PCo-A ANALYSIS OF STRAWBERRY GERMPLASM USED IN EUROPEAN BREEDING PROGRAMS, BASED ON EVALUATION OF DNA POLYMORPHISM OF INVESTIGATED PLANTS

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A B S T R A C T

The level of genetic relatedness of ninety-six strawberry cultivars, released in different breeding centres of seventeen countries, was estimated based on analysis of their DNA polymorphism. Five hundred fifty-eight polymorphic amplicons, with a size range from 80 to 2600 bp, were generated in PCRs carried out on the template of DNA isolated from plants representing all analyzed cultivars. In RAPD reactions, polymorphic bands covered 58% of the total number of PCR products, while in ISSR, SSR and selective AFLP, the polymorphic DNA fragments covered 75%, 70% and 67% of all amplicons, respectively. Data concerning DNA polymorphism were assembled using the PCo-A method (*Principal Component Analysis*), and then referred to information about country of origin and pedigree described by the breeders. The results showed that contemporary breeding uses genetic resources in a very narrow range. Consequently, the cultivars released in individual breeding centres presented a very close relationship and were grouped in one, or at most two, genetic clusters.

Key words: genetic relatedness, *F.* x *ananassa*, cultivars, RAPD, SSR, ISSR, AFLP markers

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.), an accidental hybrid of two native species *F. chiloensis* and *F.virginiana*, is one of the most important small fruit crops cultivated in almost all regions of the world (Staudt, 1989; Hancock et al., 2008). Breeding of the species was initiated

with selection of hybrids of *F. chiloensis* x *F. virginiana* and hybrids obtained from crosses with other wild representatives of *Fragaria* genus. The breeding was undertaken by amateurs and private firms in England in the XIX c. to obtain plants producing big fruits (Staudt, 1989; Darrow, 1966). Since the middle of the XX c., strawberry breeders have been focused on improving the plant's resistance to diseases, productivity and local adaptation, as well as on improving fruit quality (Hancock et al., 2008). However, in contrary to the first works, the contemporary breeding is mainly based on cultivars that have been previously released by breeders and positively perceived on the market (Hancock et al., 2008; Hummer and Hancock, 2009).

At present, several hundred strawberry cultivars are grown commercially. Their sharing in the total fruit production is strongly correlated with acceptance of their phenotypical traits by producers and consumers at the national levels (Korbin and Mezzetti, 2010). From the breeder's point of view, phenotypic characteristics are very useful for planning introgression of novel traits. Molecular dissections of potential parents and obtained progenies, however, have been recognized as a very important strategy in plant breeding, considering that inheritance process is based on molecular fundamentals (Tanksley et al., 1989; Nybom, 1990; Bassil and Lewers, 2009; Peace and Norelli, 2009). Revealing advantages of molecular tools, such as the relatively short time of assays and independence from environmental conditions, as well as the abilities for investigations of the genetic capability often invisible in the phenotypes, have additionally reinforced the status of molecular tools in modern agriculture (Weising et al., 2005; Hummer and Janick, 2009; Hummer and Hancock, 2009).

Since 2000, in the Research Institute of Pomology and Floriculture (RIPF), Skierniewice, Poland, numerous molecular techniques, known as useful for plant breeders, have been applied. Such techniques have been used at the Institute, for identification of fruit and ornamental plants, for estimation of their relationship and the level of genetic distance between genotypes, as well as for searching the donors of genes coding desired traits. In this paper, we showed the results of the study on molecular relatedness of 96 strawberry cultivars that have been released in the breeding centres of seventeen countries and used as the parental forms in previous and current crossing programs.

MATERIAL AND METHODS

Plant material

Ninety-six cultivars grown in the strawberry field collection of the Department of Fruit Plant Breeding (RIPF) were used for the study (Tab. 1). Each genotype was represented by three plants. Two grams of young leaves were collected separately from each plant and kept at a temperature of -70 ˚C until the DNA extraction.

