USE OF SSR MOLECULAR MARKERS FOR SWEET CHERRY (*Prunus avium* L.) SPECIES

FOLOSIREA MARKERILOR MOLECULARI TIP SSR LA CIREŞ (*Prunus avium* L.)

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Abstract. *Sweet cherry (Prunus avium L.), belonging to Rosaceae family, is a very popular fruit tree species and it is cultivated worldwide. Recent advances in molecular biology research have brought to light new data, including the identification of molecular markers related to traits of interest. Microsatellite (simple sequence repeats or SSR) markers have been used extensively for a variety of purposes, such as the study of genetic variability, DNA fingerprinting, molecular identification, etc. The present review proposes to bring together data referring to the use of SSRs for the sweet cherry species.*
Key words: cherry, *Prunus avium*, SSR, microsatellite

Rezumat. *Cireşul (Prunus avium L.), aparţinând familiei Rosaceae, este o specie foarte populară de pomi fructiferi şi este cultivată în întreaga lume. Progresele recente în cercetare în domeniul biologiei moleculare au adus la lumină date noi, inclusiv identificarea de markeri moleculari asociaţi unor trăsături de interes. Microsateliţii (secvenţe simple repetate sau SSR) au fost folosiţi pe scară largă pentru o varietate de scopuri, cum ar fi studiul variabilităţii genetice, amprentarea ADN, identificarea moleculară etc. Studiul de faţă propune reunirea datelor referitoare la utilizarea markerilor tip SSR la cireş.*
Cuvinte cheie: cireş, *Punus avium*, SSR, microsatelit

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a popular fruit tree, believed to have originated around the Caspian and Black Seas (Dirlewanger *et al.*, 2007). It is a diploid species (2n=2x=16) with a small (338 Mb) haploid genome (Dirlewanger *et al.*, 2009). Sweet cherry is cultivated throughout Europe, as well as worldwide, in temperate climate zones, and it is characterized by a moderate need for winter low temperatures and resistance to high temperatures during summer (Dondini *et al.*, 2018). In Romania, sweet cherry is a traditional fruit crop, and new sweet cherry varieties are continuously developed and studied (Asănică *et al.*, 2012; Sîrbu *et al.*, 2015; Iurea *et al.*, 2016; Ungureanu *et al.*, 2018; Zlati *et al.*, 2018).

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Current objectives in sweet cherry breeding are yield, fruit size, fruit firmness, crunchiness, juiciness, and taste. Just as important are also the tolerance to rain-induced fruit cracking, resistance to winter frost, tolerance/resistance to bacterial canker, caused by *Pseudomonas* spp., and blossom blight and brown rot, caused by *Monilinia* spp. (Garcia *et al.*, 2019). Classical breeding of sweet cherry has been greatly limited due to the long generation time and the large size of the trees. Therefore, the recent advances in molecular biology research, including the identification of molecular markers linked to the traits of interest, offer new tools to plant breeders, decreasing the time needed to develop novel varieties (Dirlewanger *et al.*, 2009).

Among the molecular markers identified to date, microsatellites or SSRs (*Simple Sequence Repeats*). SSR molecular markers have been discovered and developed in the last decades of the 20th century. They are single locus markers, are highly polymorphic, and are co-dominant, so they can distinguish heterozygous from homozygous individuals. Their reliability and high level of polymorphism let to their use to a variety of purposes, such as genetic variability studies, DNA fingerprinting, molecular identification, etc. (Ben-Ari and Lavi, 2012). The present review aims to summarize data related to the use of SSRs for the sweet cherry (*Prunus avium*) species.

GENETIC DIVERSITY STUDIES

High genetic variability is essential for breeding programs, especially in the context of growing climate changes (abiotic stress) and the increased susceptibility of present varieties to various pests and diseases (biotic stress), hence the need for development of novel varieties that can cope with these challenges (Bhandari *et al.*, 2017). Genetic variability assessment studies in sweet cherry populations/collections using SSR molecular markers have been performed worldwide.

In Romania, Berindean *et al.* (2013) studied the polymorphism between 36 sweet cherry accessions from I.C.D.P. Iași, using 15 primer pairs developed for peach, sweet cherry, tetraploid cherry, and sour cherry SSRs, and concluded that SSR primers are transferable across *Prunus* genus.

