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Przygotowanie populacji mapującej uzyskanej ze skrzyżowania odmian ‘Elsanta’ i ‘Senga Sengana’ dla analizy regionów QTL sprzężonych z wybranymi cechami użytkowymi gatunku *Fragaria*

Preparation of the mapping population derived from the cross of ‘Elsanta’ and ‘Senga Sengana’ suilable for analysis of QTL regions linked to selected *Fragaria* traits

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Celem przeprowadzonych badań było przygotowanie populacji mapującej uzyskanej w wyniku skrzyżowania odmian ‘Elsanta’ i Senga Sengana’, przydatnej do badań genotypowo-fenotypowych, poprzedzonych sporządzeniem ‘szkieletu’ mapy genetycznej.
Pierwszym etapem badań była ocena molekularna roślin form rodzicielskich pod kątem stopnia polimorfizmu genetycznego. Na postawie analizy wytypowanych 450 markerów SSR w genomie odmiany ‘Elsanta’ zidentyfikowano łącznie 418 alleli polimorficznych, natomiast w genomie odmiany ‘Senga Sengana’ – 337 alleli. Przeprowadzone badania potwierdzają wysoki stopień heterozygotyczności genomów obu wytypowanych do badań odmian.
Kolejnym etapem prac była analiza molekularna siewek uzyskanych w wyniku krzyżowania obu heterozygotycznych form rodzicielskich oraz ocena statusu genetycznego genotypów potomnych. Badania te potwierdziły, że w obrębie roślin populacji mapującej ‘Elsanta’ i ‘Senga Sengana’ występują genotypy pochodzące tylko z kontrolowanego zapylenia. Ponadto, analiza segregacji, w populacji mapującej, alleli heterozygotycznych, zidentyfikowanych w genomach form rodzicielskich, umożliwiła sporządzenie ‘szkieletu’ zintegrowanej mapy obu badanych genomów truskawki. Wstępna mapa genetyczna, do sporządzenia której zastosowano 44 markery SSR, zawiera łącznie 27 grup sprzężeń, na których zidentyfikowano loci 53 alleli polimorficznych, pokrywających 1 033 cM genomu truskawki.
W wyniku przeprowadzonych testów potwierdzono, że uzyskana populacja stanowi wartościowy materiał do badań związanych z opracowaniem mapy genetycznej truskawki. Ponadto, sporządzony ‘szkielet’ mapy ‘Elsanta’ × ‘Senga Sengana’ poszerza bazę do dalszej lokalizacji genów i identyfikacji regionów QTL sprzężonych z ważnymi cechami użytkowymi truskawki.

Słowa kluczowe: allele heterozygotyczne, genom *Fragaria*, mapa genetyczna, SSR

The aim of the study was to generate a mapping population derived from an 'Elsanta' and ‘Senga Sengana’ cross, so as to be useful for genotypic-phenotypic studies, and subsequently, to construct a 'skeleton' of the integrated strawberry genetic map.
The first stage of the research was based on molecular assessment of parental plants for genetic polymorphism. After analysis of 450 selected SSR markers, 418 polymorphic alleles were identified in the genome of the 'Elsanta', and 337 alleles in the genome of the 'Senga Sengana'. The study confirms the high degree of genetic heterozygosity of both of the strawberry varieties.
In the next stage of the work, molecular analysis of seedlings resulted from the cross of the heterozygous parental forms, as well as the confirmation of genetic status of hybrid genotypes were conducted. These studies confirm that the origin of the prepared mapping population was the result of the controlled pollination. Moreover, segregation of heterozygous alleles in the mapping population enabled the preparation of the 'skeleton' of an integrated map of 'Elsanta' x 'Senga Sengana'. Herein, the initial genetic map was found to contain 27 linkage groups representing the loci of 53 polymorphic allele, covering 1 033 cM of the strawberry genome.
Generally, as a result of the tests, we confirmed that the obtained population represents valuable material for research related to the strawberry genome mapping trial. Additionally, the 'skeleton' of 'Elsanta' x 'Senga Sengana' genetic map enlarged the database for further gene localization and for identifying QTL regions linked to important strawberry traits.

Key words: *Fragaria* genome, genetic map, heterozygous alleles, SSR

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Introduction

Strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) is an important species grown in temperate regions. Globally, about 9 million tonnes of strawberry fruits are produced each year, and European countries account for half of this amount (FAOStat, 2019).

The conventional breeding of *F.* × *ananassa* is focused on development of new varieties, representing the most favourable combination of phenotypic traits, adapted to different cultivation conditions (e.g. salinity), different agroclimatic conditions (i.e. frost, drought), different pressure from biotic factors (e.g. fungal diseases, including *Verticillium wilt,* anthracnose) and abiotic factors, as well as different consumer preferences (fruit firmness and persistence, ascorbic acid and sugars content) (Darrow, 1966; Hancock, 2008).

Genetic studies supporting the global breeding of strawberry, led to the identification of valuable molecular markers, including those associated with fruit quality, flowering or resistance to pathogens. For many of them, loci on linkage groups of genetic maps of various genomes of strawberry have also been identified (Denoyes-Rothan et al. 2004; 2005, Sargent et al. 2011, Sargen et al. 2012).

Genetic and breeding experiments revealed both monogenic (resistance to phytophthorosis and alternaria) and polygenic of strawberries traits inheritance (Lerceteau-Kohler et al., 2002). Because of the additive (polygenic) interaction of genes, their alleles usually accumulates in the genome, and each genotype may show a different phenotypic features, depending on its own genetic potential (Semagn et al., 2006; Hancock et al. 2008). This is particularly important for research focused on mapping of different genomes. The prerequisite for starting such work is to generate a relevant collection of progeny plants, called the mapping population (including at least 100 – 150 hybrid genotypes), in which all genotypes come from a controlled crossing, and the parental forms represent a high degree of heterozygosity (Liebhard and Gessler 2000). This approach ensures the correct analysis of the segregation of the identified alleles of molecular markers, useful for the saturation of genetic maps. The appropriate selection and preparation of plant material are therefore key steps in research directed for identification of genome regions, determining important functional traits of *Fragaria* species.

The aim of the study was to generate a mapping population suitable for the construction of a 'skeleton' of the strawberry genetic map and identification of genomic regions linked with important strawberry traits (QTL, quantitative trait loci) such as resistance to fungal diseases (*V. dahliae, Colletotrichum acutatum, Phytophthora cactorum*); fruit quality (content of health-benefits); and resistance to abiotic factors, i.e. cold hardiness or water deficit).

Materials and Methods

The research material consisted of F_1 hybrid plants obtained from crossing between two parental cultivars, 'Elsanta' and 'Senga Sengana' (CP – cross pollination). The selected parental cultivars are extremely different in terms of many traits, including susceptibility to biotic and abiotic factors, and fruit quality.

Preparation of plant material

The seeds obtained from a cross pollination programme (2014) were dried, stratified, and planted in pots filled with a mixture of peat substrate and sand in a ratio of 3:1. Young progeny seedlings with at least two leaves (200 plants), constituting the mapping population, were transferred into multi-pot trays (54 pots, size 55 × 55 × 62 mm and volume of 90 cm^3). Plants were kept for 3-4 weeks in a greenhouse and later planted on a field plot. Regular cultivation and field nursery works were carried out, such as watering and fertilization of plants, soil loosening, and disease and pest control (Plant Protection Program, spraying to control powdery mildew; Signum 33 WG, for spider mite; Nissorum Strong 250 SC and other pests and pathogens hazardous for the plantation), including both parental forms and F_1 hybrids.

Additionally, young runners were collected from parent plants and planted in order to maintain a pool of the same clones of hybrid genotypes, which were further used as material for phenotypic experiments.

Material for molecular studies

Genomic DNA of the 'Elsanta' and 'Senga Sengana' parental forms and 200 plants from the mapping population was isolated following a procedure described by Doyle and Doyle (1990). Leaf tissue (2g) was ground in liquid nitrogen, suspended in CTAB extraction buffer and incubated at 65ºC for 30 minutes. For the precise separation of the upper phase (containing the nucleic acids) the suspension of disintegrated tissue was mixed with an equal volume of chloroform/

isoamyl alcohol mixture (24:1 v/v) and centrifuged (15 000 g). DNA was then precipitated with isopropyl alcohol (1:3 v/v), dried at room temperature and dissolved in TE buffer (pH=8, Sigma). RNA molecules were removed from the samples by RNase A treatment (10 µg ml^{-1}, 37°C/1 h). The purity of the DNA templates was assessed based on electrophoresis fractionation in a 0.9% agarose gel and by measuring the extinction coefficients at 230, 260 and 280 nm (Gene Quant Pro Amersham Pharmacia Biotech). The DNA concentration was determined by comparison with the λ phage DNA with known concentrations of 315, 157 and 79 ng $µl^{-1}$.