DNA extraction

Total DNA was extracted from plant samples using the method described by Doyle and Doyle (1990). Plant tissues ground in liquid nitrogen were incubated for 30 minutes at 65 ˚C in extraction buffer (2% CTAB, 100 mM Tris HCL, pH 8.0; 1.4 M NaCl, 20 mM EDTA, 2% PVP-40 and 0.2% β-mercaptoethanol). Nucleic acids were purified with chloroform/isoamyl alcohol (24:1) and phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with isopropanol, pelleted by centrifugation (30 min, 12 000 rpm, 4 ˚C), and then dissolved in 500 µl of TE buffer. RNA was degraded with 8 M LiCl at a temperature of -20 ˚C. Final DNA concentration in prepared samples was measured spectrophotometricaly at 260 nm (Gene Quant pro Amersham Pharmacia Biotech). Additionally, sample quality and nucleic acid concentration were estimated by electrophoresis in 0.8% agarose gel and comparison with λ DNA concentration standards (Invitrogen).

Polymerase chain reactions (PCR)

All enzymatic reactions were performed according to previously described protocols in a MJ research thermocycler (Kuras, 2010). Twenty six primers were used for RAPD amplifications (OPA: 03, 05, 07, 08, 09, 15; OPB: 04, 05, 06, 10, 12; OPC 08; OPF: 01 - 05; OPG: 02, 03, 05, 07, 08, 11, 12, 13, 16), twenty primers for ISSR (810, 811, 814, 818, 822, 823, 824, 825, 827, 836, 840, 841, 845, 848, 850, 853, 854, 857, 864, 895), eight primers for SSR (Fvi9 and 11, EMFv1, 2, 3 and 6, ARSFL33 and 35) and the eight primer combinations for selective AFLP reactions (*Eco*RI-act/*Mse*-cta, *Eco*RI-act/*Mse*-cac, *Eco*RI-acc/*Mse*-cat, *Eco*RI-acc/*Mse*-cag, *Eco*RI-acc/*Mse*-ctt, *Eco*RI-agc/*Mse*-cac, *Eco*RI-acg/*Mse*-cac, *Eco*RI-agg/*Mse*-ctc). RAPD and ISSR products were separated in 1.5% agarose gel, dyed with ethidium bromide and observed under UV light. AFLP products were visualized in white lights after electrophoresis in 6% denaturizing polyacrylamide gel and dyeing with silver nitrate. SSR products were separated in Bioanalyzer Agilent 2100, according to the manufacturer's protocol. All informative products of the PCR-based reactions were subjected to statistical analysis.

Molecular database analysis

The level of genetic relatedness among the analyzed genotypes was estimated by comparing DNA band patterns using Jaccard's coefficient of similarity (Tan et al., 2005). Matrices based on similarities were subjected to non-hierarchical clustering with the PCo-A method (*Principal Component Analysis*) (Davis, 1986). The NTSYSpc 2.2 program was utilized for cluster generating.

RESULTS AND DISCUSSION

Four types of PCR-based assays, that were conducted on a template of DNA isolated separately from each of the 96 genotypes, made it possible to identify some polymorphism within the analyzed genetic pool.

Table 1. Germplasm collection with pedigree and the country of origin described by breeders

