comparison with foreign collections.

FIGURE 2. ADMIXTURE analysis of apple genotypes from Kazakhstan in comparison with foreign collections.

We compared genotyping data for 25 domestic apple samples (23 local cultivars from FVRI and IPBB collections; two ‘Golden Delicious’ and ‘Starkrimson’ samples from the collection of the Institute of Plant Biology and Biotechnology, were used as control samples) with 21 *Malus sieversii* samples from the IPBB and FVRI collection. To place our specimens into a wider genetic context, we used external data provided by RosBREED project participants that included domestic apple cultivars from the U.S.A. collection having known identity (Vanderzande et al.) and Sievers apple from the New Zealand collection (Kumar et al.). Additionally, ‘Golden Delicious’ and ‘Granny Smith’ cultivars were selected from the New Zealand dataset to check concordance of genotypes between our and U.S.A. datasets (‘Golden Delicious’) and between U.S.A. and New Zealand datasets (‘Granny Smith’). A total of 99.8% of markers were concordant between ‘Golden Delicious’ samples from our collection and from U.S.A., 99.8% of markers were concordant between the two ‘Granny Smith’ samples present in both datasets from U.S.A. and New Zealand. Confirmed control samples (‘Golden Delicious’ in our data and U.S.A. dataset; ‘Granny Smith’ in U.S.A. and New Zealand datasets) had sufficiently high rates

of matching SNPs to justify pairwise data comparison between these datasets. Such pairs form indirect links between all three datasets, and thus we considered all data to be compatible and suitable for merging and further analysis.

Principal component analysis (PCA) (Figure 1) revealed four overlapping clusters corresponding to four groups according data sources and apple species. The first principal component (PC1) explaining 15.68% of total variation differentiates *M. domestica* and *M. sieversii*, while the second (PC2) with 6.4% shows high dispersion within both species (left panel). The cultivars from Kazakhstan (blue) are closely related to foreign domestic apple varieties (purple), but form distinguishable sub-clusters. The third component (PC3) explains 4.5% of total variance and shows the distinction of most Kazakh samples from foreign cultivars (right panel). *M. sieversii* formed a distinct, compact sub-cluster consisting mostly of samples from the Institute of Plant Biology and Biotechnology (green). *M. sieversii* samples from the New Zealand collection form a more heterogeneous group between wild and domestic apple from Kazakhstan (yellow).

ADMIXTURE analysis was applied for potential cluster numbers (K) from 2 to 20 to discover the genetic structure

FIGURE 3. Neighbor joining tree of genetic distance of apple genotypes from Kazakhstan and foreign collections: K: data by Kumar et al.; V: data by Vanderzande et al.; IPBB: plant material from the Institute of Plant Biology and Biotechnology, Almaty; FVRI: plant material from the Fruit and Vegetable Research Institute, Almaty.

of Kazakh apple cultivars and Sievers apple (Figure 2; see also Supplemental Figure S1 for all original images). *M. sieversii* forms a distinct group across all *K*s, and two sub-groups can be observed that presumably include pure Sievers apple specimens (orange) and mixed genotypes with *M. domestica*. Most of the mixed individuals originated from the New Zealand collection. Comparing domestic apple varieties from Kazakhstan with foreign cultivars, the presence of specific genetic pool persistent across all *K*s (shown as dark green) can be observed. The German cultivar 'Geheimrat Dr. Oldenburg' displays the highest similarity with this group outside the set of Kazakh varieties (see Supplemental Figure S1 for details). Some cultivars (e.g., 'Ainur', 'Tulpan', 'Makpal') show a well expressed admixture with 'Golden Delicious'. As was expected by their diversity, the group of foreign cultivars generally shows high heterogeneity, particularly in comparison with the Kazakh group. As a result, the ADMIXTURE cross-validation test did not converge to the true *K* value across all 20 *K*'s. Nevertheless, the clustering pattern of *M. sieversii* and Kazakh cultivars was consistent across all *K* values, which supports high stability of the revealed groups.

A neighbor joining tree displays Kazakh apple cultivars as three separate groups (Figure 3). The first group is in proximity to 'Golden Delicious' and includes varieties known to be descendants of this cultivar in the breeding process. This group includes varieties such as 'Ainur', 'Voschod' and 'Danalykt'. The second group includes 'Saltanat', 'Zailiyskoe', 'Kazakh Jubilee', etc., as well as 'Geheimrat Dr. Oldenburg'. This sub-cluster is closest to the cluster of *M. sieversii*. The third group includes three cultivars ('Anuar', 'Aizhan', 'Zarya Alatau') and belongs to the cluster of diverse foreign varieties as a sub-cluster. *M. sieversii* samples from the Kazakhstan and New Zealand collections form a separate mixed cluster. A specimen from the U.S.A. collection designated as 'Russian seedling' is also in this cluster and so can be attributed as a *M. sieversii* sample with high certainty.

