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GENETIC DIFFERENTIATION OF HISTORIC CULTIVARS OF HERBACIOUS PAEONIA BASED ON SRAP MARKERS: DOCUMENTATION AND CONSERVATION OF BOTANIC COLLECTIONS

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Introduction

Paeonia L. (family Paeoniaceae) comprises about 35 species of shrubs and perennial herbs distributed widely in the northern hemisphere [20, 26, 29]. The genus possesses great ornamental and medicinal value, which is a reason for its extensive culture, breeding and wide representation in botanical garden collections. Section Paeonia has the most taxa (about 27 herbaceous taxa, including P. lactiflora Pall.) and the most diverse geographic range (from East and Central Asia, the Western Himalayas to the European Mediterranean region). This section has about 1/3 rare to endemic species as well as evidence of complex reticulated evolution that results in incompletely-understood phylogenetic relationships between species [27].

Contemporary cultivated herbaceous peonies mainly belong to P. lactiflora, although there is a great diversity of interspecific and intersectional hybrids. Over 3,000 cultivars have been introduced or bred outside of Eastern Asia since 1820s, half of which are presumed extinct [D. Michener, communication from R. Jakubowoski – ICRA Registrar, unpublished]. Many points of the origin and phylogenetic relationships among P. lactiflora cultivars (and other species) are unclear since their documented history is inconclusive or absent and synonymy is present. To understand the cultivated peony phylogeny, it's domestication history and breeding potential of desirable ornamental characteristics as well as resistance to diseases and adaptability to environmental factors, it is critical to identify, profile, and assess the genetic diversity across the field genebanks of available historical accessions.

Botanical mentioned collections are a national heritage asset and much of it likely now unique. Botanical garden collections are essential for research related to genotypes that will be needed during the pending global climate change [6, 22]. Specifically, botanical gardens collections can function as field genebanks where their rich but selected genetic pool reflects significant artificial selection from complex socio-cultural historical factors as well as acquisition of new genotypes for subsequent educational or research objectives. Effectively understanding this captured diversity and its phylogenetics, evaluating cultivars for their bio-cultural conservation value, and predicting which cultivars carry useful traits for future breeding requires using contemporary molecular genetics approaches [23].

Use of molecular genetics and genomic approaches to resolve fundamental questions on the phylogenetics and origin of cultivated plants from their wild ancestors though domestication has become accepted [3, 5, 13, 17, 18, 28]. Molecular markers for cultivar

identification, genetic map construction, genetic diversity assessment, and molecular marker-assisted selection (MAS) have been found useful in many horticultural plants [16], including ornamentals [7]. Within the genus Paeonia, several recent studies document the genetic diversity of cultivated and wild species of tree and herbaceous peonies and show the high resolution power of different types of molecular markers for phylogenetic and domestication aims [8, 31, 32]. In particular, sequence related amplified polymorphism (SRAP) markers were successfully applied for genetic diversity documentation in various plant species and groups [15, 24], including tree and herbaceous peonies [9, 10, 12]. SRAPs spot coding regions of the genome, for up to 20% are co-dominant, possess capacity to elucidate markers with inherent biological significance, and therefore could facilitate the construction of linkage maps [15, 24].

Molecular certification of the genetic diversity in historical collections (field genebanks) of genus Paeonia is a critical step to resolve the confounded taxonomy and phylogeny of cultivated peonies; this is the first study to survey European, American and Soviet genetic resources of cultivated peonies for analytical depth. These methods provide a unique opportunity to distinguish genotypes/cultivars rigorously, which is intractable when based only on morphological characteristics – especially when historical documentation is lacking. The resultant datasets will help resolve and reconstruct the sequence and geographically dispersed history and process of herbaceous peony domestication in the important regions of its selection – Europe, USA, former USSR.

The aim of this research was to develop SRAP molecular marker systems effective for large-scale fingerprinting of herbaceous Paeonia genetic resources, mainly P. *lactiflora* cultivars, and possessing enough resolution power to discriminate the intraspecific (cultivars), interspecific (hybrids) and species levels, to conduct analysis on the first set of samples and reveal their relationships. Complete molecular profiling of the historically-deep collections of Central Botanical Gardens NAS of Belarus (CBG) and Matthaei Botanical Gardens and Nichols Arboretum of the University of Michigan (MBGNA) and wild parent species should help breeders in their work towards desired characteristics.