No.	cultivar	Pedigree	Country of origin
1	'Aga'	'Honeoye' x 'Dukat'	Poland
2	'Alice'	'Korona', 'Totem', 'Holiday', 'Tioga', 'Redgauntlet', 'Gorella', 'Redchief', 'Wiltguard', 'Surecrop'	United Kingdom
3	'Ananasowa' (Surpise des Halles')	N – pedigree unknown	France
4	'Annapolis'	(K74-5) x 'Earliglow'	Canada
5	'Astra'	'Dana' x 'Real'	Poland
6	'Bogota'	('Climax' x 'Deutsch Evern') x 'Tago' Zb.53116 x 'Tago'	The Netherlands
7	'Bolero'	'Selva' x 'Rapella' or ('Redgauntlet' x 'Wiltguard') x ('Gorella' x 'Cardinal') x 'Selva'	United Kingdom
8	'Bounty'	'Jerseybelle' x 'Senga Sengana'	Canada
9	'Calypso'	'Selva' x 'Rapella'	United Kingdom
10	'Camarosa'	'Douglas' x Cal 85.218-605	USA
11	'Camino Real'	Cal. 89.230-7 x Cal. 90.253-3	USA
12	'Capitola'	Cn25(ca75121101) x 'Parker'	USA
13	'Carisma'	'Camarosa' x 'Parker'	Spain
14	'Cortine'	'Addie' x 'Pajaro'	Italy
15	'Cigaline'	'Gariguette' x 'Earliglow'	France
16	'Coral'	('Sunrise' x 'Gorella') x 'Earliglow'	Romania
17	'Dagmar'	'Festivalnaya' x 'Senga Sengana'	Czechoslovakia
18	'Dukat'	'Koralowa 100' x 'Gorella'	Poland
19	'Elista'	Self cultivar 'Jucunda'	The Netherlands
20	'Elkat'	'Elsanta' x 'Dukat'	Poland
21	'Elsanta'	'Gorella' x 'Holiday'	The Netherlands
22	'Emily'	'Honeoye' x 'Gea'	United Kingdom
23	'Eros'	'Elsanta' x 'Allstar'	United Kingdom
24	'Favette'	('S. Deshalles' x 'Regina') x ('Pocahontas' x 'Aliso')	France
25	'Feng Xiang Ming'	N	China
26	'Filon'	'Seal' x 'Selva'	Poland
27	'Florence'	('Tioga' x 'Redgauntlet') x ('Wiltguard' x 'Gorella') x 'Providence'	United Kingdom
28	'Fortune'	N	USA
29	'Fratina'	'Valentine' x 'Senga Sengana'	Germany
30	'Gaviota'	Chandler x Camarosa	USA
31	'Geneva'	NY -316 ('Streamliner' x 'Fairfax') x 'Red Rich'	USA
32	'Gerida'	'Elvira' x 'Elsanta'	Switzerland
33	'Gorella'	'Juspa' x US-3763	The Netherlands
34	'Heros'	'Gorella' x 'Dukat'	Poland
35	'Holiday'	'Raritan' x 'New York 844'	USA
36	'Honeoye'	'Vibrant' x 'Holiday'	USA
37	'Induka'	'Puget Beauty' x 'Senga Sengana'	The Netherlands
38	'Kama'	'Senga Sengana' x 'Cavalier'	Poland
39	'Kardinal'	'Haward 17' x 'Wahington'	USA
40	'Karel'	'Kama' x 'Real'	Poland
41	'Karmen'	'Gorg Soltwedel' x 'Sparkle'	Czech Rep.
42	'Kaster'	'Senga Sengana' x 'Valentine'	Poland
43	'Kent'	('Redgauntlet' x 'Tioga') x 'Raritan'	Canada
44	'Kimberly'	'Elsanta' x 'Parker' or 'Gorella' x Handler'	The Netherlands
45	'Korona'	'Tamella' x 'Induka'	The Netherlands
46	'Lambada'	IVT-76013 ('Silvetta' x 'Holiday') x IVT-74112 ('Karina' x 'Primella')	The Netherlands