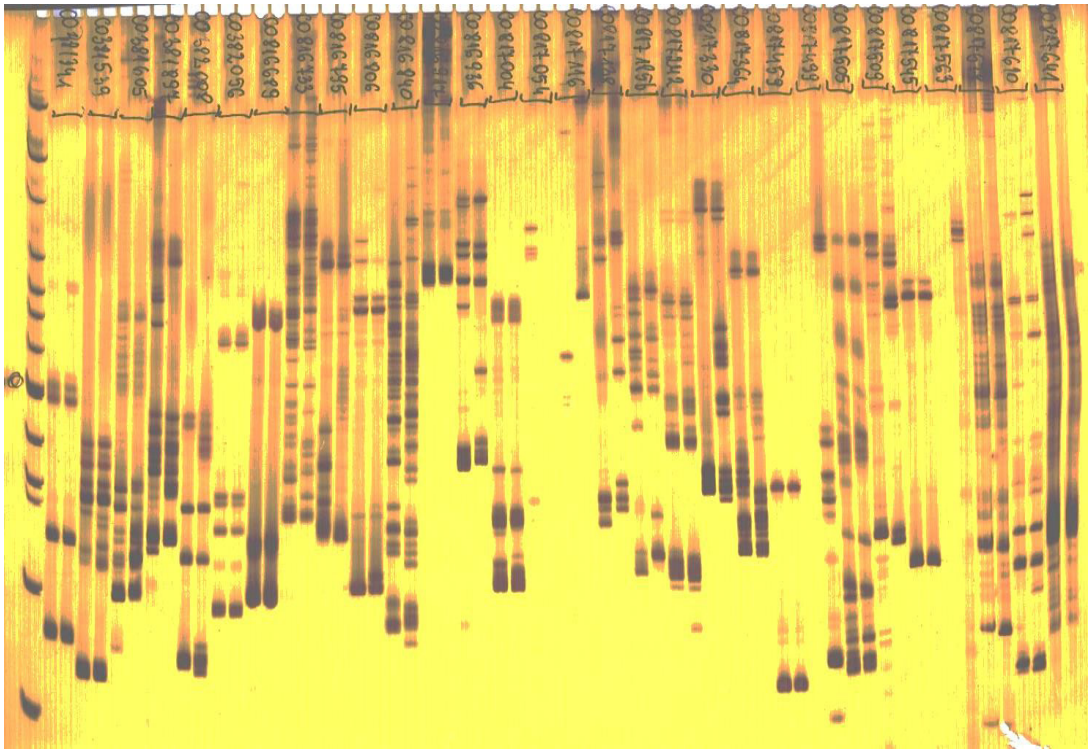
Amplification of polymorphic allele (SSR-PCR)

The Genome Database for Rosaceae (GDR, www.rosaceae.org) for *Fragaria* and olgonucleotides information from the available literature were used to perform the PCR assays based on the 450 microsatellite primer pairs, finally applied for genetic mapping procedure.

The reaction mixture (13 µl) contained 5 ng DNA isolated from 'Elsanta' and 'Senga Sengana' cultivars and progeny genotypes obtained by their cross pollination, 0.325 U polymerase (Ampli Taq, Applied Biosystems), 10 × PCR-buffer II with 1.5 mM $MgCl_2$, 1.25 mM dNTP (Applied Biosystems) and 0.35 µM SSR primers. Touchdown PCR assays were performed at optimal temperatures: 94°C – 30 s, 55°C – 45 s, 72°C – 60 s/10 cycles, 94°C – 30 s, 50°C – 45 s, 72°C – 60 s/25 cycles. The generated amplicons were separated in 7% polyacrylamide gels which were stained in 0.4% silver nitrate solution and visualized in white light.

Identification of markers loci

The mapping analysis was carried out on DNA templates isolated from parental forms and from 200 plants belonging to the 'Elsanta' × 'Senga Sengana' population with confirmed genetic status. A set of markers available in the database (www.rosaceae.org) and those described for the genetic maps of wild (diploid) species *F. nubicola*, *F. vesca* and the octoploid 'Redgauntlet' and 'Hapil' cultivars were selected to create the 'skeleton' of the genetic map for both cultivars. Specific DNA fragments were amplified using SSR-PCR tests according to the procedure presented above.-.



Rys. 1. Wzory prążkowe (reprezentują parę obu form rodzicileskich) przedstawiające wielkości alleli zidentyfikowanych dla 30 przkładowych markerów SSR, różnicujące genom odmiany 'Elsanta' (I ścieżka z pary) oraz 'Senga Sengana' (II ścieżka z pary).

Fig. 1. Band patterns (in pairs of both parental forms) representing the alleles identified for 30 SSR markers, differentiating the genome of 'Elsanta' (1st path from pair) and 'Senga Sengana' (2nd path from pair) cultivars.

The 'skeleton' of the genetic map for 'Elsanta' × 'Senga Sengana' cross was created using 44 SSR markers (ARFL002, ARSFL022, EMFn017, BFACT004, EMFn214, ARSFL011, FvH4180, FvH4153, FvH4164, FvH4160, FvH4154, ARSFL100, UDF009, BFACT017, BFFv09–11–02, EMFv018, CO816733, EMFv007, EMF132, FvH4163, BFACT045, FvH4169, BFACT036, UDF001, FvH4161, FvH4155, FvH4177, FvH4165, FvH4173, CFVC0316, BFACT002, ARSFL015, EMFn213, CFACT111, ARSFL012, CFVC015, BFACT003, FvH4171, COBRA, CO817054, BEFv14–29, EMFn049, FvC103, EMFn021).

The composition of the reaction mixture and the touch-down PCR assay parameters were the same for all oligonucleotide pairs. Annealing temperature generally ranged from 60°C to 55°C (1), and only for the markers ARSFL - from 55°C to 50°C. In both cases for each 10 cycles, temperature deceased of 0.5°C per cycle. The final annealing of the oligonucleotides was performed at a stable temperature of 55 or 50°C.

The skeleton of the genetic map was constructed using JoinMap v.3.0 software (Biometrics, Kyazma NL) (van Ooijen and Voorrips 2001). The type of segregation of alleles of the analysed genetic markers in the mapping population was verified with the χ^2 test based on the value of the deviation between their theoretical (Mendelian) and the observed distribution. Markers were allocated to particular linkage groups based on the LOD threshold (Logarithm Odd Ratio)> 3.0. Map distances (cM, centimorgans) were estimated using the *Kosambi* mapping function. The identified linkage groups were presented in a graphical format in MapChart 2.1 software.

Results

Assessment of the degree of heterozygosity of 'Elsanta' and 'Senga Sengana' genomes

PCR assays performed on the DNA template from 'Elsanta' and 'Senga Sengana' with 450 pairs of microsatellite primers revealed 228 polymorphic genome fragments (polymorphic alleles) (Tab. 1). In total, 3 532 SSR-PCR assays were performed.

The amplification pathways of polymorphic DNA fragments identified in the genomes of 'Elsanta' and 'Senga Sengana' and obtained with 30 selected microsatellite primers are presented as an example on the electrophoregram (Fig. 1). The sizes of identified polymorphic alleles differentiating the parental forms, and the sequences of oligonucleotides used in PCR assays are presented in Table 1.

The number of heterozygous alleles identified in the genomes of both parental forms is presented in Table 2.

After the analysis of 450 selected SSR markers,

Tabela 1
Table 1

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

Sizes of PCR amplicons (segregating alleles) differentiating 'Elsanta' and 'Senga Sengana' genomes and the sequences of oligos generating the above heterozygous alleles.

Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
1	FvH4004	480, 600	300, 560	cccagatctccctacctttacc	agttctacccccttttcagacc
2	FvH4005	270, 275	-	tcaagttacgtcgcttacatgg	ttgctgtttcaagaccactagc
3	FvH4006	290, 350	270, 315	cgagtactccaccttcaattcg	atgagagatcttccgtcgtagg
4	FvH4009	480	450	ttggctgttccagtttgaga	ccaaagggactggtggtaaa
5	FvH4014	150, 400	130, 300	tgacctcaatctcttgaacc	atccctactcaaaaccgtcagc
6	FvH4019	200	220, 600	catctcagttggatcagaatcg	caaggaatcaaggatcataggc
7	FvH4022	-	200, 560, 565	agttctggaacgtcctttaggc	acacaagcacatacccaaaacc
8	FvH4023	595, 600	-	tactgggggcaatatgtttgg	gccccttgtaaacttctactgg
9	FvH4025	260	-	gttcagttgagagccataatgc	gaatgatgaaaggcagctaagg
10	FvH4031	520	-	tctcacctcatccaaatcttcc	agtgggaatggttccttttttgg
11	FvH4033	150, 190	110	ctccacaaacctaataccaacg	gacgacgacttcttcaaaacg

Tabela 1 cd.
Table 1 cont.