Objects and methods of research

Individual research objects were accessions from collections of genus Paeonia of the CBG and MBGNA. The collections of the CBG comprises more than 320 herbaceous genotypes including cultivars from Soviet selection programmes as well as endangered Paeonia species; MBGNA maintains more than 250 herbaceous cultivars of American and European selection as well as Chinese origin. Institutional databases of the peony collections are on-line and contain accession name, morphological description, available information on history and origin [http://mbgna.umich.edu/peony/; http://hbc.bas-net.by/bcb/eng/].

In this study four wild Paeonia species and 50 accessions of cultivated peonies were included: cultivars of P. lactiflora and interspecific hybrids (30 of European, 4 – American and 15 – Soviet selection) and several unresolved accessions for verification. The wild species are P. lactiflora Pall., P. tenuifolia L., P. daurica subsp. mlokosewitschii (Lomakin) D.Y.Hong (further noted as P. mlokosewitschii) and P. anomala L. (Appendix Table).

Material for genotyping was collected at MBGNA and CBG (Table 1) during the growing season (June – August, 2013 and 2014; Table 1). From each analysed peony plant 3 leaves were sampled (bulked), which were dehydrated directly after the harvest using silica gel (Silicagel 60, 0.2-0.5 mm, AppliChem).

Table 1

Locations of the MBGNA and CBG Paeonia collections, sampled for SRAP-genotyping

No	Location	S*	GL, DMS/ DD		GD, km (No)	
			Latitude	Longitude	1	2
1	CBG, Minsk, Belarus	4	53°55'15.1356"N/ 53.920871	027°36'38.8224"E/ 27.610784	4250.85	–
2	MBGNA, Ann Arbor, Michigan, USA	0	42°16'51.7404"N/ 42.281039	083°43'32.3112"E /83.725642	–	4250.85

*Abbreviation: NS – number of samples taken for genotyping, GL – geographic location, GD – geographic distance, noted in km; coordinate format: DMS – degrees minutes seconds, DD – decimal degrees.

Genomic DNA was isolated from silica dehydrated plant leaves by CTAB method [4]. A weighed leaf tissue (100 mg) was ground in a homogenizer (TissueLyser LT, Qiagen), and then 2X CTAB extraction buffer was added, containing 2% w/v of cetyltrimethylammonium bromide (CTAB), 1.41 M NaCl, 0.10 M Tris-HCl, 0.02 M EDTA. RNA degradation in DNA samples was performed as described [11]. Prior the SRAP-analysis the amount of DNA in each sample was equated and its equivalent amount was used for each PCR. DNA samples were stored at -20°C. For the detection of genotypic variability between individuals of the investigated Paeonia genotypes we have tested 4 pairs of SRAP primers (PrimeTech, Belarus): Me05/Em01; Me05/Em10; Me07/Em01; Me07/Em10 (Table 2), described previously for tree and herbaceous peonies of Chinese origin [12]. The primers revealed consistent amplification and polymorphism between species, interspecific hybrids and P. lactiflora cultivars, and were used in our study.

Table 2

Forward and reverse SRAP primers used in this study

Primer	Type	Sequence (5'→3')	T m, °C
Me05	Forward	TGAGTCCAAACCGGAAG	47
Me07	Forward	TGAGTCCAAACCGGACA	47
Em01	Reverse	GACTGCGTACGAATTAAT	43
Em10	Reverse	GACTGCGTACGAATTCAG	48

The PCR reaction mixture (25 μl) contains 60 ng of genomic DNA, 200 μM dNTPs, 2.5 mM MgCl2, 20 pM of each primer, 10x buffer, and 1 U Taq DNA polymerase (Primetech, Belarus). The amplification was carried out in Sure Cycler (Type 8800, Agilent Technologies, USA) using the following program: 3 min denaturing at 94°C, eight cycles of 30 sec denaturing at 94°C, 30 sec annealing at 37°C, and 90 sec elongation at 72°C. In the following 32 cycles the annealing temperature was increased to 50°C, with a final elongation step of 7 min at 72°C. Each PCR product (15 μl) was fractionated into microchips (Bioanalizator 2100, Agilent) or into 1.2% agarose gel and visualized by staining with ethidium bromide. Electrophoresis was carried out at a constant 100 V for 120 min at room temperature. Ladder Markers (100 bp and 1kb, Primetech, Belarus) were loaded each time as the reference for fragments size estimation. Gels were documented using a Molecular Imager VersaDoc MP 4000 image system (BioRad, USA). The molecular sizes of the fragments were

calculated using specialized software Bioanalyser Expert 2100 (Agilent) or QuantityOne (BioRad) on the basis of molecular weight standards.