Discussion

The most significant efforts in apple breeding in Kazakhstan were made during the Soviet period. Due to the economic importance of the development of new productive crops, the government supported extensive breeding studies. Apple breeders were not only using traditional local and Russian varieties as a genetic basis, but also introducing foreign cultivars having known desirable qualities. Such breeding practices were continued also in independent Kazakhstan. As such, the existing Kazakh apple cultivars represent an original mix of genetic pools from the old local varieties and famous cultivars from other countries around the world. Another genetic source is wild apple *M. sieversii*, the historical progenitor of domestic apple. Gene flow between two apple species can occur both involuntary by cross-pollination between trees in orchards which are close to wild populations and artificially as part of breeding efforts. However, the extent of this admixture is unclear as there are only limited studies on Kazakh apple cultivars (Omasheva et al., 2018). Wide use of free pollination in breeding practice in the country also makes genetic relationships between cultivars less clear.

Based on the results of the present study, we can draw conclusions about the genetic compositions of our local cultivars. 'Saltanat', 'Zailiyskoe', 'Kazakh Jubilee', 'Talgar', 'Rubin' (KZ), 'Aport' ('Aport Alexander') form the most distinct group along with the German cultivar 'Geheimrat Dr. Oldenburg' in both admixture and genetic distance analyses. Two assumptions about the composition of this cluster can be made. The

first assumption is built upon traditional attribution of apple cultivars. 'Saltanat', 'Zailiyskoe' and 'Kazakh Jubilee' are known to have originated from the 'Burchardt's Reinette' (Crimean cultivar, obtained in 1863 from an unknown source) (National Fruit Collection, 2019a). 'Geheimrat Dr. Oldenburg' is considered to be a cross between 'Baumann's Reinette' and 'Minister von Hammerstein' cultivars (National Fruit Collection, 2019b). The latter descended from the 'Landsberg Reinette' cultivar (National Fruit Collection, 2019c). 'Landsberg Reinette' and 'Burchardt's Reinette' were previously shown to have high genetic similarity (Omasheva et al., 2018), and thus 'Burchardt's Reinette' could be a direct descendant of 'Landsberg Reinette'. This described group could also represent a family of varieties related to 'Landsberg Reinette'. Although the origin of the 'Aport' cultivar is unclear, it may also be related to 'Landsberg Reinette' based on its existence and its relationship to 'Talgar' in the described group.

The second assumption is based on insights from a recent study of the pedigree of a wide range of old apple varieties using a high density SNP array (Muranty et al., 2020). That study showed that the 'Dr. Oldenburg' cultivar could have arisen from a cross between 'Alexander' (syn. 'Aport' or 'Aport Alexander' (National Fruit Collection, 2019d)) and 'Reinette Ananas' instead of being descended from 'Baumann's Reinette'. As 'Aport' lies at the base of the discussed cluster, this group of varieties is likely descended from this cultivar. Such a possibility is supported by the positions of 'Dr. Oldenburg' and 'Talgar' in the neighbor joining tree. The presence of descendants of 'Burchardt's Reinette', or 'Landsberg Reinette', as discussed above, in the same cluster raises the question of the relationship between 'Landsberg Reinette' and 'Aport (Alexander)'. There is no documented data on the progenitors of these two old varieties, so this relationship requires additional studies to clarify the origin of 'Dr. Oldenburg' and the Kazakh cultivars studied here.

This group is also a part of a larger cluster including Sievers apple samples (Figure 3) and indicates a significant influence of *M. sieversii* on the origin of their presumed common progenitor cultivar, 'Aport' or 'Landsberg Reinette'. Based on history of apple breeding in Kazakhstan, a reverse influence is also possible wherein spontaneous gene flow via free pollination between apple orchards and wild apple populations occurred. Results of admixture analysis indeed indicated the influence of domestic apple on some *M. sieversii* specimens and so confirm the impact of gene flow on wild apple genotypes. Four samples of *M. sieversii* (Urzhar 358, Chilik 416, 417, Lepsy 467) showed significant admixture attributed to local cultivars of the 'Aport' cluster. Moreover, samples that were propagated *ex situ* (data by Kumar et al. (2014) and material from FVRI) show further admixture with *M. domestica* during cultivation, and the presumable sources of this are more diverse.