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

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Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
12	FvH4041	200, 250	220	cagctgcatctatttgatctgg	cgagctcaactctcttctaccc
13	FvH4042	280	-	aacaggagcttatacggaatcg	aggagctcaatctcatcatcg
14	FvH4049	250, 260	-	acggctactctagggaagatgg	ctctccacccttgcattagc
15	FvH4050	185, 200	210	tagcatccatagttccatacgc	caatacaatctcccaactcacc
16	FvH4052	170, 175	320	caagaggtcgttaggtgaaatg	gtgttcaagcccagaaatgatg
17	FvH4056	-	450	gggggtattttgaagtgtaagc	gcgaaaaatatgagaggagagagg
18	FvH4057	200, 225, 280, 310	235, 240, 300, 380	agtttggaccgggtcaagagg	agctcttcctccctcacaacg
19	FvH4058	220	-	gttagagaagcagtggatgacg	gggcatctacacatgttgacc
20	FvH4059	450	-	tgcacatagagcaacagtataggg	ttgttagcatgaagtgcaaagc
21	FvH4061	-	200	accaactccatactccacaagc	gcctccctctctagctcttcc
22	FvH4082	-	495	cggtttacctaactccaactgc	cctgcaagaaatctctactgtcg
23	FvH4083	360, 600	300, 470	aattgggggtgtgacaatgc	gcttcaaatttgggatctgg
24	FvH4086	-	600	acaccccaagcaaattacagg	tgctatttaggcaactccttcc
25	FvH4088	-	400, 450	cctcagtcttaacgaacgaacc	tagagagagcaccgactcaagg
26	FvH4090	320	330	cagttgatgtgttgctgagtcc	agtttttgttttcatctttgg
27	FvH4091	220, 300, 400	200	tcaccggttccttctttatagc	taatttcttgcaggacgttcg
28	FvH4102	600	610	tttccttttggttgaaaactcc	tcataggaagcttttattcatacc
29	FvH4104	240, 250	510	agactgctcatcctctctttgc	atcatgggagagaacattaggg
30	FvH4105	-	320, 330	cgttggagatctggtaaactcc	taaccttcccctcgtgaaatcc
31	FvH4106	155, 215	220	tacaaaggacttgcctcttagg	tcactttcgttgtcctacatcc
32	FvH4112	265, 320, 330	210, 460	tttcaacaaattaaggcaaacg	gccatagaaaaccagaaaaacc
33	FvH4113	125	110	tgactagcaatagtgcaacagc	cggcagagaacccagtaagc
34	FvH4114	200, 380, 400	-	gcagcatgagagacaagatgc	gttagcagaagggaaatgaacc
35	FvH4116	290, 405	380	ggacattgaccaagcatttacg	tacggtcaggaagattgagacc
36	FvH4117	295, 305	300	actttgcttggctagttgtcg	gagggttctggagtctaagtgc
37	FvH4121	400	-	tacacagtgacatagccgatgc	gtcctaggtttcctcgttctcc
38	FvH4123	240	245	gttgaagcaaccttgaatgc	ggaggacaactccaatttatgg
39	FvH4124	240, 285	385	gcacagttccatcagtttttca	acaattgatcccttggaaattg
40	FvH4127	180, 200, 385	170, 195, 485	accaccacaacctagtctctgc	tccctaagttttctctccatcc
41	FvH4131	135	-	cagactcagcagagtgatttgg	ctaatggcccgtatcttctcc
42	FvH4134	100	-	aagtgaaggaggggaagaagg	gcttcataaaacagccactgc
43	FvH4136	190, 580, 600	-	ttcctgatcaagctaggaatgc	ccttggtgtactctcaaactcg
44	FvH4145	205, 280, 410	300, 420	aaagtcttgggggagtaaaagg	ggtacatggtgatgtgtcatcc
45	ChFaM 149	-	165, 595, 600	ccctcttcccgtaaagtcca	cggactcaccggtcactaga
46	ChFaM 262	400	-	actcggtgaggaaagacgaa	agagaagaccgctgaggatg
47	ChFvM 232	320	330	gcttcttgggacagcaacac	tgaaccctgcatttcctctg
48	ChFvM 243	330	340	tcctttctcgctcactgtca	aaacaaaggaaaggctggtg
49	ChFvM 245	-	510	tcaaccacaccgctactcct	cttgctgagctcgtccttgt
50	ChFaM 009	410	-	tccaagttccagagctcctt	cgtcgagctcagacatgaaa
51	ChFaM 010	200, 580	210	tatcgcctgcaattcatctg	gctggctctgtggagtgagt
52	ChFaM 011	95, 300, 360	80, 90, 210, 270	tcctctccttctttcccttca	cgagatctcccgagactgag
53	ChFaM 017	80, 90, 160, 250, 295	415	ctcactctctgcgaacttgc	caactcaccttgcaccgatt

Tabela 1 cd.
Table 1 cont.

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

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Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
54	ChFaM 022	185, 200, 495	-	gggccactcctacttcttca	ttggccttgagagcttcgat
55	ChFaM 026	100	-	tcgtctctcgtttctctaaaagc	atcaagtgctcgtccacgta
56	ChFaM 030	300, 335	-	ccatgaagcagtgaagtcca	agaaaatcccgagagcctttt
57	ChFaM 031	300, 310	200, 210	gctagcaaagccctaagcaa	acggtgggcacacttaaaga
58	ChFaM 032	-	410	ggtccctgcttcttcttctttt	ttcagccccattttccagta
59	ChFaM 033	110, 505	115, 510	cacaaatccacacacagcag	cccaggaaggtaaccacaaa
60	ChFaM 036	130	135	gcagcctcaagaagtgaagg	ccatcttgatatcacaggcataa
61	ChFaM 037	505	-	acgacgatcaccacaaggac	aagctccgtcgtttcctctt
62	ChFaM 040	-	205, 300	agtggtcatcagcaccatca	taaccgggaacggtactctg
63	ChFaM 041	185	180, 280, 490	ccacacgaaggagaaggaga	aacgtgtcattcgaccacaa
64	ChFaM 044	170, 615	165, 310, 410, 595	cgctgagtcgttctaatttca	ttttgttgacgagcgagatg
65	ChFaM 046	195, 300, 310	-	ccatttccatggccttgttt	ggccttgttgggtctgagag
66	ChFaM 047	235	200, 215, 240, 260	tcatttctctctcccctcgat	gatgtgatttacggcgaagg
67	ChFaM 056	195, 260	265, 510, 600	aaaacgtcgtcgttcaggat	cgtactgctgttgctgctgt
68	ChFaM 058	140, 195, 205, 210, 300, 420, 500	550, 560, 580	gacccaaacacaccctgaac	ctccccattctgggaaatct
69	ChFaM 060	165, 550, 560, 580	280, 590, 610	tgagctaccaccaagaaccc	aataccettggtacccctcg
70	ChFaM 063	120, 300	125, 195	gacgtctccgatccgttgat	ctggctcgcgtacgactttc
71	ChFaM 064	285	190, 280	caccaacttcgacaacctca	gactcttctttggcgagctg
72	ChFaM 065	220, 245, 250, 265	200, 250	gaccgggagagataacagca	atagaagccaatgcgtgatg
73	ChFaM 066	110, 590	115, 280, 310, 580	attttggccacgaagaattg	cgatgtcaggaacgaactga
74	ChFaM 068	420	200, 425	catctccagttccttggctc	acgaccattcctcttcctca
75	ChFaM 070	280, 630	-	agcattgaattcccagttttg	ccttgcagcaggaactaagg
76	ChFaM 072	385	-	tggcagaaatttccaaaagg	ctcccccagaagtccagatt
77	ChFaM 077	250, 320, 400, 440	270, 300, 395, 410	gaaagggctggacatggata	atgttgttatttggcctgct
78	ChFaM 078	245, 285, 290, 300, 315, 390, 410	235	cagcctcattgcaaatctga	cttaccggtttcgatgtggt
79	ChFaM 080	530, 560	160, 540	ttcggtgccggtaaagatac	aagttccaccaccatgcaat
80	ChFaM 081	-	295, 300, 310, 340, 415	aactgagctctcggcaagtc	gaatactcgcggaggaagtg
81	ChFaM 082	230, 235	220, 245	agtacggcaattgaagctcg	gatctcggttgaagatcgga
82	ChFaM 083	220, 250	-	ttttctggcttttggattgg	gcacttttcatcacaggca
83	ChFaM 086	280	225	tttggagctcaatcccatctg	atttggccagcctccgtct
84	ChFaM 088	660	140	ggtggcaaaactcatggaga	gggaagcgaagttgaagagg
85	ChFaM 092	110	-	acccaagttcccttcgactc	atgcgctttgcataacaggt
86	ChFaM 093	285, 290, 300, 390, 395	-	cgccctcaaatccctctaac	gaagtgagtgttccgctgct
87	ChFaM 094	180	175, 185	atggagggcgctactgaaaa	aatggcgagcttggactttc
88	ChFaM 095	210	-	gccagaagcaaaaaccagaa	gggaagttgaaattgtcgga
89	ChFaM 097	275, 325, 250, 400, 450, 470	265	gccaaaggttggttctttga	gccattttggaagaggtgaa
90	ChFaM 098	305, 495, 520	205, 215	gtgagagtcagcccacccta	gcgacgaggatgaagaagag
91	ChFaM 100	100, 170	120, 190	ttggaacccagaaaatcgaa	cagcggagagagaaacaagg
92	ChFaM 101	150, 500	220, 240, 520	ggagtaagctgatcactctgt	actccgaggctgtaatccct

Tabela 1 cd.
Table 1 cont.

