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Relation of floral morphological characters to genetic variation at transposon level of Velkopavlovická cultivar apricot clones

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Abstract: The aim of the submitted study is to establish the relations between differences in the floral morphological characters of the apricot clones of the Velkopavlovická cultivar and the genetic variation established by means of molecular genetic analysis by the S-SAP method (Sequence-Specific Amplified Polymorphism). Twenty-six clones of the above cultivar were observed during two vegetation seasons. Two complementary genotypes genetically similar to the Velkopavlovická cultivar were chosen and observed to emphasise the relations considered.

As for the below mentioned material, the number of floral organs was counted i.e. the number of sepals (*sepala*), petals (*petalum*), stamens (*stamen*) and pistil (*pistillium*).

Statistical differences in the numbers of floral organs were identified among individual clones as well as among complementary genotypes. The transposon sequences analysis by means of molecular genetic method S-SAP made it possible to identify the clones of Velkopavlovická cultivar.

The genetic relationship and floral morphological characters dendrograms of the observed apricot group were generated on the basis of the obtained data.

The comparison of the results indicates that the identified polymorphic loci of the transposon sequences of clones have no influence on the number of floral organs in the observed apricot flowers.

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Key words: clones, apricot, *Prunus armeniaca* L., floral morphology, S-SAP, transposon

Introduction

Several authors have already studied the floral morphology of apricot flowers (Vachůn, 1973), (Surányi, 1995), (Lachkar, et. all, 2010). The authors engaged in this kind of research reported that the average apricot flower consists of 5-5.62 sepals and petals, 24.8-36.1 stamens and usually only one pistil.

The authors agree that many cultivars show statistically different numbers of floral organs, which helps to identify specific apricot cultivars (Vachůn, 1973), (Surányi, 1991), (Milatović and Stojanović, 2005), (Lachkar, et. all, 2010).

During their two-year research of the clones of Velkopavlovická cultivar (Vachůn and Lauko, 1981), the authors carried out the morphological analysis of flowers for 14 clones of the mentioned cultivar and found that the analysed flowers on average consist of 5.1-6.1 sepals and petals, 30.5-35.7 stamens and usually only 1-1.54 pistils per blossom. The authors also pointed to the frequent occurrence of anomalies regarding the flowers of Velkopavlovická cultivar. These include more than five sepals and petals, a doubled number of stamens but most importantly the occurrence of double-pistil flowers. The anomalies were established in both research years and a statistically significant difference in the number of observed floral organs was confirmed among the clones. However, the reason for their development has not been clarified yet.

The temperatures during each blooming stage are supposed to have an impact on floral organs quality, in particular the pistil size (Surányi, 1995). The correct development of the pistil is disrupted by late spring frosts as well as by higher spring temperatures typical mainly for the southern countries (Legave, et. all, 2004).

The study (Surányi, 1995) mentions that the number and quality of floral organs suggest the adaptability range of each cultivar to the conditions in which they are grown for a long time.

The above-mentioned studies refer to the significant effect of climatic conditions on some of the morphological modifications in apricot flowers but only a few studies are concerned with the clarification of the cause of these morphological differences on the basis of genetic characters regarding apricot cultivars or their cultivated clones.

Retrotransposons are kind of retroelements which can be found in the genome of many eukaryotic organisms (Labrador and Corces, 1997), (Kumar and Bennetzen, 1999). They are able to generate protease, reverse transcriptase and integrase and, therefore, are able to amplify and integrate themselves back into the genome of the organism. Thus they share similarities with the retroviruses

(Wessler, et. all, 1995), (Labrador and Corces, 1997), (Waugh, et. all, 1997), (Friesen, et. all, 2001). The retrotransposons or rather their different loci within the genome were noticed to have an influence on some of the important plant characters such as grapes colour or the shape of grapes bunch, beta-amylase content in rice or the differences in blooming of Arabidopsis species (Kobayashi, et. all, 2004), (Saika, et. all, 2005), (Fernandez, et. all, 2010), (Strange, et. all, 2011). On this account, the methods focused on transposable loci in apricot genome seem to be ideal for the search of the genetic relations to the observed differences in the floral morphology. At present, there are several retransposon-based methods and this study applies the S-SAP method (Sequence-Specific Amplified Polymorphism).