Table 1			
47	’Laroma’	N	Germany
48	’Luna’	‘Selva’ x ‘Real’	Poland
49	‘Madeleine‘	‘Miranda‘ x ‘Addie‘	Italy
50	‘Magura’	(’S. Sengana’ x ’Talisman’) x ’Gorella’	Slowakia
51	‘Majoral’	‘Harvester’ x ‘Gariguatte’	France
52	‘Mara des Bois‘	(‘Gento’ × ’Ostara’) × (‘Redgauntlet’ × ’Korona’) or (‘HummiGento’ x ‘Ostara’) × (‘Redgauntlet’ × ‘Korona’)	France
53	’Maraline’	‘Sequoia’ x ‘Redgauntlet’	France
54	’Maria’	N	Slowakia
55	’Marianna’	N	Switzerland
56	’Marilyn’ (Maj 197)	‘Elsanta’ x ‘Marmolada’	Poland
57	’Marmolada’~’Onebor’	Nr 15 x ‘Gorella’	Italy
58	’Maxima’	N	Belgium
59	’Maya’	N	Italy
60	’Melody’	Nr 66 M1 x ‘Senga Sengana‘	United Kingdom
61	‘Miss’	(‘Honeoye’ x ‘Comet’) x ‘Dana’	Italy
62	‘Nadina’	N	Switzerland
63	‘Oda’	‘Inga’ x ‘Onebor’	Norway
64	‚Onda‘	Sel.83.52.1 x ‚Marmolada‘	Italy
65	‘Onebor’~’Marmolada’	Nr 15 x ‘Gorella’	Italy
66	‘Ostara‘	‘Redgauntlet‘ x ‘Macherauch’s Dauerente‘	
67	‘Otlichnica’	N	Russia
68	’Patty’	‘Honeoye’ x ‘Marmolada’~’Onebor’	Italy
69	’Pavana’	’Senga Tigaiga’ x ’Merton Dawn’	The Netherlands
70	’Pegasus’	’Redgauntlet’ x ’Gorella’	Switzerland
71	’Petrina’	N	
72	’Plena’	‘Senga Sengana’ x ‘Merton Dawn’	Poland
73	’Polka’	’Tuduka’ x ’Sivetta’	The Netherlands
74	’Priswita’	N	Russia
75	‘Purpuratka’	N	Germany?
76	‘Real’	(’Senga Sengana’ x ’Midway’) x ’Dukat’	Poland
77	‘Redgauntlet’	NJ-1051 x ’Climax’	United Kingdom
78	‘Roreal’	’Premial’ x ’Brio’	Romania
79	‘Rosie’	’Honeoye’ x {‘Cardinal’ x (‘Belrubi’ x ‘Holiday’)}	United Kingdom
80	‘Senga Gigana’	’Senga 341’ x (’Rotkappchen’ x ’Hansa’) x ’Fin’ (seedling of ’Fairfax’)	Germany
81	‘Senga Precosa’	’Regina’ x ’Senga 1260’	Germany
82	‘Senga Sengana‘	‘Markee‘ x ‘Sieger‘	Germany
83	‘Salut’	‘Selva’ x ‘Dukat’	Poland
84	‘Seal’	‘Senga Sengana’ x ‘Real’	Poland
85	‘Settler’	‘Guardian’ x ‘Holiday’	Canada
86	‘Shi Mei no 2’	‘Chunxiang’ x ‘Haiguan Zaohong’	China
87	’Shortcace’	N	N
88	’Spadeka’	N	Germany
89	’Syriusz’	’Troubadour’ x ’Kama’	Poland
90	’Ustoćnik’	N	Russia
91	’Velshebnica’	‘Festivalnaya’ x ‘Vola’	Russia
92	’Vikat’	‘Tarda Vicoda’ (‘Vicoda’) x ‘Dukat’	Poland
93	‘Vima Tarda’	‘Vima Zanta’ x ‘Tarda Vicoda’	The Netherlands
94	‘Vima Zanta’	‘Korona’ x ‘Elsanta’	The Netherlands
95	‘Vega’	‘Senga Sengana’ x ‘Valentine’	Poland
96	’Quang Ming Xing’	N	China

Among 396 DNA fragments sized from 80 to 1240 bp, and obtained in selective AFLP reactions with eight primer combinations, two hundred sixty-five bands were polymorphic. One hundred twenty-eight of 221 amplified bands showed polymorphism in random amplification with 26 selected RAPD primers. In PCRs amplifying microsatellites (SSR) and inter-satellite regions (ISSR), twenty-five (70% of all obtained bands with size 128-370 bp) and respectively, one hundred forty polymorphic DNA fragments (75%, size: 150-2030 bp) were generated for the 96 investigated genotypes. All total, over five hundred fifty polymorphic DNA bands diversifying the cultivars were found. Techniques allowing estimation of the genetic polymorphism in our experiments have been described by many authors as useful for molecular analysis of the plants, including *F.* x *ananassa* (Graham et al., 1996; Degani et al., 2001; Korbin et al., 2002; Tyrka et al., 2002; Arnau et al., 2003; Kuras et al., 2004; Kashyap et al. 2005; Levers et al., 2005). Each method represents different "affinity" to diverse DNA regions, ability of heterozygote distinguishing, and level of technical hardiness. This is why, different molecular techniques used for the same plant material may reveal either similar or different patterns of diversity, and genetic relationships (Powell et al., 1996; Mylborne et al., 1997; Nybom, 2004). In general, gathering molecular data from several systems that generate polymorphism increases the chance for proper interpretation of genetic relations (Weising et al., 2005).