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

Sizes of PCR amplicons (segregating alleles) differentiating 'Elsanta' and 'Senga Sengana' genomes and the sequences of oligos generating the above heterozygous alleles.

Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
93	ChFaM 105	170, 180	160, 175	cctccaaacacaatccacca	tctgaggtttatgcgggact
94	ChFaM 106	170, 190, 230, 335	120, 410	accaaccgaggcgagagag	cgtcatctgcacctgcttc
95	ChFaM 107	-	500	tgccaaacaaacaaatgttga	catatcgatgtccttcataggg
96	ChFaM 108	520	170	catggaaagagagcgaaagc	cgaggaatgagggaggaatc
97	ChFaM 109	-	420, 450	aagctgcttgcgaagaacaa	ggctgatgccagtccattta
98	ChFaM 110	310, 605, 615	-	ttttcctttggtggttttgg	ccagagaaagccatttaggc
99	ChFaM 111	145, 180	135	gcccaaccgagtctctctct	cgggcttcaatttgctcaat
100	ChFaM 112	495	485	ttcaagcttttcttcttcctctc	catcttcacctgacccctct
101	ChFaM 114	-	145, 275, 495	tgtggtgccttggtttacaa	cgcctctctcctctctaatcc
102	ChFaM 115	460, 480	280, 285	cctcggcttcttcatctttg	accacactgacaggacgatg
103	ChFaM 120	130, 200, 210, 420	255, 300, 320, 330	ggtttcatcagagggcgtctt	taaagctcccagcaggcatt
104	ChFaM 122	245, 255, 260	-	gactcacagtctctcccagtgt	tgatattgagcaacgggtga
105	ChFaM 126	270	-	gttagggagtccgggaatga	ttccaatcccatctgacgac
106	ChFaM 129	195	-	agatcaacatcgcctccaac	tgctcgttgtccataacctg
107	ChFaM 130	240, 260, 300	195, 310	gccagtcacaagaagccaaa	tgctctgaaaccctttatctg
108	ChFaM 144	390,	-	cacgtcttggcttcctcttc	cggagcagattttcttggag
109	ChFaM 146	170	150, 155	acgagggaagaatggagcac	agatggtcctgactggatgg
110	ChFaM 147	350, 360	-	acgagggtcacctgagactg	ccaggagaaggtaccgaagg
111	ChFaM 148	-	210	ccctccatcaaagccagtt	cattagaccccgacttgtca
112	ChFaM 151	200	-	accaccaccgttttctcctc	accaccgactcgtccttctt
113	ChFaM 159	285	-	tctctctcatcgccccagag	acccatccacagggttcttg
114	ChFaM 160	-	300, 480	ccactccccaaaagagcaca	ctgcctccacaatcctcacc
115	ChFaM 161	210, 510, 615	270	cgaggccttgtcttctttgt	gcggaggtagctgttgtagc
116	ChFaM 163	150, 265	155	ttcgggtcttgtattgctttga	ttcaattccgaaggcacaac
117	ChFaM 164	235	200	cactcagccagatccagagc	gcgccaaggatggtcttaat
118	ChFaM 174	140, 175	160, 195	gagggagatggcacggagat	ctccggcattgaaatcgaga
119	ChFaM 177	245, 265, 400	-	cctgcagagtgcaagagaga	cactcacaaggcatgagagcta
120	ChFaM 178	165, 280, 325	320	aaacgaagaaaggaagtcttcaa	agctgtgcaggggtttatgt
121	ChFaM 194	-	175, 195	cccttccttgccagtcattt	cccatcccacagttgttctc
122	ChFaM 196	-	305	cctctcacctctttctctctaaaa	agcgcatgagtctgctgtta
123	ChFaM 203	480	-	cgagggtcacggctactaaa	tgagtatgaccaatccgaaaga
124	ChFaM 209	-	295	cccccaaaaccctctattct	atcattcccaagccactgtc
125	ChFvM 028	168, 505	170, 507	aatggcatcaacttctgcac	cagcctgctgctgtagttct
126	ChFvM 049	-	500, 505	atggttggtgatcaatggtg	ttcatatgcaatttgatggaac
127	ChFvM 087	280, 295	300	gaaaggggaagccttttcat	tgggacgaaagttcccaata
128	ChFvM 125	-	150, 520	gcgactgccatcgtaaccta	tctccaatgttttcgccaag
129	ChFvM 140	-	185	ccacttcccataccactgga	cgtcgtaggttcggtctctg
130	ChFvM 181	175	155, 165	ggagaactgctttggtaggg	acgtacaccagcagatgagc
131	ChFvM 182	400, 435, 515	470	ggaaccaaacgaacaccaac	gcggaggagtgagtgaagac
132	ChFvM 184	255	235, 240, 340, 350, 600	gcactttggggttttgggta	ttgcaagaaccccccttccta
133	ChFvM 191	245, 535	255, 285, 300, 350	ccagcagaatcctcaaatgc	gtgccaacaagcccattg
134	ChFvM 192	-	265, 275, 450	tgaggtcagtctgtgagaattg	tttccgagagtggagagcat

Tabela 1 cd.
Table 1 cont.

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

Sizes of PCR amplicons (segregating alleles) differentiating 'Elsanta' and 'Senga Sengana' genomes and the sequences of oligos generating the above heterozygous alleles.

Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
135	ChFvM 193	-	140	catcagaaccatcaatcatcg	tactgccggagaatgaaca
136	ChFvM 201	-	245, 285, 325, 335	tgattcaactccagcgaagc	atcagcaggcgaatccttct
137	ChFvM 202	225, 235	-	caaagggctccagctatctc	aggatcgtcagctaagaagca
138	ChFvM 205	220	125, 185	gcgaaaccctatggattgtt	acaaccaccaaattcccatt
139	ChFvM 210	-	185, 265, 540	tccccacattttccttgttt	gtggtgttggtgagtggaga
140	ChFvM 212	150, 170, 190	195	caaatcttcaacggtctctcc	acggaggaggaggaagtcat
141	ChFvM 213	195, 245, 330, 350, 450	280	aaccctaggaggctgaaacc	atacccgggtggtacttgt
142	AW06432	250, 270, 280, 500, 570	-	tgagctgaagaaggtgctga	aagggaaactggaggtagcaa
143	CO378933	150, 500	-	cgaggcttgtctctgtgttg	cacgaccttgacacccttct
144	CO379009	105	-	tgtgattgggagagaggagg	ctgccccaaacttggtttta
145	CO379012	-	145	cacgaggattgtttgaaccc	accaacacacaaagctgctg
146	CO379659	250, 400	140, 200	aggggaggcctcacttagag	tatccatgagatcccagcct
147	CO380151	-	150	cgagggtttgatcgagtctg	aacatgatcacaaggccaca
148	CO381174	275	445, 455	ccacaagaaaggagacgagc	tcaggagcatgaatcaatcg
149	CO381539	272	270, 265	gtctgcccctgttacgctac	ctgtgtagctccggcacata
150	CO381605	-	300	ccacccctttacctttcaca	caattccgaaggcacaactt
151	CO381897	230	-	agaggctgaggatcatggtg	ggcaaatacaatgctaaacca
152	CO382008	-	125, 145, 385	gcccttgcttagttgctttg	atgcatggctatggcttgtt
153	CO816733	275, 300, 400, 480, 570	310, 350	tcccaacacctcacttgtcc	attcagccaggtctgagcat
154	CO816806	-	205	cgagggagaaaccctaacct	ggacgatcccttgtagtgga
155	CO816840	270, 340	150, 215, 295, 305	cagccttgatgtctcgttga	ccatgacatcctctgccttt
156	CO816936	300	380, 520	ttctctccgatcttctccga	catcgactggcttctccttc
157	CO817004	220, 240	225	cgtcagccctaagaagatgg	acgaccaatacagaccaggg
158	CO817138	250	285, 500, 590	tgaaaacttttgctctgggc	tcaggccatgaaacactctg
159	CO817185B	195, 400, 510, 565	260, 500	tcatccactgggaagaaagg	catcaatcatgcacacacga
160	CO817242	-	150, 520, 525	aatccccaaatcctcaaacc	ctccacgctcttcttgttcc
161	CO817330	-	270, 405, 430, 505, 510, 550	gcttcatggcttcgttcttc	gtggcattcagttgggctat
162	CO817364	285, 295	260	gccttccccttcttcaaatc	gtccattttccagtggtgct
163	CO817505	120, 280	-	tcctgaagcaacgatgactg	cacttgccgcagaagaaaa
164	CO817509	130, 140, 195	215	tcaccgtcctccttctcaac	cgaagaggaaattgagccag
165	CO817772	-	500	tcacaaccgacgagtttcag	tttcttcactgccctgctct
166	CO817850	-	300	gtgttgagcaagaatggggt	tagtgcccagaatgaggctt
167	CO817919	270, 280	-	cagaatccaccggcttacat	cgctagcttttctgctcgat
168	CO818131	250, 315	295	ccttcctccgaaaccctact	gggctcaggttatacgagca
169	CX661091	180	235	aagccatgactacctccacg	atgaagccgaaatcgaaatg
170	CX661274	-	215	tataacaacgttgggccctc	tacgccagctcataccacag
171	CX661428B	295, 330	-	gaagacggtggatgaggtgt	ctgctgaaacccgaatccta
172	CX661749	-	500	tagatttttcccattccccc	atctgacccaacaaaaccca
173	CX661752	-	165, 175	acctgacctgaccaaaccag	tggggatgaggatgagagtc
174	CX661843	245, 515	-	ctcccatagatgcctcgaac	ttgaacagcgagaagtggtg