This method is derived from more common AFLP (Amplified Fragment Length Polymorphism). Contrary to AFLP, only one specific primer derived from one of conservative sites of the given retrotransposone type is used for the fragment amplification instead of two primers derived from the sites of restriction by two restriction enzymes. These highly conservative sequences are often found in the retransposon coding area called Long Terminal Repeat (LTR) (Waugh, et. all, 1997), (Labra, et. all, 2004).

A more frequent application of retransposone-based marker types is mainly limited by the fact that for individual plant species the conservative sequences from these areas, suitable for designing of complementary primers, have to be already described. The transferability of primers derived from LTR sequences among various plant species is quite doubtful due to the variability of these areas. On this account, the conclusion of the article was a group of new universal primers introduced as iPBS (Kalendar, et. all, 2010), seems to be valuable. The mentioned iPBS primers were designed according to the highly conservative sequences called Primer Binding Site (PBS) to which tRNA binds, acting as a primer for reverse transcriptase during the replication cycle of retroviruses and LTR retrotransposons.

Material and Methods

Plant material

This study observed the clones of Velkopavlovická cultivar together with two random selected genotypes related to this cultivar.

The group of 26 clones (Doc. Blatný, Goliáš, Chersonskij 239/1-8, Jubilejnyj, K 2, LE-13, LE-47, LE-57, LE-89, LE-103, LE-108, LE-111, LE-115, LE-120, LE-130, LE-267, LE-285, VP-LE-12/2, Maďarská C 235, M.30, M.44, M.48, M.72A, M.90, VP-LE-11/2, VP 126) and genotypes LE-97 and NS-2 were analysed. LE-97 is registered as a genotype obtained by random pollination of Velkopavlovická cultivar from the area of Pouzdřanská step in the Czech

Republic (Zhebentyayeva, 2010). Genotype NS-2 comes from Novy Sad (Serbia) and is supposed to be related to the Hungarian cultivar Ligety óriás.

All the observed plants were in their tenth year after the planting. The above-mentioned plant material came from local apricot gene pools situated in Lednice in the premises of Horticulture Faculty, Mendel University, Czech Republic.

Methodology of floral morphology

For the purposes of floral morphology research, branches were cut off from different parts of three trees of each clone or genotype in the early spring. The branches were stored in laboratory conditions at 18 – 22 °C where they were kept until the floral buds sprouted. In the D-E blooming stage, 50 random flowers were removed from each clone or genotype and the specific organs (sepals and petals, stamens and pistils) were separated and counted (Vachůn and Lauko, 1981).

Methodology of molecular genetic analysis S-SAP

For the purposes of the S-SAP method, internal phloem was taken from three trees of each clone or genotype and was used for DNA isolation. The methodological procedure described by the authors (Waugh, et. all, 1997) was chosen and slightly adjusted for DNA isolation and analysis. Firstly, the template DNA (300 ng) was digested by restriction endonucleases *Eco*RI and *Mse*I. The adapters with known sequences were consequently ligated to such emerged sticky ends and a primary template was thus created. The PCR pre-amplification was then carried out with primers complementing the ligated adaptors (Vos, et. all, 1995). A secondary template was made by dilution to 5 $ng.ml^{-1}$ DNA concentration and then was used in the selective amplification. The following three types of *Mse*I primer were used in the selective amplification - *Mse*-SA-GC, *Mse*-SA-CT and *Mse*-SA-AG (Vos, et. all, 1995) combined with six fluorescently labelled iPBS primers (FAM - 2242, 2232; JOE – 2238, 2224 and NED – 2415, 2237) described by the authors in (Kalendar, et. all, 2010). Eighteen primer combinations were used in total. The PCR reaction for the selective amplification contained 0.5 µl of labelled primer (10 µM), 0.5 µl of *Mse*I-SA-XY primer (10µm), 1.5 µl 10x PCR buffer, 0.12 µl dNTP (25mM), 2U Taq-polymerase Dynazyme (Finnzymes) and 6.58 µl H_2O. A secondary template was added into this mixture in a volume of 5 µl. Temperature setting for selective amplification was used as described in (Waugh, et. all, 1997). Amplified samples were consequently analysed on the genetic analyser ABI PRISM 310. The occurrence of amplified DNA fragments within each clone was evaluated in the form of binary matrix (0= present product, 1= absent product). Genetic similarity among observed clones was evaluated on the basis of the obtained S-SAP spectra similarity while the coefficient of similarity was counted according to (Nei and

Li, 1979). The UPGMA method was used to construct a dendrogram of genetic similarity among the analysed clones and genotypes.