Data analysis. The profiles of amplified DNA fragments obtained by SRAP-PCR analysis were the basis for the creation of binary matrices, where the presence of the amplicon was designated as "1" and the absence - as "0". Only distinct, discrete and reproducible amplicons were scored. A marker was considered as polymorphic if fragment was absent in at least one of the accessions. Reproducibility was estimated by scoring and comparing fragments profiles produced under identical conditions of at least two biological repetitions. Polymorphism information content of each primer (PIC) was calculated according by the following equation: PIC = 1 - ∑pi2, where pi is the frequency of the ith allele for each SRAP marker locus in the set of 54 peony accessions investigated [2].

Genetic similarities between cultivars were measured by the Nei similarity coefficient based on the proportion of shared alleles [19]. The NJ (neighbor-joining), UPGMA (unweighted pair-group method with arithmetic averages) trees were constructed using the Treecon software [30]. The wild species Paeonia daurica subsp. mlokosewitschii (Lomakin) was used as an outgroup in the NJ trees as a most distant species based on known phylogeny [25]. The number of 1,000 replicates was used for all bootstrap tests. Calculation of genetic diversity indices and the number of rare alleles, principal coordinate analysis (PCoA) were performed using GenAlex [21].

Predicting the morphologic characteristics and origin data beyond genotyping data. Predicting the following parameters: type of flower (single, semi-double, double), season of blooming (very early, early, early midseason, midseason, late midseason, late and very late), year of introduction, region (country of introduction) was performed using simple linear regression model and Poisson regression model.

Results and discussion

1. Levels of polymorphism and molecular identification of Paeonia cultivars revealed by SRAP markers. Iteratively selected informative SRAP primers were used to detect polymorphisms at the intra- and interspecific levels, i.e. to show the variability of genomic DNA of different P. lactiflora cultivars and Paeonia species, (see Table 2). The method produced discrete reproducible amplicons; their set were unique to each studied genotype differentiate every genotype. The amplicons' profiles obtained using SRAP primers are shown in Table 3.

Table 3

Characteristics of the amplicons of genotypes of Paeonia obtained with SRAP markers

Primer pair	No markers	Diapason of fragments length, bp	No of fragments per sample (min/max/aver)	No of polymorphic markers/ %	PIC
Me05 / Em01	30	74–1908	3/16/9.5	30/100	0.325
Me05 / Em10	22	84–869	3/13/8	22/100	0.329
Me07 / Em01	36	98–1756	6/16/11	36/100	0.247
Me07 / Em10	25	97–1065	5/13/9	23/92	0.159
Mean	28,3	–	4.25/14.5/9.4	27.8/98	0.265
Total	113				

The selected primers pairs generated amplicons in the size range from 74−1908 bp, the number of received markers varied from 22 to 36. The percentage of polymorphic loci identified with primers Me05/Em01, Me05/Em10 and Me07/Em01 was 100%, with primer Me07/Em10 − 92%. The total number of generated SRAP markers for the studied genotypes

of Paeonia was 113, with an average of 9.38 markers per sample. Percentage of polymorphic content of primer pairs varied from 0.159 (for Me07/Em10 combination) to 0.329 (for Me05/Em10), with an average of 0.265. Primers revealed number of genotype-specific markers. For P. daurica subsp. mlokosewitschii (Lomakin) D.Y.Hong applied SRAP primers detected 4 specific markers; for P. anomala L. – 3; for P. tenuifolia L. – it was revealed 3 unique bands. Among P. lactiflora Pall. cultivars by 1 individual markers possesses 'Albert Crousse' (Crousse, 1893), Augustin D'Hour (Calot, 1867), Suruga (Millet, 1955) and 'Vesennii', 3 individual markers were revealed for 'Novost Altaja' (Lutchnik, 1963).

2. Genetic similarity and cluster analysis of Paeonia species and cultivars. The values of Nei's genetic distance for analysed genotypes (based on the frequency of 113 SRAP alleles) were used to construct a cluster maps using the UPGMA and NJ algorithm. NJ phylogram of pruned genetic distances data (50 accessions) is presented in Fig. 1. (In this analysis accessions with unresolved labels were excluded). We interpret Fig. 1 to represent the genetic relationships among accessions. On NJ dendrogram (see Fig. 1) all accessions are distinctly separated from each other and 2 major groups are evident and assigned as cluster I and II. Cluster I contains species P. mlokosewitschii, P. teniufolia, P. anomala, and interspecific hybrid 'Orlenok'. Cluster II contains wild P. lactiflora itself and its domesticated cultivars. For this analysis P. mlokosewitschii served as an outgroup for the phylogeny data [25, 14]. Its role as an outgroup from the other taxa is also supported by this research (genetic distances; data not presented). Similarly, the clustering of P. lactiflora x tenuifolia hybrid 'Orlenok' between its parent species is consistent with its breeding history (Fomitcheva, 1963) [1] and further confirms the power of developed SRAP markers to resolve hybrid interspecific origin of Paeonia cultivars.