Tabela 1 cd.
Table 1 cont.

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

Sizes of PCR amplicons (segregating alleles) differentiating 'Elsanta' and 'Senga Sengana' genomes and the sequences of oligos generating the above heterozygous alleles.

Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
175	CX661893	-	190, 610	cgggttcttctacttcgtcg	gcccaaagacaggcctagat
176	CX661895A	100	-	gtagccatggaatcgtccac	actgcgagcaagtgtcttga
177	CX662065	170, 495	-	acagagagccagaaacggaa	agcgagagagagagcgaatg
178	CX662065A	-	190, 235, 360, 500	atgaccacagcaacctctcc	gcgtgtttgggattgagact
179	CX662153	115, 120, 175	130	gaaactcccccgaatttacg	tctcgggtgagctgagaaat
180	CX662162	290, 505	-	tcaccacatgaaaagttgcc	atagaaagagcggcacgaaa
181	CX662184	155	145, 160	acgctcttctttcactccca	gagagagatgtgcggagagg
182	CX662207	265,285, 290	310	agcagccggtcagatgatag	atcacggtcaaagccaactc
183	CX662235	220, 255, 340, 345	-	cctctcttccccacactcaa	gtagctctttccgaacgtgg
184	FvA 108	150	-	acaaagagaccaccaactacc	gatagccaaaagagcagtga
185	FvA 110	185, 245	255, 265	caacctttcctagcatcaactc	actcgatctgaaatcgttgtct
186	FvA 114	120, 270, 315	150, 295	attggctaatgaatctccg	ggcaagggaatggataata
187	FvA 115	98, 370, 450	100, 445	acaagttcgcagggtatga	gggaaaacacagaaatctcc
188	FvA 117	110, 295	-	ccattttacattgtcacaacg	gttagggtttcatgtggactc
189	FvA 119	280, 400	120, 150, 265	gcccttgatgacagaaaag	cccttcgttggagataatg
190	FvA 123	-	310	gcaatgttgttactgaaatgtg	agcatctcttagtgcctcatc
191	FvA 125	420	-	atccaagggctaagagagg	cagaaatacccaccaaagttc
192	FvA 127	95, 215	195	tctccctcatccacaatcaac	accggagtgaaaccctaattc
193	FvA 129	145	-	gaaaggagtctgttttgtgtcc	actgggctcatcatacgc
194	FvA 2	250, 270	252, 262	gttccatcacttacagacatcc	cctgattatgtgcctttattc
195	FvA 5	120	85, 135	aagggagagaagagagaaagtg	acaacctatcggctcattaac
196	FvA 7	-	550	cagtgaagacttccgcactag	gcatatcgtcattatgtgc
197	FvA 9	570, 580	610	gaggtgctccttgtgaatg	aacccatcaaccagtttgg
198	FvB 1	290, 315, 450, 480	620	agagcagaagaaaaccatagc	cctccctggaaatagatacac
199	FvB 101	175, 195, 480, 485	120	tcaagtctcgaccaaaataact	cgttgtcacttccattagagg
200	FvB 108	300, 455, 495	130, 215	ggttgacatgagtgcatattc	gaaggtataggagtgcaaagtg
201	FvB 110	215, 225	-	ctaccgcaccataatttagaag	catccaaatgaacctattcaag
202	FvB 111	270, 275, 280, 520	190, 195	ttaggttttgcgaagttgaag	aggagaaccgcgctagtg
203	FvB 112	165, 195, 205, 285, 600	290, 335, 250	ggcaacgaagagtaaggc	caaccataataccctcattttc
204	FvB 113	-	230	tgacccagaaatgaaagaagg	agaaagaacctgctgcttgag
205	FvB 116	200	215, 225	cctccatccctaactttcc	gctctttgtcttcgtcagc
206	FvB 119	330, 385	380, 390	acccattgccatccctact	ccacctgaacaaccaccac
207	FvB 120	220, 240	-	ataccctcatctcttgctcttg	gattcttcatgtgatcgtctga
208	FvB 123	-	360, 370, 390	gaaacacttttgcccttgtg	ggtttactgggggtcag
209	FvB 124	190, 220, 250, 260	205, 240	agacaaacaagcagcaatagtg	agaaagggaagtgcctaagtg
210	FvB 126	125	110, 180	gagcatcggcactacacc	catggctggcttcagatc
211	FvB 127	420, 500	265, 270	tttcaaggcaaatccactatc	gcatcggaagcatcaagt
212	FvB 129	510, 550	-	gcgatgaaacagtagcagc	gaagaagggggaagaatc
213	FvB 132	220, 250	260	aaggctccccctgtttag	gaacctgtaggctgtgtagaaa
214	FvB 2	230, 340, 350	600	ttgaggcacttgaaaaaagac	aagggaggtggaaaggtg
215	FvB 8	280, 300, 400, 420	275, 290, 320, 410	ggtgagtggtatttgcctaac	cttcattacggtggagtctg

Tabela 1 cd.
Table 1 cont.

Wielkości produktów różnicujących odmiany 'Elsanta' i 'Senga Sengana' (allele segregujące) w reakcji SSR oraz sekwencje starterów generujących powyższe allele heterozygotyczne.

Sizes of PCR amplicons (segregating alleles) differentiating 'Elsanta' and 'Senga Sengana' genomes and the sequences of oligos generating the above heterozygous alleles.

Lp. No.	Mrker Mol. marker	produkty różnicujące (pz) Size of the amplicons (pb)		starter F Farward Primer	starter R Revers Primer
		Elsanta	Senga Sengana		
216	FvC 10	295, 350, 380	255, 285, 300, 460	cctccatgtcgatcctcttatc	gccaattccgattattcttgag
217	FvC 103	200, 215, 250, 395	-	tcctccaactccaacttctc	cgaatctatgtcccctatcg
218	FvC 105	270, 350	-	gggtggtcaagtctccag	actccgattactgttttccag
219	FvC 107	205, 480	180	ggcaaattacatgatgaaccag	gaatggagtccttggagaactc
220	FvC 108	275	345, 255, 495	tcccgtgaacagtgtcag	agcaaagaggtagagaacgag
221	FvC 109	165, 210, 520, 590	260	agggcgagacttgaaatatg	aagccttacctggaaattacc
222	FvC 110	420	450	ctgtttcttcctcagattgttc	gtcattgtaactgctgtgtg
223	FvC 111	215, 260, 270, 290, 340, 415	200, 240, 315, 400	ccattcttcgcccccttaac	caaaactgtcactgcctcaaa
224	FvC 112	145, 160, 170	147, 150, 280	ggtacgaattgtgggaattg	cggcctgattgtattatctg
225	FvC 113	220, 250, 260, 270	180	ccctcctctctttctctttcc	ggcagacagatccgaga
226	FvC 12	-	190, 250	ggaggacagcgatagagatatg	gggtccatttctgtttaacaac
227	FvC 122	320, 385	300	tcttcatacccatacccactac	ccagacacccatctaattctac
228	FvC 123	170	140, 250	gaccacaaacccactaatc	caaacaccgagacttctgag

Tabela 2
Table 2

Liczba produktów SSR-PCR różnicujących genomy odmian 'Elsanta' i 'Senga Sengana'.

Number of SSR-PCR products differentiating 'Elsanta' and 'Senga Sengan' genomes.