The other distinct group include cultivars known to be related with 'Golden Delicious': 'Ainur', 'Voschod', 'Danalyk', 'Kuanys', 'Makpal'. These varieties form a separate sub-cluster inside the cluster of 'Golden Delicious' and its relatives. Groups of these varieties are distinct from the foreign cultivars based on 'Golden Delicious' because of involvement of other local cultivars in the breeding process. Admixture analysis reveals that the 'Aport' group had the largest impact as the most common cultivars in apple breeding and production in Kazakhstan. The remaining three cultivars, 'Aizhan', 'Anuar' and 'Zarya Alatau', have mixed origin from foreign cultivars and are included in one of the large clusters along with 'Granny Smith', 'Gala' and other varieties.

Genetic characterization of local varieties and wild apple is important for finding and developing new genotypes having economically important traits. Kazakhstan has a broad genetic pool for marker-assisted selection of apples that is practically unknown among the international research community and only partially reflected in the present study. Here we showed that the apple collection of Kazakhstan contains varieties having unique admixture between cultivars that are well known across the world and original local specimens, including the wild Sievers apple. The group of varieties related to 'Golden Delicious', the most popular and well characterized apple cultivar in the world, were shown to share significant genetic similarity to their progenitor. Thus, these varieties can potentially form a good basis for apple selection, compatible with existing breeding practices for 'Golden Delicious'. The 'Aport' group can be a valuable source of new genetic material from *M. sieversii*, because of their genetic proximity.

Together, our results show that the local apple varieties of Kazakhstan are of potential interest for apple breeding as an intermediate between *M. domestica* and *M. sieversii*. Not only do these varieties represent promising, but understudied, genotypes, they also play a role as a breeding platform compatible with both species, for further work on introgression of potentially important genes from wild to domestic apple.

Conclusion

In this study we obtained, for the first time, SNP genotyping data for multiple apple varieties in Kazakhstan, and compared this data with a collection of well-known cultivars to reveal two groups related to 'Golden Delicious' and 'Aport' and/or 'Landsberg Reinette'. 'Aport' and its relatives are close to *M. sieversii* specimens and as such are interesting as an intermediate form between the two species. Together with results from previous studies, our findings show relationships between these cultivars and form the foundation for future studies to examine the relationship between 'Aport' (Alexander) and 'Landsberg Reinette' cultivars.

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The authors would like to dedicate the present paper to the memory of Dr. Sergey Chekalin (1954–2019). Research and conservation of plant biodiversity in Kazakhstan was the work of his life. The demise of Dr. Chekalin is a huge loss for plant biology and ecology in Kazakhstan. *Malus sieversii* was an object of his particular interests, and the collection of wild apple samples in the IPBB would not exist without his efforts.

Conflict of interest

The authors have no conflicts of interest to declare.

Availability of data

Genotyping data on apple varieties from Kazakhstan can be accessed from the Open Science Foundation website using following link: https://osf.io/hwrtx/?view_only=c5b1fdbb-9ca64aeca22f95cc97682cb5.