Cluster II has an internal hierarchy: P. lactiflora is relatively separated while clusters A, B and C are suggested. It reflects cultivar-landraces of European (fundamentally French and English), American and Soviet selection. Further subclusterization is observed. Cluster A includes the cultivars 'Albert Crousse' (Crousse, 1893; Double, pink, midseason), 'Arcturus' (Auten, 1933; Single, red, very early) and 'Arlequin' (Dessert & Mechin, 1921; Anemone, pink, midseason). Given the presumed later parentage of the single/anemone forms (if derived from Japanese selections introduced after the mid- to late-1800s), the anomaly is 'Albert Crousse', indicating research with additional related cultivars is needed.

Cluster B1 holds 'Novost Altaya' (CBG accession), 'Arkady Gaidar' (syn. 'Arkadij Gaydar'), 'Pamiati Gagarina', 'Mirnyi', and 'Belyi Parus'. Their breeding history suggests a common derivation. 'Mirnyi' and 'Belyi Parus' (Sosnovets) were selected from open-pollination work involving intervarietal and interspecific parents at the Botanical Garden of the Moscow State University starting in 1951 [1]. Cultivars 'Pamiati Gagarina' and 'Arkady Gaidar' were both bred by Krasnova in 1957 and 1958, respectively; all are historically congruent.

Assessing authenticity of all samples of 'Novost Altaya'(CBG, A, K) – an interspecific hybrid of P. anomala and P. lactiflora, has been requested from this study. When plotted all three 'Novost Altaya' samples from different original sources all placed differently on the dendrogram (data not presented). The accession 'Novost Altaya_A' clusters with the interspecific hybrid 'Orlenok', and is likely authentic. Based on clustering analysis Novost Altaya CBG was likely mislabeled. This shows the method's power to resolve identification queries where only morphological features are suggestive.