In Turkey, Aka Kaçar *et al.* (2005) genotyped 7 local varieties using 13 SSR markers (7 developed for sweet cherry and 6 developed for sour cherry and peach). For 2 SSR loci, B4C3 (peach) and GA65 (sour cherry) there was no amplification. PMS49 (sweet cherry) and pchpgms3 (sour cherry) proved to be monomorphic. The remaining loci were polymorphic, with an average of 4 alleles per locus. The highest polymorphism was observed for the PceGA34 locus, developed for sour cherry. Ercisli *et al.* (2011) analyzed 18 wild sweet cherry genotypes from northern part of the country, using 10 SSRs, out of which 6 were developed for plum, apricot and peach. All 10 molecular markers proved to be polymorphic (between 3 and 7 alleles amplified), with the highest number of alleles amplified in the PS12A02 locus (developed for sweet cherry), followed by

markers Pchgms1 and UDP96001, both developed for peach, with 6 alleles amplified, proving the transferability of SSR markers across *Prunus* genus. Turkoglu *et al.* (2012) also studied the genetic variability of wild cherry genotypes, selecting for their study 37 *Prunus avium*, 8 *Prunus cerasus* and 7 *Prunus mahaleb* accessions collected from the Ordu region. 12 SSR markers were chosen for this study, all of them proving to be polymorphic, amplifying between 6 (UDP96-019) and 12 (PS12A02) alleles. Őz *et al.* (2013) genotyped 24 local sweet cherry accessions using 9 SSR markers (5 from cherry, 3 from peach and 1 from plum). All markers proved to be polymorphic, amplifying between 5 (UCD-CH13 and UCD-CH21, developed for cherry, and CPSCT010, developed for plum) and 10 alleles (PS12A02, UCD-CH17, and UCD-CH31, developed for cherry).

In Greece, Ganopoulos *et al.* (2011) assessed 19 Greek traditional cultivars and two international sweet cherry cultivars using 15 SSR and 10 ISSR markers and used stepwise multiple regression analysis (MRA) to associate the molecular markers with phenotypical traits. They identified 6 SSR linked to harvest time, 4 SSRs linked to fruit polar diameter, and one SSR linked to fruit weight.

Guarino *et al.* (2010) screened 60 Campanian sweet cherry accessions near Eboli, Salerno, Italy, with 28 SSR markers, to assess genetic diversity within the collection. Only one of the markers studied (EMPA021) proved to be monomorphic. The dendrogram generated using the unweighted pair group method with arithmetic average (UPGMA) classified all Campanian varieties into one group, proving their autochthonous character.

Wünsch and Hormaza (2004) used 12 SSR markers to study the genetic variability within a group of 28 local varieties from Jerte Valley in Western Spain. Except for two cultivars, 'Pico Colorado' and 'Pico Colorado Cirino', all other cultivars produced unique SSR profiles. UPGMA cluster analysis revealed a clear separation between the old traditional cultivars and the most recent introduced cultivars.

Patzak *et al.* (2019) analyzed the genetic variability of 123 current, old, and local sweet cherry cultivars from the Research and Breeding Institute of Pomology in Holovousy, Czech Republic, using 19 SSR and 2 EST-SSR molecular markers. All markers proved to be polymorphic, and the number of alleles per locus varied between 2, for CN911135, and 9, for EMPaS006. The dendrogram constructed using unweighted Neighbor-Joining (NJ) clustering revealed 3 main clusters with 10 subgroups. Cultivars were grouped based on their geographical origin, such as Switzerland cultivars in cluster Ia, Germany cultivars in IIa and IIb, and Canada and USA cultivars in IIc and IId. Old Czech cultivars, however, were spread through the whole cluster, depending on their genetic origin.

The variability of sweet cherry cultivars from Latvia and Sweden was analyzed by Lacis *et al.* (2009) using only 3 SSR markers: PceGA25, PMS3, and PMS49. From Latvia were analyzed 58 accessions, whereas from Sweden were

analyzed 68 accessions. The markers were highly polymorphic and identified between 4 and 10 alleles for each locus, most probably due to the high number of accessions analyzed and the fact that the two collections contain diverse material with unknown origin.

In Lithuania, Stanys *et al*. (2012) analyzed 31 sweet cherry cultivars, 20 local traditional cultivars and 11 common cultivars of foreign origin, using 14 SSR markers in a multiplex approach. In the case on local varieties, the number of alleles for each locus varied between 2 and 11, whereas for the foreign cultivars the number of alleles varied from 2 to 8 for each locus. Also, in the study were identified 20 unique alleles for the Lithuanian cultivars, pointing to an adaptation to local stringent ecological conditions.