Statistical evaluation of morphological data

The obtained results were subject to the statistical analysis of variance (ANOVA) and the significance of differences between the individual means was carried out by LSD-test at 0.05 and 0.01. This data were further processed into a matrix in NTSys PC in 2.1., where the comparison of matrices of the data from floral morphology analysis and molecular genetic analysis was carried out using the Mantel test.

Results and Discussion

Floral morphology

This study presents results of the floral morphology analysis conducted in 2010 and 2011. The obtained data are listed in Table 1. They indicate that the average number of floral organs in the observed clones of Velkopavlovická cultivar ranged between 4.96-5.23 sepals, 5.00-5.34 petals, 28.50-33.20 stamens and 1.0-1.1 pistils. As regards the complementary genotypes, LE-97 proved statistically higher numbers than the average values of sepals and petals in both years and in the number of stamens in 2010. As regards genotype NS-2, higher average numbers of stamens were established in both years and also this was the only genotype with the occurrence of anomalies in the number of pistils in both years. However, the number of anomalies concerning the pistils was not statistically significant. In the course of each year, the numbers of floral organs were found to be very different for each clone as well as among the clones and the complementary genotypes. Important differences depending on the year of observation are also evident for many of the clones.

The clones with the lowest number of sepals and petals can be listed as follows Doc. Blatný, LE-115, LE-130, LE-267, M.30, M.48, M.72A, M.90. On the other hand, the clones with significantly higher number of sepals and petals include Chersonskij 239/1-8, Jubilejnyj, LE-57. The clones K-2, LE-111, LE-285 a VP-LE-12/2 showed considerable reduction of numbers of sepals and petal unlike the clones Goliáš, Jubilejnyj, LE-120 and genotype NS-2 which proved an increase in the number of floral organs during 2011 as compared to 2010.