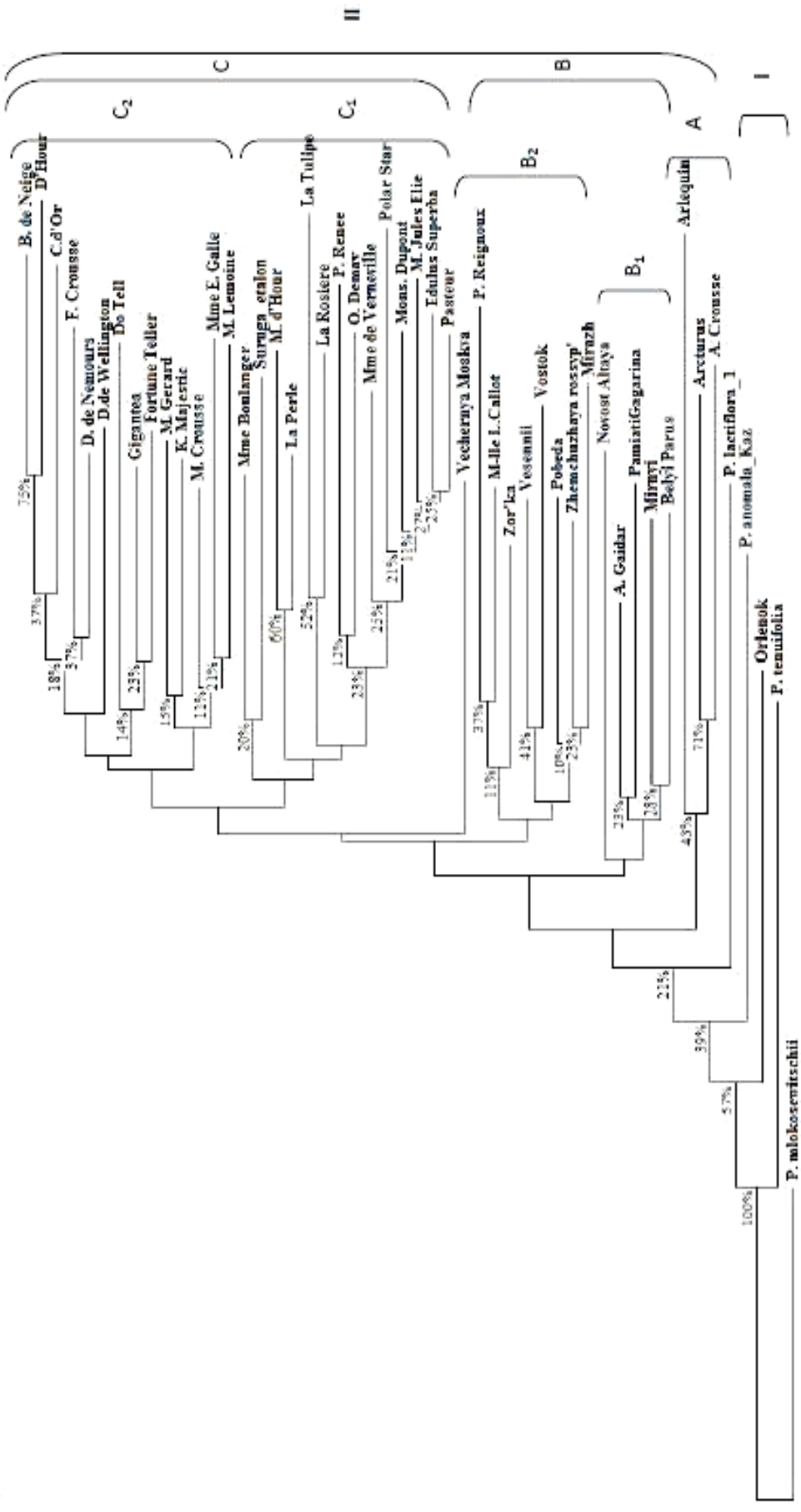

Fig. 2 Consensus Neighbor-joining dendrogram depicting genetic relationships (Nei) between *Paeonia* genotypes generated from 113 SRAP markers. *P. daurica mlokosewitschii* is used as an outgroup. Numbers above branches represent bootstrap values. For genotypes full names see Appendix Table

The rest of Soviet selections and two historical French cultivars (‘M-lle Leonie Callot’ and ‘Pierre Reignoux’) are grouped at cluster B2. A future goal is to much better resolve the historic French phylogeny with additional samples and taxa. One possibility is these cultivars were bred from different parental cultivars than the others, thus grouping them with the Soviet cluster. Lacking breeding records, expanding study to include contemporary sister-cultivars is likely the best approach. Intriguingly in the Soviet group, ‘Vecheriaya Moskva’ was separated yet it; closely distributed ‘Zhemchuzhnaya rossyp’ and ‘Mirazh’ are of Japanese flower type.

The distant position of cluster C from the species (Cluster I) based on developed SRAP polymorphic genomic regions is significant. Although the historically oldest French cultivars are here, these were likely bred from (or were simply renamed) old Chinese landraces-cultivars that were then “new” in Western Europe. The long history of domestication in China, presumably not involving repeated breeding with wild P. lactiflora or any other herbaceous peony species, would account for the genetic distance indicated here. This is counter-intuitive since one would expect the historically “old” cultivars to cluster basally with the species. However, the history of Paeonia domestication in Western Europe and America is doubtlessly based on highly-developed Chinese (and later Japanese) domesticates as ancestral, not the wild species. Thus the unexpected “old” French cultivars are removed from species and opens new research questions.

To examine fine relationships among the peony accessions employed, Principal coordinates analysis (PCoA) was performed using standardized molecular data. It is graphically presented in Fig 3. In general, the relationships between genotypes revealed by PoCA was conceptually consistent with the data obtained in this study by both UPGMA and NJ clustering analysis. The total variance explained by the first, second and third principal coordinates using PoCA was 6.18%, 5.12% and 4.49%, respectively. PC2 differentiates a subset of species and interspecific hybrids from most of the P. lactiflora cultivars; PC1 reflects a clear geographic European-USSR gradient in Paeonia, with spatially dispersed (unclustered) US cultivars.

All analysed wild herbaceous peony species such as P.mlokosewitschii, P. anomala, P. lactiflora and P. tenuifolia were clearly distant from all other peony accessions. The interspecific hybrids ‘Orlenok’ and ‘Novost Altaya’ (A and K accessions) are close to their parent species, so PCoA data are in congruent with previously presented cluster analysis data.

Compact and close enough distribution of Soviet cultivars to species, compared to another groups, may be evidence of breeding that included wild Paeonia species, while the broad range of other cultivars is more of a continuum with the exception of a cultivar cluster centered around the problematic ‘A Crousse’ (as already discussed). The scattered distribution of American cultivars among European may reflect gene flow through additional introductions directly from China and Japan, and may also provide indication for a desire for phenotypically different forms driving novel breeding. The small sample size for the American group (4 accessions) indicates a bigger study is needed.