References

- Alexander, D.H., and Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics* 12, 246. <https://doi.org/10.1186/1471-2105-12-246>.
- Chagné, D., Crowhurst, R.N., Troggio, M., Davey, M.W., Gilmore, B., Lawley, C., Vanderzande, S., Hellens, R.P., Kumar, S., Cestaro, A., et al. (2012). Genome-wide SNP detection, validation, and development of an 8K SNP array for apple. *PLoS ONE* 7, e31745. <https://doi.org/10.1371/journal.pone.0031745>.
- Doyle, J.J., and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus (Madison)* 12, 39–40.
- Dzhangaliev, A.D. (2010). The wild apple tree of Kazakhstan. In *Horticultural Reviews* (Oxford, U.K.: John Wiley & Sons, Inc.), p. 63–303. <https://doi.org/10.1002/9780470650868.ch2>.
- Feurtey, A., Guitton, E., De Gracia Coquerel, M., Duvaux, L., Shiller, J., Bellanger, M., Expert, P., Sannier, M., Caffier, V., Giraud, T., et al. (2020). Threat to Asian wild apple trees posed by gene flow from domesticated apple trees and their "pestified" pathogens. *Mol. Ecol.* 29, 4925–4941. <https://doi.org/10.1111/mec.15677>.
- Isin, M.M. (2016). The degeneration (degradation) of the Aport apple variety due to non-transmissible plant disease and its control methods. *Plant Prot. News* 3, 78–79.
- Jombart, T., and Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics* 27, 3070–3071. <https://doi.org/10.1093/bioinformatics/btr521>.
- Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., and Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* 15, 1179–1191. <https://doi.org/10.1111/1755-0998.12387>.
- Kumar, S., Raulier, P., Chagné, D., and Whitworth, C. (2014). Molecular-level and trait-level differentiation between the cultivated apple (*Malus domestica* Borkh.) and its main progenitor *Malus sieversii*. *Plant Genet. Resour. Charact. Util.* 12, 330–340. <https://doi.org/10.1017/S1479262114000136>.
- Muranty, H., Denancé, C., Feugey, L., Crépin, J.L., Barbier, Y., Tartarini, S., Ordidge, M., Troggio, M., Lateur, M., Nybom, H., et al. (2020). Using whole-genome SNP data to reconstruct a large multi-generation pedigree in apple germplasm. *BMC Plant Biol.* 20, 1–18. <https://doi.org/10.1186/s12870-019-2171-6>.
- National Fruit Collection (2019a). Burchardt's Reinette. <http://www.nationalfruitcollection.org.uk/full2.php?id=829> (accessed May 18, 2021).
- National Fruit Collection (2019b). Geheimrat Dr. Oldenburg. <http://www.nationalfruitcollection.org.uk/full2.php?id=2219> (accessed May 18, 2021).
- National Fruit Collection (2019c). Minister von Hammerstein. <http://www.nationalfruitcollection.org.uk/full2.php?id=3990> (accessed May 18, 2021).
- National Fruit Collection (2019d). Alexander. <http://www.nationalfruitcollection.org.uk/full2.php?id=99> (accessed May 18, 2021).
- Omasheva, M.E., Pozharsky, A.S., Smailov, B.B., Ryabushkina, N.A., and Galiakparov, N.N. (2018). Genetic diversity of apple cultivars growing in Kazakhstan. *Russ. J. Genet.* 54(2), 176–187. <https://doi.org/10.1134/S1022795418020138>.
- Omasheva, M.Y., Chekalin, S.V., Ryabushkina, N.A., and Galiakparov, N.N. (2014). Evaluation of the molecular genetic diversity of *Malus sieversii* populations in the Dzungarian Alatau and Tarbagatai mountains. *Biotechnol. Theory Pract.* 1, 26–34. <https://doi.org/10.11134/btp.1.2015.3>.

Omasheva, M.Y., Flachowsky, H., Ryabushkina, N.A., Pozharskiy, A.S., Galiakparov, N.N., and Hanke, M.-V. (2017). To what extent do wild apples in Kazakhstan retain their genetic integrity? *Tree Genet. Genomes* 13, art. 52. <https://doi.org/10.1007/s11295-017-1134-z>.

Paradis, E., Claude, J., and Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290. <https://doi.org/10.1093/bioinformatics/btg412>.

Pereira-Lorenzo, S., Fischer, M., Ramos-Cabrer, A.M., and Castro, I. (2018). Apple (*Malus* spp.) breeding: Present and future. In *Advances in Plant Breeding Strategies: Fruits* (Springer Intl. Publishing), p. 3–29. https://doi.org/10.1007/978-3-319-91944-7_1.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., et al. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575. <https://doi.org/10.1086/519795>.

R Core Team (2019). R: A Language and Environment for Statistical Computing. <https://www.r-project.org>.

Rambaut, A. (2018). FigTree. <http://tree.bio.ed.ac.uk/software/figtree>.

Richards, C.M., Volk, G.M., Reilley, A.A., Henk, A.D., Lockwood, D.R., Reeves, P.A., and Forsline, P.L. (2009). Genetic diversity and population structure in *Malus sieversii*, A wild progenitor species of domesticated apple. *Tree Genet. Genomes* 5, 339–347. <https://doi.org/10.1007/s11295-008-0190-9>.

Vanderzande, S., Howard, N.P., Cai, L., Da Silva Linge, C., Antanaviciute, L., Bink, M.C.A.M., Kruisselbrink, J.W., Bassil, N., Gasic, K., Iezzoni, A., et al. (2019). High-quality, genome-wide SNP genotypic data for pedigreed germplasm of the diploid outbreeding species apple, peach, and sweet cherry through a common workflow. *PLoS ONE* 14(6), e0210928. <https://doi.org/10.1371/journal.pone.0210928>.

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis (New York, U.S.A.: Springer-Verlag). https://doi.org/10.1007/978-3-319-24277-4_9.

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SUPPLEMENTAL INFORMATION – FIGURE S1

For Supplemental Information see www.ishs.org/eJHS