Table 1. Average number of floral organs in 2010 and 2011

Clone/Genotype	Sepals		Petals		Stamens		Pistil	
Year	2010	2011	2010	2011	2010	2011	2010	2011
Doc. Blatný	5.10a	5.12a	5.16a	5.12a	30.58ab	30.82c	1.00a	1.00a
Goliáš	5.08a	5.22b	5.12a	5.28b	30.22a	29.92b	1.00a	1.00a
Chersonskij 239/1-8	5.18b	5.26b	5.20ab	5.32b	31.62c	30.36b	1.00a	1.00a
Jubilejnyj	5.14ab	5.30b	5.18ab	5.34b	30.78b	32.00cd	1.00a	1.02a
K.2	5.26b	4.98a	5.28b	5.08a	30.26a	29.84b	1.04a	1.00a
LE-103	5.14ab	5.02a	5.14a	5.06a	30.28a	28.92a	1.00a	1.00a
LE-108	5.12ab	5.12a	5.12a	5.14a	30.36a	31.20c	1.00a	1.04a
LE-111	5.28bc	5.10a	5.28b	5.08a	32.30c	28.50a	1.08b	1.00a
LE-115	5.08a	5.08a	5.08a	5.12a	30.06a	29.90b	1.00a	1.04a
LE-120	5.04a	5.32bc	5.04a	5.38bc	29.86a	30.80c	1.00a	1.00a
LE-13	5.16ab	5.04a	5.20ab	5.08a	31.06b	29.26a	1.04a	1.00a
LE-130	5.08a	5.04a	5.10a	5.04a	31.56c	29.54ab	1.00a	1.00a
LE-267	5.06a	5.08a	5.06a	5.12a	31.18b	30.78bc	1.00a	1.00a
LE-285	5.34c	5.02a	5.34b	5.04a	31.62c	29.26a	1.08b	1.00a
LE-47	5.04a	5.14a	5.00a	5.16a	31.24b	31.12c	1.00a	1.04a
LE-57	5.24b	5.32bc	5.24b	5.24b	30.90b	31.52c	1.00a	1.10b
LE-89	5.10a	5.18ab	5.12a	5.18ab	30.26a	30.20b	1.00a	1.00a
M.30	4.96a	5.00a	5.04a	5.04a	30.70ab	31.82cd	1.00a	1.00a
M.44	5.10a	5.16ab	5.18ab	5.20ab	30.92b	33.20d	1.00a	1.04a
M.48	5.00a	5.12a	5.02a	5.14a	29.78a	28.76a	1.00a	1.02a
M.72A	5.00a	5.02a	5.08a	5.00a	30.86b	28.86a	1.00a	1.00a
M.90	5.00a	4.98a	5.00a	5.06a	30.36a	30.26b	1.00a	1.00a
Maďarská C235	5.10a	5.20ab	5.12a	5.22b	30.74ab	30.24b	1.00a	1.00a
VP.126	5.04a	5.02a	5.04a	5.04a	30.52a	29.06a	1.00a	1.00a
VP-LE-11/2	5.12ab	5.00a	5.14a	5.00a	30.76ab	29.38a	1.00a	1.02a
VP-LE-12/2	5.24b	5.02a	5.26b	5.04a	32.12c	29.22a	1.02a	1.00a
Average No. for Clones	5.12ab	5.11a	5.14a	5.14a	30.80b	30.18b	1.01a	1.01a
LE-97	5.54d	5.46c	5.48c	5.48c	32.46c	30.84c	1.02a	1.00a
NS-2	5.08a	5.34bc	5.08a	5.34b	32.30c	30.82c	1.04a	1.02a

a-ab, ab-b, b-bc, bc-c, c-cd, cd-d = statistical difference $\alpha = 0,05$
a-b, ab-bc, b-c, bc-cd, c-d = statistical difference $\alpha = 0,01$

The comparison of the number of stamens proved significant differences among the clones. There has also been a slight decrease in the average number of stamens per flower in most of the clones. The below-average number of stamens was recognised in the following clones - LE-103, M.48, VP.126. The biggest decrease was observed in clones LE-111, LE-285, M.72A, but also in genotypes

LE-97 and NS-2. On the contrary, an increase in the number of stamens of the clones Jubilejnyj, M.30 and M.44 was shown. The anomaly occurrence of double pistils was statistically significant only in clones LE-111, LE-285 in 2010 and in clone LE-57 in 2011. Their occurrence was not however confirmed in both years of research.

In spite of the statistically significant differences in individual characters of floral morphology, the clones of Velkopavlovická cultivar as well as two genotypes proved relatively low or medium variability of the mentioned characters. The variability of each character in the clones ranged between 0 and 11.7% for sepals, 0 and 12.6% for petals, 3.87 and 12.42 % for stamens and 0 – 27.3% for pistils during two years of research (these data are not stated in the table).

The comparison of all the numbers of individual floral organs by means of correlation proved a high dependence between the numbers of sepals and petals in both years. Other floral organs also proved a medium dependence but this fact was not observed in 2011. The correlation results are stated in Table 2.

Table 2. Number of mutual correlations of all particular floral organs

Correlation	Sep./Pet	Sep./Stam.	Pet./Stam.	Sep./Pis.	Pet./Pis.	Stam./Pis.
2010	0.97**	0.55*	0.54*	0.57*	0.58*	0.53*
2011	0.96**	0.47	0.49	0.30	0.18	0.45
2010-2011	0.97**	0.49	0.51*	0.37	0.28	0.26

* - medium dependence
** - high dependence

On the basis of the obtained data of floral morphology, a dendrogram of genetic similarity of clones and complementary genotypes in the total number of all observed floral organs from the average values for both monitored years was drawn up in order to facilitate the comparison of the data from the molecular genetic analysis (Figure 1).