Further analysis of relationship of Paeonia accessions by their region of origin (wild species, Europe, USSR and USA) by Nei genetic distance/ similarity indices is summarized in Table 4. The closest regions are Europe and USSR (genetic distance – 0.024), and most distant are Wild and USA groups (genetic distance – 0.103). At the same time USA and USSR groups are relatively equally distant to the European group analysed (genetic identity 0.97 and 0.98, respectfully).

This finding is consistent with the suggestion that US breeders by early 1900s were using novel source material rather than re-breeding only from existing European cultivars.

What is needed is a better understanding of what was being sought by the breeders and what material was available.

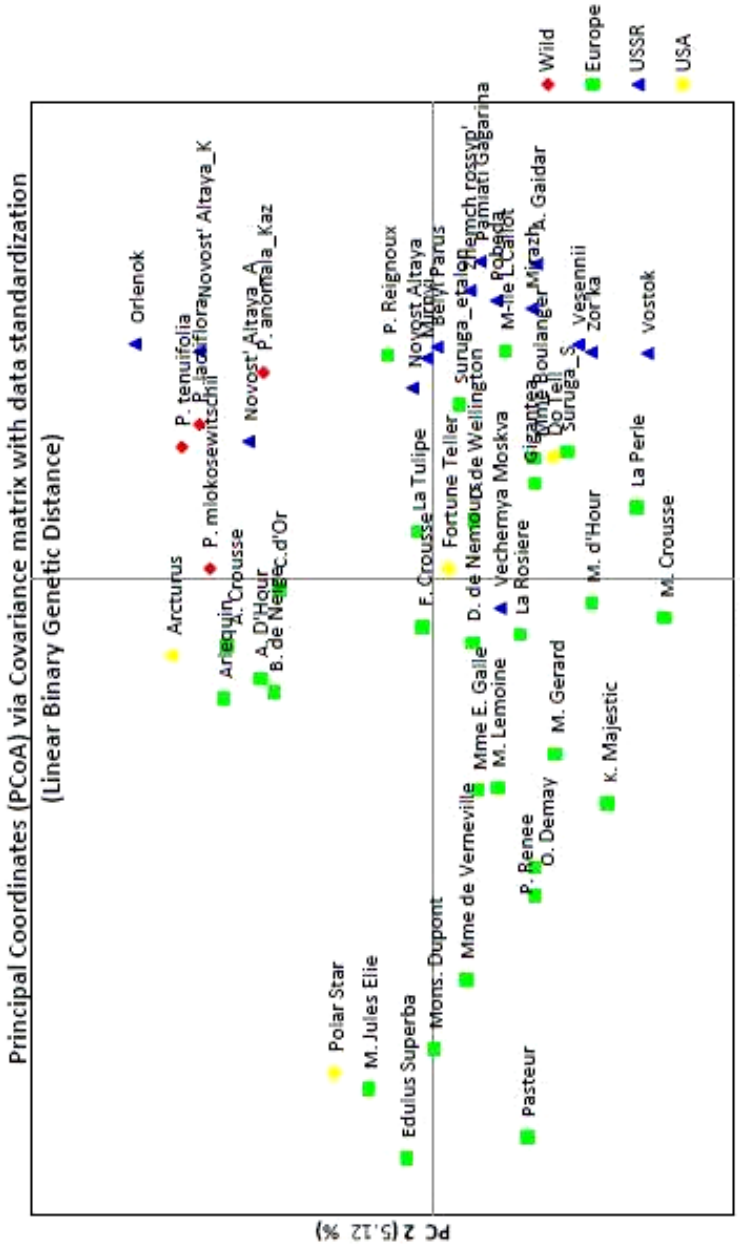

Fig. 2 Diagram showing the relationships among 53 accessions of *Paeonia* based on PCoA using 113 SRAP markers
Visualization of genetic relationships among Paeonia cultivars and species. PC axis 1 and PC2 were calculated from 49 *Paeonia* cultivars samples, and 4 *Paeonia* species were subsequently projected onto these axes. The proportion of the variance explained by each PC is shown in parentheses along each axis. The wild species samples are represented by diamond, European cultivars – as rectangles, USSR – as triangles, USA as circles and the region of their origins are indicated in the legend. Full names, description and geographic origin of the samples are described in the Appendix Table.

In USSR the peony breeding program began in 1949 based on about 200 cultivars, mostly of French selection; wild species hybridization was widely applied [1].