OTHER USES FOR SSR MOLECULAR MARKERS

Molecular identification of cultivars using SSR markers can be used to avoid synonyms and homonyms, and to accurately identify fruit trees clones at seedling stage. In the case of sweet cherry, Struss *et al.* (2003) used 15 SSR markers to fingerprint 15 sweet cherry varieties. They used as template in their study gDNA extracted from both leaves and fruits and proved that there were no differences between the cultivar fingerprints based on the tissue used for gDNA extraction. The usage of fruit as starting material is helpful when leaves are not available for analysis. This type of fingerprinting is valuable not only for monitoring cultivar identity, but also to protect plant breeder's rights and for quality control in the market. In Turkey, Turet-Sayar *et al.* (2012) used this technique to clarify the genetic identity of a registered cultivar, 0900Ziraat that was grown in various nurseries with different names due to morphological and pomological differences observed between trees. Following the analysis of 17 different trees from 8 nurseries, the authors concluded that there was no genetic variation between the individual trees studied and the phenotypic differences were due to local adaptations and variations in rootstocks. In China, Liang *et al.* (2018) used a set of 10 SSR markers to fingerprint 63 cherry cultivars, both varieties and rootstocks, succeeding to differentiate between cultivars that have very similar phenotypes, especially at seedling stage.

With the introduction of next generation sequencing (NGS), more and more genomic data becomes available for numerous cherry varieties, allowing for the development of novel DNA tests to be used in sweet cherry breeding. Fruit color is an important commercial trait for sweet cherry, so Sandefur *et al.* (2016) developed a DNA test, Pav-Rf-SSR, based on a single SSR marker, to routinely predict the fruit color. The test proved to differentiate individuals that would produce mahogany and blush fruits, and it is already used routinely in The Pacific Northwest Sweet Cherry Breeding Program.

CONCLUSIONS

1. Genetic variability of sweet cherry populations/collections using SSR molecular markers have been performed worldwide, helping to preserve the local varieties, and providing best cultivar combinations for sweet cherry breeders.

2. SSR markers may be used to accurately fingerprint sweet cherry accessions, allowing for accurate identification at the seedling stage or in case of phenotypic variation due to different location or rootstock variation.

3. SSR-based PCR tests linked to valuable commercial traits such as fruit color may be developed to differentiate in early growth stages individuals to be used in breeding programs.

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REFERENCES

1. Aka Kaçar Y., Lezzoni A.F., Çetiner S., 2005 - *Sweet cherry cultivar identification by using SSR markers.* Journal of Biological Sciences, 5(5), pp. 616-619.

2. Asănică A., Hoza D., Tudor V., Temocico G., 2012 - *Evaluation of some sweet cherry cultivars to winterfreeze in different areas of Romania.* Scientific Papers, Series B, Horticulture, pp. 23-28.

3. Ben-Ari G., Lavi U., 2012 - *Marker-assisted selection in plant breeding.* In Plant biotechnology and agriculture. Academic Press, pp. 163-184.

4. Berindean I.V., Tămaş E., Cardei E., Pamfil D., 2013 - *Optimization of PCR Conditions to Amplify SSR Markers in Sweet Cherry Cultivars.* Bulletin UASMV serie Agriculture, 70(1), pp. 201-207.

5. Bhandari H.R., Bhanu A.N., Srivastava K., Singh M.N., Shreya H.A., 2017 - *Assessment of genetic diversity in crop plants-an overview*. Advances in Plants & Agriculture Research, 7(3), pp. 279–286.

6. Dirlewanger E., Claverie J., Iezzoni A.F., Wünsch A., 2009 - *Sweet and sour cherries: linkage maps, QTL detection and marker assisted selection.* In Genetics and genomics of Rosaceae Ed. Springer, New York, NY, pp. 291-313.

7. Dirlewanger E., Cosson P., Tavaud M., Aranzana M., Poizat C., Zanetto A., Arus P., Laigret F., 2002 - *Development of microsatellite markers in peach [Prunus persica (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (Prunus avium L.).* Theoretical and Applied Genetics, 105(1), pp. 127-138.

8. Dondini L., Lugli S., Sansavini S., 2018 - *Cherry breeding: sweet cherry (Prunus avium L.) and sour cherry (Prunus cerasus L.).* In Advances in Plant Breeding Strategies: Fruits Ed. Springer, Cham, pp. 31-88.

9. Ercisli S., Agar G., Yildirim N., Duralija B., Vokurka A., Karlidag H., 2011 - *Genetic diversity in wild sweet cherries (Prunus avium) in Turkey revealed by SSR markers.* Genetics and Molecular Research, 10(2), pp. 1211-1219.

10. Ganopoulos I.V., Kazantzis K., Chatzicharisis I., Karayiannis I., Tsaftaris A.S., 2011 - *Genetic diversity, structure and fruit trait associations in Greek sweet cherry cultivars using microsatellite based (SSR/ISSR) and morpho-physiological markers.* Euphytica, 181(2), pp. 237-251.