According to the data in Figure 1, it is possible to divide the observed clones and genotypes into the following groups. The clones Goliáš, LE-89, LE-120 and Maďarská C235 form a group of rather identical clones in most of the observed morphological characters. The clone Doc. Blatný is very similar to the first group but in 2011 the statistically lower number of sepals and petals was proved contrary to the other clones from this group.

Another group is formed by clones LE-130 and LE-267 which are statistically identical with the exception of the number of stamens per flower.

The group comprising clones LE-103, M.72A, M.90 and VP.126 showed only a relatively low variability in the number of stamens. These clones also showed only a small number of the other observed organs.

The fourth group includes clones LE-47, LE-108 and VP-LE-12/2 which were significantly different in the numbers of stamens in each year of the

research. Moreover, LE-47 also proved statistical differences in the number of sepals and petals which were statistically lower in 2010 than in the other two clones.

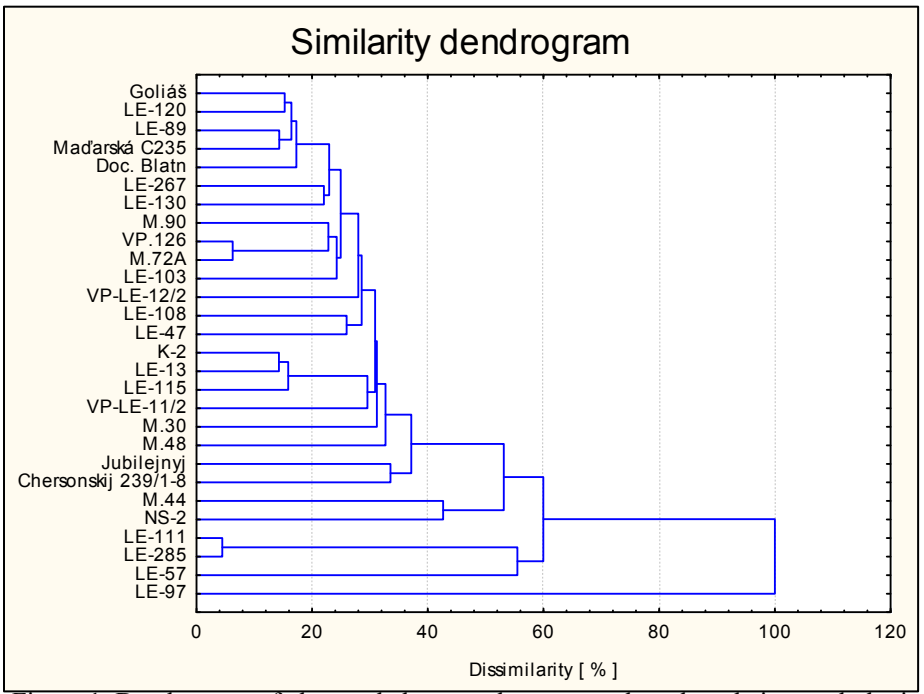

Figure 1. Dendrogram of observed clones and genotypes based on their morphological characters of flowers

The fifth group was made up of clones K-2, LE-13, LE-115 where the differences were observed in sepals and petals and clone LE-115 achieved a much lower number of floral sepals and petals than the other two clones in 2010. Certain variability could be described in the number of stamens in LE-13 against clones K.2 and LE-115.

The dendrogram derived from floral morphology (Figure 1) allows comparison of most of the other clones and genotypes separately due to numerous differences in floral morphology during 2010 and 2011. Another small group includes clones LE-111 and LE-285 which are nearly identical in the numbers of floral organs per flower.

Figure 1 and Table 1 indicate that, like in the (Vachůn and Lauko, 1981) studies, the clones of Velkopavlovická cultivar showed statistically significant differences when the numbers of floral organs were compared. Statistical differences were also evident in comparison of the complementary genotypes

with the average values of the observed clones. This confirms the fact that the number of floral organs can be used for cultivar identification purposes (Vachůn, 1973), (Surányi, 1991), (Milatović and Stojanović, 2005), (Lachkar, et. all, 2010).