Grupa starterów Group o teste markers	liczba par starterów testowanych z danej grupy Number of primer pairs tested from the merker group	liczba starterów w grupie, w reakcji z którymi obserwowano produkty polimorficzne Number of primers in the group in which polymorphic products were observed in the reaction	liczba produktów różnicujących odmiany Number of polimorfic PCR products	
			Elsanta	Senga Sengana
FvH	151	44	80	52
ChFvM	135	97	178	155
CO	48	27	35	42
CX	33	15	20	16
FvA	29	14	23	17
FvB	27	18	45	30
FvC	27	13	37	25

418 polymorphic alleles were identified in the genome of 'Elsanta', and 337 alleles in the genome of 'Senga Sengana'. The study confirmed the high degree of genetic heterozygosity of these two strawberry cultivars.

Analysis of the mapping population. Genetic map of the studied genomes.

SSR allele segregation analysis performed for the mapping population confirmed high degree of genetic heterozygosity of the progeny plants. PCR reactions with 44 selected SSR markers led to the identification of 53 polymorphic alleles, segregating in all generated 'E x SS' seedlings. The distribution and recombination frequency were analysed to identify the location and genetic distance of these alleles (Tab. 3). The collected data were used to construct a 'skeleton' of genetic maps of 'Elsanta' and 'Senga Sengana' cultivars.

The integrated genetic map constructed

for the 'Elsanta' × 'Senga Sengana' population contained a total of 27 linkage groups (LG), showing a high degree of homology to: chromosomes II and VI (LG2 and LG6 included a-d homologs), chromosomes I, III and VII (LG1, LG3 and LG7 included a-c homologs) and chromosomes IV and V (LG4 and LG5 included a and b homologs) of *Fragaria* genome.

In total, loci for 7 SSR markers on chromosome I, 8 on chromosome II, 10 on chromosomes III and VI, 5 on chromosomes IV and V, and 4 on chromosome VII we identified. The size of the mapped genome fragment (bin map) of stawberry was about 1 033.50 cM (Fig. 2).

Tabela 3
Table 3

Wyniki analizy rozkładu alleli i frekwencji rekombinacji w populacji mapującej oraz dystans genetyczny zidentyfikowanych loci markerów SSR.

Allele distribution, recombination frequency in the mapping population and genetic distance of identified SSR markers.

Grupa sprzęż. Linkage group	Locus1	Locus2	Frekwencja rekombinacji/odległość mapowa (cM) Recombination frequency (cM)	Typ segregacji alleli w populacji Segregation type of alleles	Test X2 Chi-square test	Rozkład alleli w populacji Alleles distribution in mapping population
LG1A	EMFn049	FvC103	19	nnxnp	0.8	3:1
	EMFn049	COBRA	25	nnxnp	2.2	3:1
	FvC103	COBRA	6	mlxll	7.8	3:1
LG1B	COBRA	CO817054	24,3	nnxnp	0.3	3:1
	CO817054	FEFv14–29	20,7	mlxll	4.9	3:1
LG1C	BFACT003	FvH4171	21,7	mlxll	0.2	3:1
	FvH4171	ARSFL002	28,3	nnxnp	0.6	3:1
	BFACT003	ARSFL002	50	nnxnp	10.4	3:1
LG2A	ARSFL012	CFVCT015	30	abxab	3.0	1: 2: 1
	ARSFL012	BFACT002	42	abxab	3.5	1: 2: 1
	CFVCT015	BFACT002	12	nnxnp	0.4	3:1
LG2B	EMFn214	CFACT111	34,3	mlxll	3.5	3:1
LG2C	BFACT002	ARSFL015	18,4	mlxll	0.1	3:1
LG2D	FvH4173	CFVC0316	24,3	mlxll	1.7	3:1
LG3A	UDF0001	FvH4161	18,4	nnxnp	4.3	3:1
	UDF0001	FvH4155	26,6	mlxll	0.2	3:1
	UDF0001	FvH4177	46,4	mlxll	0.4	3:1
	UDF0001	FvH4165	70,7	mlxll	2.1	3:1
	FvH4161	FvH4155	8,2	nnxnp	0.2	3:1
	FvH4161	FvH4177	28	nnxnp	0.0	3:1
	FvH4161	FvH4165	51,6	mlxll	0.8	3:1
	FvH4155	FvH4177	19,8	mlxll	0.7	3:1
	FvH4155	FvH4165	43,4	nnxnp	2.3	3:1
LG3B	FvH4177	FvH4165	23,6	mlxll	2.6	3:1
	FvH4169	BFACT036	44,3	mlxll	0.4	3:1
LG3C	FvH4163	FvH4153	36	nnxnp	3.3	3:1
	FvH4163	BFACT045	68	nnxnp	1.8	3:1
	FvH4153	BFACT045	32	abxab	5.8	1: 2: 1
LG4A	EMFn132	FvH4164	34,3	abxab	0.3	1: 2: 1
	EMFn132	FvH456	42	abxab	3.6	1: 2: 1
	FvH4164	FvH4156	8	nnxnp	0.4	3:1
LG4B	CO816733	EMFv007	21,7	nnxnp	0.0	3:1

Tabela 3 cd.
Table 3 cont.

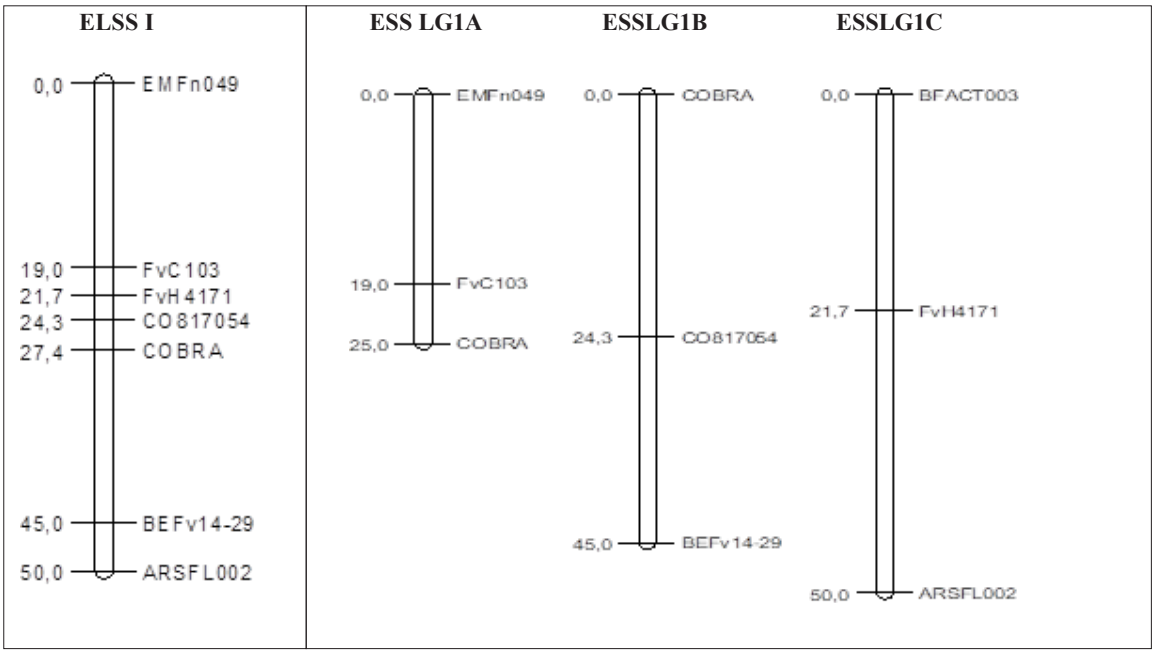
Wyniki analizy rozkładu alleli i frekwencji rekombinacji w populacji mapującej oraz dystans genetyczny zidentyfikowanych loci markerów SSR.