11. Garcia J.Q., 2019 - *Cherry breeding in the world: current analysis and future perspectives.* Italus Hortus, 26(1), pp. 9-20.

12. Guarino C., Santoro S., De Simone L., Cipriani G., 2010 - *Molecular characterization of ancient Prunus avium L. germplasm using sweet cherry SSR markers.* The Journal of Horticultural Science and Biotechnology, 85(4), pp. 295-305.

13. Iurea E., Sîrbu S., Corneanu G., 2016 – *Coralis - a new sweet cherry cultivar for commercial plantations.* Lucrări Științifice, Universitatea de Științe Agricole Și Medicină Veterinară "Ion Ionescu de la Brad" Iași, Seria Horticultură, 59(2), pp. 213-218.

14. Liang C., Wan T., Xu S., Li B., Li X., Feng Y., Cai Y., 2018 - *Molecular identification and genetic analysis of cherry cultivars using capillary electrophoresis with fluorescence-labeled SSR markers*. 3 Biotech, 8(1), pp. 1-10.

15. Öz M.H., Vurgun H., Bakir M.E.L.İ.K.E., Buyuk I., Yuksel C., Unlu H.M., Cukadar K., Karadogan B., Kose O., Ergul A., 2013 - *Molecular analysis of East Anatolian traditional plum and cherry accessions using SSR markers.* Genetics and Molecular Research, 12(4), pp. 5310-5320.

16. Patzak J., Henychová A., Paprštein F., Sedlák J., 2019 - *Evaluation of genetic variability within sweet cherry (Prunus avium L.) genetic resources by molecular SSR markers.* Acta Scientiarum Polonorum-Hortorum Cultus, 18(3), pp. 157-165.

17. Sandefur P., Oraguzi N., Peace C., 2016 - *A DNA test for routine prediction in breeding of sweet cherry fruit color, Pav-R f-SSR*. Molecular breeding, 36(3), p. 33.

18. Sîrbu S., Iurea E., Corneanu M., 2015 - *Evaluation of sweet and sour cherry cultivars useful in genetic improvement.* Lucrări Științifice, Universitatea de Științe Agricole Și Medicină Veterinară" Ion Ionescu de la Brad" Iași, Seria Horticultură, 58(2), pp.105-110.

19. Stanys V., Baniulis D., Morkunaite-Haimi S., Siksnianiene J.B., Frercks B., Gelvonauskiene D., Stepulaitiene I., Staniene G., Siksnianas T., 2012 - *Characterising the genetic diversity of Lithuanian sweet cherry (Prunus avium L.) cultivars using SSR markers*. Scientia Horticulturae, 142, pp. 136-142.

20. Struss, D., Ahmad, R., Southwick, S.M. and Boritzki, M., 2003. *Analysis of sweet cherry (Prunus avium L.) cultivars using SSR and AFLP markers.* Journal of the American Society for Horticultural Science, 128(6), pp. 904-909.

21. Turet-Sayar M., Turkec A., Demir T., 2012 - *Identification of sweet cherry cultivars (Prunus avium L.) and analysis of their genetic relationship using microsatellite DNA fingerprinting.* Journal of Agricultural Science, 4(8), pp. 134.

22. Turkoglu Z., Bilgener S., Ercisli S., Yildirim N., 2013 - *Simple sequence repeat (SSR) analysis for assessment of genetic variability in wild cherry germplasm.* Journal of Applied Botany and Food Quality, 85(2), pp. 229-233.

23. Ungureanu I.V., Iurea E., Sîrbu S., Istrate M., 2018 - *Researches concerning the behaviour of cherry varieties with different rippening periods in the conditions of the Northeast of Romania.* Lucrări Ştiinţifice, Universitatea de Ştiinţe Agricole Şi Medicină Veterinară" Ion Ionescu de la Brad" Iaşi, Seria Horticultură, 61(2), pp. 87-92.

24. Wünsch A., Hormaza J.I., 2004 - *Molecular evaluation of genetic diversity and S-allele composition of local Spanish sweet cherry (Prunus avium L.) cultivars*. Genetic Resources and Crop Evolution, 51(6), pp. 635-641.

25. Zlati C., Istrate M., Dascălu M., Paşcu R., Bernardis R., Iacob F.L., 2018 - *Evaluation of some new sweet cherry cultivars adapted to the Romanian north-east conditions.* Lucrări Ştiinţifice, Universitatea de Ştiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" Iaşi, Seria Horticultură, 61(2), pp. 209-214.