Table 3

Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between groups of Paeonia by region of origin

Group	Wild	Europe	USSR	USA
Wild	****	0.9290	0.9363	0.9018
Europe	0.0736	****	0.9763	0.9683
USSR	0.0658	0.0240	****	0.9544
USA	0.1034	0.0323	0.0467	****

3. Predicting morphologic and non-morphological characteristics for future breeding work. The sets of genetic markers, characteristic to each accession were analyzed for significant interactions with standard morphological descriptors of this genotype that could be logically (including biologically) coded as a numeric value. The ‘year of introduction’ and ‘season of bloom’ (early, middle, late) revealed the significant correlation with several markers at analyzed genotypes (data not shown). The other parameters as type of flower (single, semidouble, double) and ‘region’ (country of introduction) did not reveal significant correlation with genetic data. Since all floral forms have been bred in all regions, this was anticipated. For “year” simple linear regression model revealed significant relationship with SRAP marker 1.6_410bp (p= 0.0171), indicating genetic diversity in the cultivars increases over time, which makes sense if new genotypes were available. Poisson regression analysis of genotypic data and ‘season of bloom’ produced a significant relationship for 1 SRAP marker 1.16_829bp (p= 0.036). Both correlations suggest that a deeper survey of the molecular markers could help finding genetic linkage with favorable traits and will be useful in both applied and theoretical work on herbaceous peony breeding and certification.

Conclusions

Applied SRAP analysis allow to generate 113 markers (in average 9.4 loci per primer), and demonstrated high resolution power for effective discrimination herbaceous Paeonia on the specific level, interspecific (hybrids), and intraspecific (P. lactiflora cultivars). This is well supported by the fact, that species, interspecific hybrids ‘Orlenok’ and ‘Novost Altaya’ and P. lactiflora cultivars were found to be characterized by several unique genotype markers.

Clusterization analysis using UPGMA and NJ algorithm, and also results of principal coordinate analysis allow for the first time to generate relationship between the studied genotypes, which revealed its consistency with the region of origin of genotypes, as well as with available data on the pedigree. Specifically, European landraces and Soviet cultivars of P. lactiflora were clustered distinctly by groups; interspecific hybrids ‘Orlenok’ and ‘Novost Altaya’ were located between their parent species, although it is necessary to study the contribution of each parent more precisely.

Developed markers and genotypic passports of all studied genotypes could be thus used for the delimitation and identification of P. lactiflora cultivars including interspecific hybrids, revision of the unresolved origin issues, and relationship calculation, exchange of the certified material. Regression analysis in combination with SRAP markers is a powerful tool to produce markers important for MAS (such as SCAR, SNP, SSR, QTL), useful to construct linkage map of valuable traits of Paeonia cultivars [15, 24]. When added with markers of

chloroplast genome regions, large-scale capability of next-generation techniques, and on a wider set of samples from the studied regions (Europe, USA, USSR, and China as an initial center of domestication), it could be used for solving phylogeography of cultivated P. lactiflora.

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Appendix Table
Characteristics of 54 Paeonia cultivars and species in this study

o*	Name (Transliteration; variants)	Originator	Year	Country	Description**
	Белый Парус (Belyi Parus)	Sosnovets	1961	USSR	Lac. Double, white, semi early
	Памяти Гагарина (Pamiati Gagarina)	Krasnova	1957	USSR	Lac. Double, pink, midseason
	Новость Алтая (Novost' Altaya; Novost Altaja)	Lutchnik	1963	USSR	P.lactiflora x anomala hybrid, Single, lilac-rosy, early
	Мираж (Mirazh; Miraj, Mirage)	Krasnova	1959	USSR	Lac. Japanese, pink, midearly
	Мирный (Mirnyi, Mirnij, Mirnii)	Sosnovets	1952	USSR	Lac. Double, pink white, early
	Аркадий Гайдар (Arkady Gaidar; Arkadij Gaydar)	Krasnova	1958	USSR	Lac. Double, red, very late
	Победа (Pobeda)	Kupoljan	1957	USSR	Lac. Double, red, late
	Suruga_etalon	Cyt.: Millet	before 1955	France	Lac. Japanese, red, late
	Жемчужная россыпь (Zhenchuzhnaya rossyp)	Gorobetz-Tyran	1989	USSR	Lac. Japanese, pink, late
	Вечерняя Москва (Vechernya Moskva)	Sosnovets	1961	USSR	Lac. Double, crimson, magenta, late
	Восток (Vostok)	Krasnova	1957	USSR	Lac. Double, dark violet, midseason
	Зорька (Zor'ka; Zorka)	Sosnowets – Fomitschewa	1965	USSR	Lac. Double, light pink, late
	Весенний (Vesennii; Vesennij, Wesennij)	Krasnova	1959	USSR	Lac. Double, light pink, midseason
	M-lle Leonie Callot (Syn. Mons. Charles Levêque')	Calot	1861	France	Lac. Double, pink, late midseason
	Pierre Reignoux	Dessert	1908	France	Lac. Double, pink, midseason
	Paeonia anomala L.	N/a	N/a	N/a	Pink form
	Paeonia anomala L.	N/a	N/a	N/a	White form
	Suruga	Millet	1955	NL	Lac. Japanese, red, late. R.
	Новость Алтая_K (Novost' Altaya, Novost Altaja)	Lutchnik	1963	USSR	P.lactiflora x anomala hybrid, Single, lilac-rosy, early. R
	Новость Алтая_A (Novost' Altaya, Novost Altaja)	Lutchnik	1963	USSR	P.lactiflora x anomala hybrid, Single, lilac-rosy, early. R
	Орленок (Orlenok; Orlionok)	Fomitcheva	1963	USSR	Lac. Single, red, early
	Paeonia lactiflora Pall.	N/a			
	Paeonia tenuifolia L.	N/a			
	Paeonia daurica subsp. mlokosewitschii (Lomakin) D.Y.Hong	N/a			
	Albert Crousse	Crousse	1893	France	Lac. Double, pink, midseason
	Arcturus	Auten	1933	USA	Lac. Single, red, very early
	Arlequin	Dessert & Mechin	1921	France	Lac. Anemone, pink, midseason