In another comparison included in already published studies, the authors (Vachůn and Lauko, 1981) determined considerably higher numbers of floral organs per flower in the clones of Velkopavlovická cultivar 30 years ago. The development of the number of floral organs is intriguing mainly from the view of climatic changes during the second half of the 20th century, when the global warming was scientifically confirmed (Hansen, et. all, 2000), (Legave, et. al, 2004). A possible explanation of this trend is that the course of climatic changes could have a huge impact on the development of floral organs.

Molecular genetic analysis S-SAP

The results from molecular genetic analysis of clones of Velkopavlovická cultivar using S-SAP method confirmed the occurrence of retrotransposons in the genome of the observed clones. This is in agreement with other authors (Kalendar, et. all, 2010) who have stated that the transposons are present in most of the plant species. By means of the above-mentioned primers the DNA fragments were amplified using the S-SAP method and they are very likely derived from the areas containing retrotransposons. In addition, the spectra generated by this method provided a certain level of polymorphism when comparing the clones. Similar conclusions were reached by other authors (Labra, et. all, 2004), (Wegscheider, et. all, 2009), (Kalendar, et. all, 2010) who used the S-SAP method for the clonal differentiation of other cultivated plants such as *Vitis vinifera* or *Malus domestica*. Finally, the obtained polymorphism enabled to divide the observed clones into five groups.

The obtained data are shown in the form of dendrogram in Figure 2.

The first group of clones can be classified as the largest group which includes the clones with only the minimum of variability ranging from 0 to 4 loci out of 785 observed ones. Such a low variability can be considered as the minimum value of deviation which is at the level of the measurement error of the applied method. Seventeen clones are included in this largest group.

The second group which is very similar to the first one differs in approximately 4-6 loci. This group contains clones such as LE-130, M.48 and M.72A.

The third group involves the clones Chersonskij 239/1-8, LE-89, LE-115 and M.30. Similarly as the previous one, this group differs from the first group in about 4-6 observed loci.

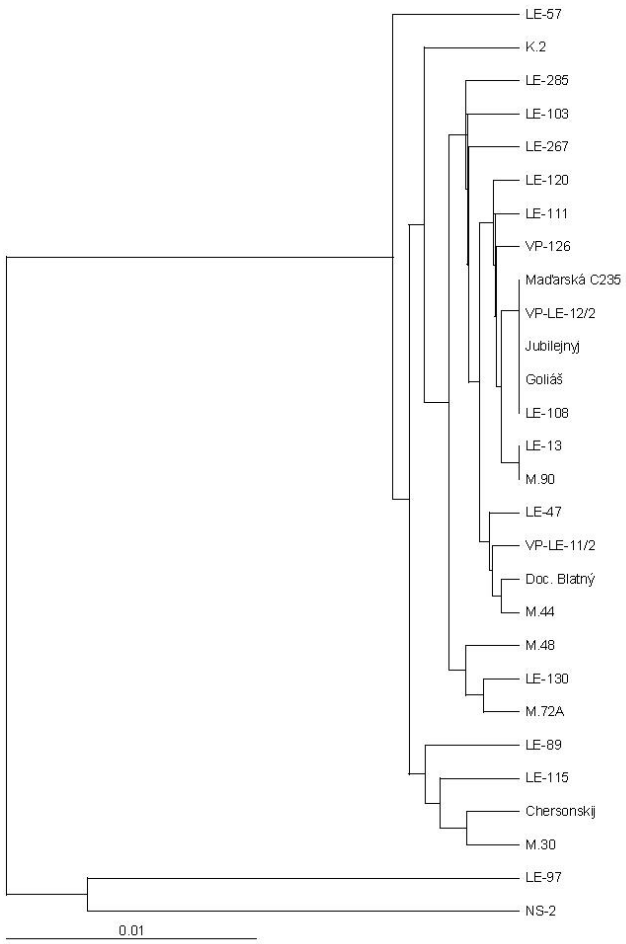

Figure 2. Dendrogram of genetic similarities of clones and genotypes using S-SAP

The last two clones each representing a separate group also differ from the main group. This concerns the clones K.2 and LE-57 which differ in 5 loci on average from the first group but their mutual polymorphism in 6 loci excludes their classification into one common group. The greatest differences can be seen among the third group of clones and clone LE-57 which reach up to 9 loci.