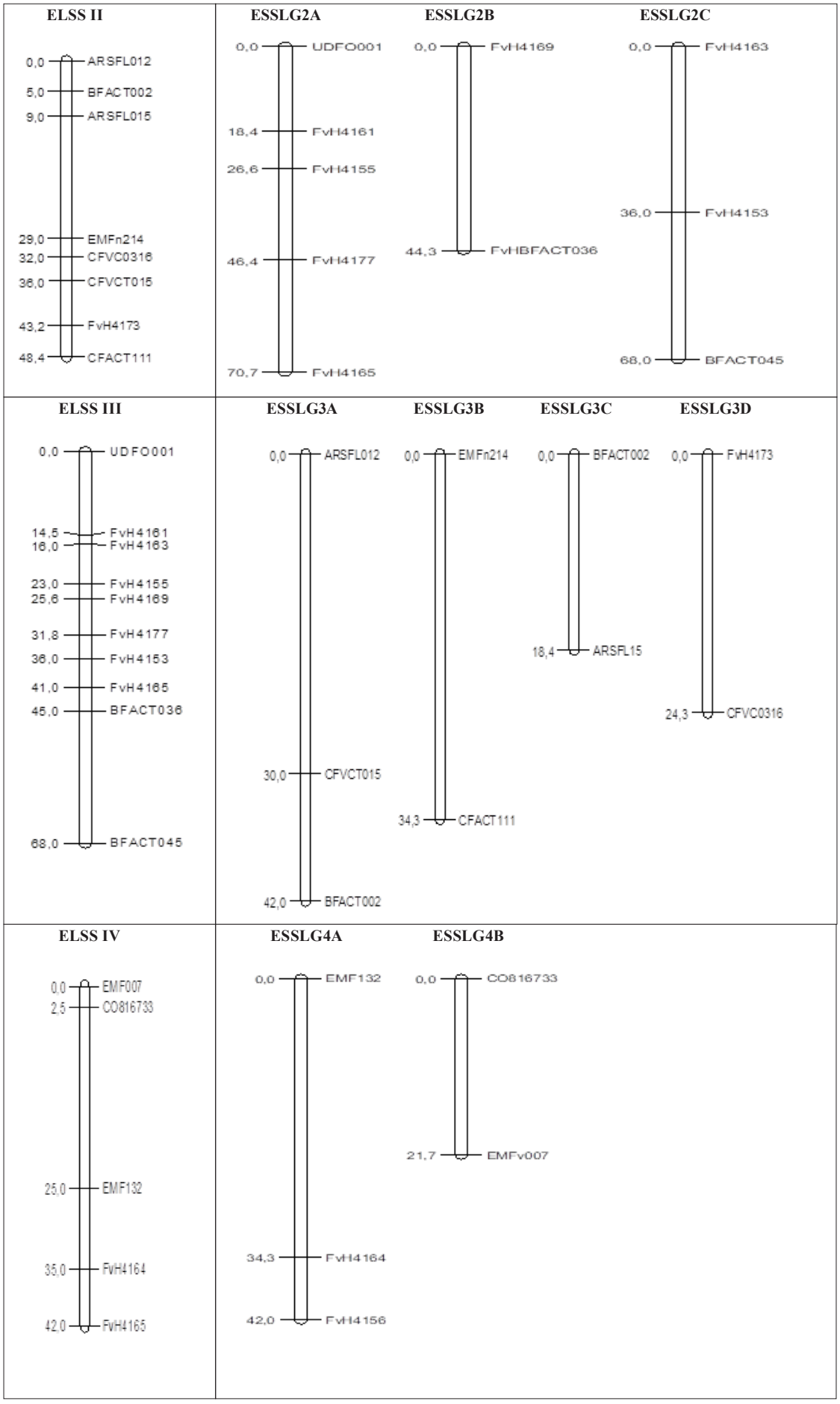
Allele distribution, recombination frequency in the mapping population and genetic distance of identified SSR markers.

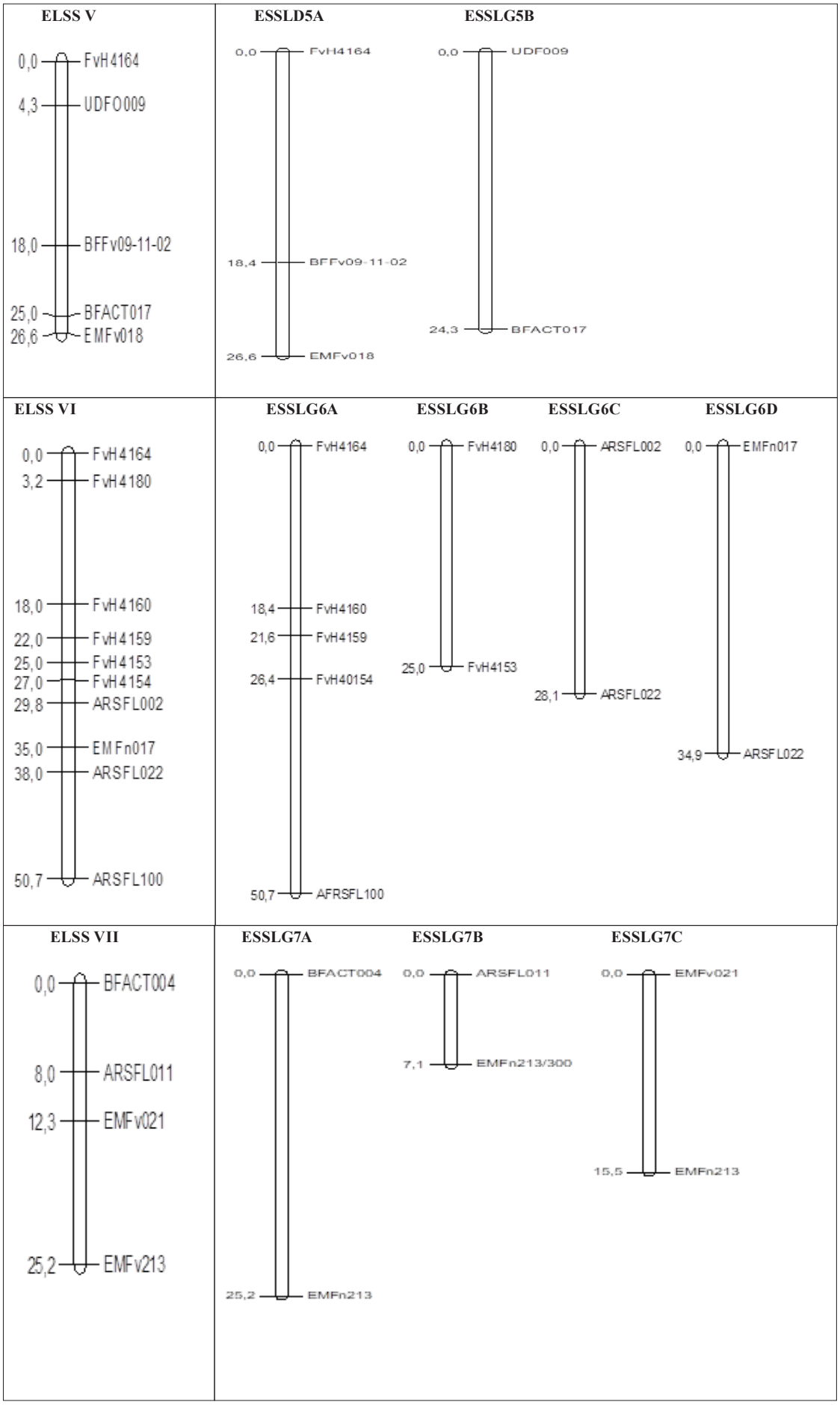
Grupa sprzęż. Linkage group	Locus1	Locus2	Frekwencja rekombinacji/odległość mapowa (cM) Recombination frequency (cM)	Typ segregacji alleli w populacji Segregation type of alleles	Test X2 Chi-square test	Rozkład alleli w populacji Alleles distribution in mapping population
LG5A	FvH4164	BFFv09–11–02	18,4	nnxnp	4.1	3:1
	FvH4164	EMFv018	26,6	mlxll	0.2	3:1
	BFFv09–11–02	EMFv018	8,2	mlxll	5.5	3:1
LG5B	UDF009	BFACT017	24,3	nnxnp	1.7	3:1
LG6A	FvH4164	FvH4160	18,4	mlxll	12.6	3:1
	FvH4164	FvH4159	21,6	nnxnp	2.0	3:1
	FvH4164	FvH4154	26,4	nnxnp	4.9	3:1
	FvH4164	ARSFL100	50,7	mlxll	0.5	3:1
	FvH4160	FvH4159	3,2	nnxnp	0.3	3:1
	FvH4160	FvH4154	8	mlxll	2.9	3:1
	FvH4160	ARSFL100	32,3	mlxll	0.6	3:1
	FvH4159	FvH4154	4,8	nnxnp	0.1	3:1
	FvH4159	ARSFL100	29,1	mlxll	4.3	3:1
	FvH4154	ARSFL100	24,3	abxab	0.6	3:1
LG6B	FvH4180	FvH4163	25	mlxll	2.5	3:1
LG6C	ARSFL002	ARSFL022	28,1	abxab	1.1	1: 2: 1
LG6D	EMFn017	ARSFL022	34,9	nnxnp	0.0	3:1
LG7A	BFACT004	EMFn213	25,2	nnxnp	2.4	3:1
LG7B	ARSFL011	EMFn213	7,1	nnxnp	5.2	3:1
	EMFv021	EMFn213	15,5	mlxll	0.2	3:1

Rys. 2. Schemat szkieletu zintegrowanej mapy 'Elsanta' × 'Senga Sengana'.

Fig. 2. The 'skeleton' (bin map) of the integrated genetic map of 'Elsanta' × 'Senga Sengana'.