	Augustin D'Hour (Syn. General MacMahon)	Calot	1867	France	Lac. Double, red, midseason
	Boule de Neige	Calot	1862	France	Lac. Double, white, early midseason
	Couronne d'Or	Calot	1873	France	Lac. Double, white, late
	Do Tell	Auten	1946	USA	Lac. Japanese, pink, midseason
	Duc de Wellington	Calot	1859	France	Lac. Double, white, NL, but midseason
	Duchesse de Nemours	Calot	1856	France	Lac. Double, white, early
	Felix Crousse	Crousse	1881	France	Lac. Double, red, late midseason
	Fortune Teller	Auten	1936	France	Lac. Single, red, not listed
	Gigantea	Calot	1860	France	Lac. Double, pink, early midseason
	Kelway's Majestic	Kelway	1929	England	Lac. Japanese, red, early
	La Perle	Crousse	1886	France	Lac. Double, pink, midseason
	La Rosiere	Crousse	1888	France	Lac. Semi-double, white, midseason
	La Tulipe	Calot	1872	France	Lac. Double, pink, early midseason
	Marguerite Gerard	Crousse	1892	France	Lac. Double, pink, midseason
	Marie Crousse	Crousse	1892	France	Lac. Double, pink, midseason
	Marie d'Hour	Calot	1883	France	Lac. Double, pink, midseason
	Marie Lemoine	Calot	1869	France	Lac. Double, white, late
	Madame Emile Galle	Crousse	1881	France	Lac. Double, pink, late
	Madame Boulanger	Crousse	1886	France	Lac. Double, pink, late midseason
	Madame de Verneville	Crousse	1885	France	Lac. Double, white, early
	Monsieur Dupont	Calot	1872	France	Lac. Double, white, late midseason
	Monsieur Jules Elie	Crousse	1888	France	Lac. Double, pink, early
	Octavie Demay	Calot	1867	France	Lac. Double, pink, early
	Pasteur	Crousse	1896	France	Lac. Double, pink, late midseason
	Petite Renee	Dessert & Mechin	1899	France	Lac. Japanese, pink, midseason
	Polar Star	Sass & Interstate	1932	USA	Lac. Japanese, white, midseason
	Edulus Superba	Lemon	1824	France	Lac. Double, pink, early

Note: *Accessions 1-24 – are from the collection of CBG; 25-54 – from MBGNA. ** R – accessions for revision; Lac. – P. lactiflora cultivar; N/a – information not applicable; NL – information not listed in available sources

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The article presents data of triennial studies in using of microbial preparations with various spectrum and organo-mineral fertilizer for growing seedlings of *Spiraea x vanhouttei* (Briot) Zab. in an industrial nursery. It was found that integration of these elements in *S. x vanhouttei* seedling growing technology increased a survival rate of the hardwood cuttings, improved their growth, stimulated lateral shoots formation, intesified flowering and increased the output of standard seedlings. The best results on all these parameters were obtained when hardwood cuttings were priviously treated with Fosfoenterin and complex of microbial preparations together with Component 2.

Key words: *microbial preparationss, Spiraea x vanhouttei (Briot) Zab., industrial nursery, technology elements*