According to the analysis, the complementary genotypes LE-97 and NS-2 differed greatly from the clones of Velkopavlovická cultivar and also great differences were noticed between these two genotypes.

The comparison of the initial matrices for each dendrogram using the Mantel test proved that the correlation coefficient between the polymorphism obtained in the molecular genetic analysis by the S-SAP method and the differences observed in the floral morphology of the observed clones and the complementary genotypes is r =

0.0806. The results from the Mantel test indicate that the presented characters are independent. This implies that the occurrence of polymorphic transposon sequences obtained with the help of selected primers does not cohere with the numbers of floral organs of the observed genotypes group. As regards genotype LE-97, both floral morphology variation and genetic similarity of the observed group are significant but in this case it is not a clone of Velkopavlovická cultivar. On the other hand, a typical clone of Velkopavlovická LE-57 showed both its floral morphology and genetic similarity more distant from the average of the observed group. The genetically related clones (Velkopalovická LE-12/2, Goliáš, Maďarska C.235, LE-108 and Jubilejnyj) differed greatly in the number of floral organs. The probable reason is that the obtained polymorphic transposon sequences do not have any impact on gene expression which controls the morphological characters in apricot flowers.

Conclusions

The floral morphological analyses of 28 clones of Velkopavlovická cultivar and their subsequent comparison with their genetic study indicate the following:

The average numbers of floral organs (sepals and petals, stamens and pistils) for each clone during one year are not identical.

Correlation was not confirmed among the numbers of organs except for the floral sepals and petals during both years of the study. High correlation was confirmed between the number of sepals and petals during both years.

The clones of Velkopavlovická apricot cultivar showed the occurrence of anomalies in the number of floral organs. However, the number of these anomalies for specific clones is not identical in the two years.

The molecular genetic analysis by the S-SAP method facilitated the genetic relationship evaluations of the apricot clones of Velkopavlovická cultivar. The S-SAP method seems to be applicable to catch the genetic differences within apricot clones.

The polymorphism in the transposon sequences established by means of selected primers does not correlate and is unlikely responsible for the different number of floral organs of Velkopavlovická cultivar clones.

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ODNOS IZMEĐU MORFOLOŠKIH OSOBINA CVETA I GENETIČKE VARIJACIJE NA NIVOU TRANSPOZONA KLONOVA KAJSIJE SORTE VELKOPAVLOVICKÁ

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Rezime

Cilj ovog rada jeste utvrđivanje odnosa između razlika u morfološkim osobinama cveta klonova kajsija sorte Velkopavlovická i genetičke varijacije utvrđene pomoću molekularne genetičke analize primenom metode amplificiranog polimorfizma sekvenci (eng. *Sequence-Specific Amplified Polymorphism* - S-SAP). Tokom dve vegetacione sezone ispitivano je 26 klonova dole pomenute sorte. Dva komplementarna genotipa genetski slična sorti Velkopavlovická odabrana su i posmatrana za potrebe ocenjivanja pomenutog odnosa.

Kad je reč o primenjenom materijalu, utvrđen je broj cvetnih organa, kao što su broj čašičnih listića (*sepala)*, cvetnih listića (*petalum*), prašnika (*stamen*) i tučkova (*pistilium*). Statističke razlike u broju cvetnih organa utvrđene su između pojedinačnih klonova, kao i između komplentarnih genotipova. Analiza sekvenci transpozona, izvršena pomoću molekularne genetičke SSAP metode, omogućila je identifikovanje klonova sorte Velkopavlovická.

Na osnovu dobijenih podataka dobijeni su dendogrami genetskog odnosa i morfoloških osobina cveta ispitivane grupe kajsija. Poređenjem rezultata zaključuje se da su identifikovana polimorfna mesta transpozonskih sekvenci klonova nemaju nikakav uticaj na broj cvetnih organa kod ispitivanih cvetova kajsije.

Ključne reči: klonovi, kajsija, *Prunus armeniaca* L., morfologija cveta, S-SAP, transpozon