ELSS II
0,0 ARSFL012
5,0 BFACT002
9,0 ARSFL015
29,0 EMFn214
32,0 CFVC0316
36,0 CFVCT015
43,2 FvH4173
48,4 CFACT111
ESSLG2A
0,0 UDFO001
18,4 FvH4161
26,6 FvH4155
46,4 FvH4177
70,7 FvH4165
ESSLG2B
0,0 FvH4169
44,3 FvHBFACT036
ESSLG2C
0,0 FvH4163
36,0 FvH4153
68,0 BFACT045
ELSS III
0,0 UDFO001
14,5 FvH4161
16,0 FvH4163
23,0 FvH4155
25,6 FvH4169
31,8 FvH4177
36,0 FvH4153
41,0 FvH4165
45,0 BFACT036
68,0 BFACT045
ESSLG3A
0,0 ARSFL012
30,0 CFVCT015
42,0 BFACT002
ESSLG3B
0,0 EMFn214
34,3 CFACT111
ESSLG3C
0,0 BFACT002
18,4 ARSFL15
ESSLG3D
0,0 FvH4173
24,3 CFVC0316
ELSS IV
0,0 EMF007
2,5 CO816733
25,0 EMF132
35,0 FvH4164
42,0 FvH4165
ESSLG4A
0,0 EMF132
34,3 FvH4164
42,0 FvH4156
ESSLG4B
0,0 CO816733
21,7 EMFv007


ELSS V
0,0 FvH4164
4,3 UDFO009
18,0 BFFv09-11-02
25,0 BFACT017
26,6 EMFv018
ESSLD5A
0,0 FvH4164
18,4 BFFv09-11-02
26,6 EMFv018
ESSLG5B
0,0 UDF009
24,3 BFACT017
ELSS VI
0,0 FvH4164
3,2 FvH4180
18,0 FvH4160
22,0 FvH4159
25,0 FvH4153
27,0 FvH4154
29,8 ARSFL002
35,0 EMFn017
38,0 ARSFL022
50,7 ARSFL100
ESSLG6A
0,0 FvH4164
18,4 FvH4160
21,6 FvH4159
26,4 FvH40154
50,7 AFRSFL100
ESSLG6B
0,0 FvH4180
25,0 FvH4153
ESSLG6C
0,0 ARSFL002
28,1 ARSFL022
ESSLG6D
0,0 EMFn017
34,9 ARSFL022
ELSS VII
0,0 BFACT004
8,0 ARSFL011
12,3 EMFv021
25,2 EMFv213
ESSLG7A
0,0 BFACT004
25,2 EMFn213
ESSLG7B
0,0 ARSFL011
7,1 EMFn213/300
ESSLG7C
0,0 EMFv021
15,5 EMFn213

Discussion

Strawberry (*Fragaria* × *ananassa* Duch. ex Rozier) is an allopolyploid (octoploid 2n=8x=56) (Davis et al. 2007), and its polyploidization during evolutionary processes probably led to disturbances in the number of chromosomes finally formatted in gametes (Hancock 1999). In addition, the strawberry genome probably was formed from an accidental hybridization of two species of wild strawberry *Fragaria chiloensis* Ehrh. (Chile) and *Fragaria virginiana* (USA).

Cytogenetic and molecular studies developed several models of allopolyploid genomes of strawberry. According to annotations Y1Y1Y1Y1ZZZZ/ Y1Y1Y1'Y1'ZZZZ (Rousseau-Gueutin et al. 2009) and AAAABBCC (Fedorova 1946), the *F.* × *ananassa* genome may consist of two (Y1, Z) or three types of sub-genomes (Y1, Y1', Z or A, B, C), and the annotation AAA'A'BBB'B' (Bringhurst 1990) even suggests the possibility of four (A, A', B, B') existing ones (Hummer and Janick 2009).

Because of the high complexity and random arrangement of the genome, strawberry is a very difficult object for advanced genetic and breeding research, and therefore reports on the interactions between the genes determining different traits of *F.* × *ananassa* are lacking. Nevertheless, attempts to construct saturated genetic maps with "located" genes, determining specific traits on relevant chromosomes have been made in recent years in several cooperating centres of Europe, USA and Japan (Kole and Abbott, 2008, Sargent et al., 2011). These resulted of generation of strawberry reference maps, for diploid plants *F. vesca* and *F. bucharica* FV × FB, (Sargent et al. 2006, 2007, 2008; Zorrilla-Fontanesi et al. 2011) and octoploid *F* × *ananassa* (Isobe et al. 2013, Sargent et al. 2012; Davik et al. 2015). These maps are saturated with numerous microsatellite markers which (taking into account the principle of collinearity from 15 to 80%, Mohamed 2014) developing the database for the construction of genetic maps of new *Fragraia* varieties.

The critical point in generating genetic maps is the selection of the proper segregating population. The use of the plant set created by crossing parental forms without sufficient divergence regarding phenotypic and genotypic terms leads to problems with the interpretation of the type of allele segregation, as well as incorrect detection of loci on the map (Liebhard and Gessler, 2000; Rungis et al., 2005; Semagn et al., 2006; Kole and Abbott, 2008; Myles et al., 2009). The simplest method of development of specific mapping populations is the cross pollination between highly heterozygous parental forms (encoded as CP: cross pollination, also used in our study). Although, depending on the type of inheritance of allele for the traits of interests, other populations, such as F_2, BC or $F_{2:3}$, created by crossing homozygous genotypes also have been used (Knapp et al. 1990; Reiter et al., 1992; Yu et al., 2000; Philips and Vasil, 2001; Vinod, 2006; Hittalmani et al., 2008; Kole and Abbott, 2008). The CP population is usually created by controlled intraspecific crossing of genotypes of known origin. An insufficiently high degree of polymorphism of parental forms and the presence of progeny from uncontrolled pollination (associated with non-selfing) may be critical for the analysis of alleles, co-segregating in mapping populations (Van Ooijen and Voorrips, 2001). Therefore, starting the genetic mapping work, the genomes of 'Elsanta' and 'Senga Sengana', were firstly: assessed regrarding the degree of heterozygosity and secondly: the genetic status of hybrids obtained by crossing of 'Elsanta' and 'Senga Sengana' was analysed.

The comparative analysis of band patterns obtained in the SSR-PCR assays on the DNA templates from the parental forms and hybrid genotypes confirmed that all analysed seedlings originated from controlled pollination. In addition, the high degree of polymorphism between the crossed parental genotypes observed in the SSR-PCR assays indicates a significant level of heterozygosity for the 'Elsanta' and 'Senga Sengana' cultivars, which confirms their suitability for the development of CP population used for the construction of genetic maps.

Another important element of population analysis useful for the construction of genetic maps of plants is the selection of molecular markers (Ritter et al., 1990, Liebhard and Gessler 2000).

According to the definition proposed by Schulman et al. (2004), markers should be linked to the trait, easy to detect in the analysed plant material, and inherited according to Mendelian principles. Another definition also assumes that these markers should reflect the alleles of each of the genes/genome fragments they represent (Griffiths et al. 1996). Therefore, in our study we used only molecular markers that clearly differentiated the crossed parental forms, and the heterozygous alleles identified for them were segregated in the hybrid genotypes.

The created 'E x SS' genetic map contains 27 linkage groups representing the loci of 53

polymorphic alleles, covering 1 033 cM of the strawberry genome. The reference map prepared for the octoploid 'Redguntlet' and 'Hapil' cultivars contains 30 linkage groups (homologous chromosomes) (Sargent et al. 2012), and for 'Sonata' and 'Babette' – 31 linkage groups representing seven chromosomes of the *Fragaria* genome (Davik et al. 2015).

The created map is an important basis for further research on the identification and confirmation of regions with Quantitative Trait Loci (QTL) in the genomes of the analysed 'Elsanta' and 'Senga Sengana' cultivars.

Knowledge of the location of precise sequences in the genome allows for the identification of potential molecular markers regulating important traits of *Fragaria*, useful for the early selection of breeding genotypes and improving the conventional breeding methods through the implementation of Marker-Assisted Selection (MAS) procedure (Sargent et al. 2012).

Previous studies conducted, for example, for 'Hanoye' cultivars confirmed the presence of QTL regions containing markers linked to the quality of strawberry fruit, identified within chromosomes II, IV, V and VII (Zorrilla-Fontanesi et al. 2011, Verma et al. 2017) and resistance to various pathogens, confirmed in the regions of chromosomes III and VI (Denoyes Rothan et al. 2004, 2005; van Dijk et al. 2014; Davik et al. 2015).

Conclusions

1. The obtained population of progeny plants originating from the 'Elsanta' and 'Senga Sengana' cross is a valuable material for research related to the development of the strawberry genetic maps.
2. All seedlings in the mapping population represented genetic patterns indicating the phenomenon of segregation of alleles derived solely from the parental forms of 'Elsanta' and 'Senga Sengana'.
3. The created 'skeleton' map of 'Elsanta' × 'Senga Sengana' can be used for further saturation, gene localization and identification of QTL regions associated with important functional traits of strawberry.

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