The Principal Component Analysis (PCo-A), introduced in the next step of our investigations, has been successfully applied to study the relationship of genotypes belonging to different plant species e.g. pear (Monte-Corvo at al., 2000), mulberry (Vijayan et al., 2005), apple (Garkava-Gustavsson and Nybom, 2007), and raspberry (Debnath, 2007). In our study, the PCo-A of data characterizing the DNA polymorphism within the germplasm collection, resulted in creating nine clusters of genetic similarity. Two big clusters contained seventeen closely related genotypes that were known as cultivars derived from 'Senga Sengana' (1st cluster: 'Fratina', 'Korona', 'Kaster', 'Kama', 'Filon', Luna', 'Karel', 'Bounty', 'Induka', 2nd cluster: 'Seal', 'Real', 'Syriusz', 'Vega', 'Magura', 'Mara des Bois', 'Alice', 'Plena') (Fig. 1, Tab. 1). The next four clusters covered 21 derivatives of 'Gorella' (1st cluster: 'Aga', 'Dukat', 'Luna', 'Karel', 2nd cluster: 'Elkat', Heros', 'Coral', 'Kimberly', 3rd cluster: 'Seal', 'Real', 'Magura', 'Salut', 'Pegasus', 'Marilyn', 'Plena', 'Alice', 4th cluster: 'Gerida', 'Eros', 'Elsanta', 'Bolero', 'Astra'). One PCo-A cluster grouped five cultivars which originated from the genotype 'Gorella', and was related with 'Marmolada' ('Patty', 'Onda', 'Oda, 'Onebor', 'Marilyn'). The last two clusters of similarity contained nine derivatives of 'Holiday' (1st cluster: 'Alice', 'Rosie', 'Marilyn', 'Miss',

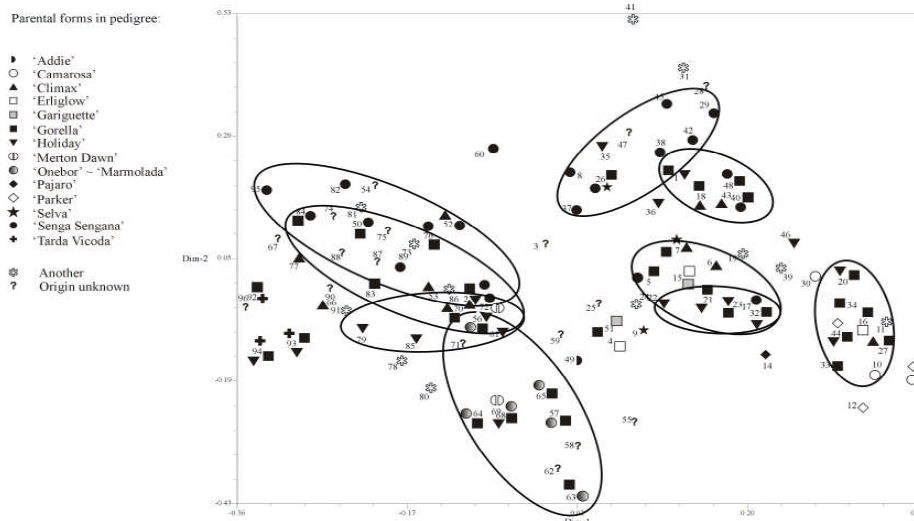

Figure 1. Relatedness of 96 analyzed cultivars estimated with the PCo-A referred to available information about their pedigree

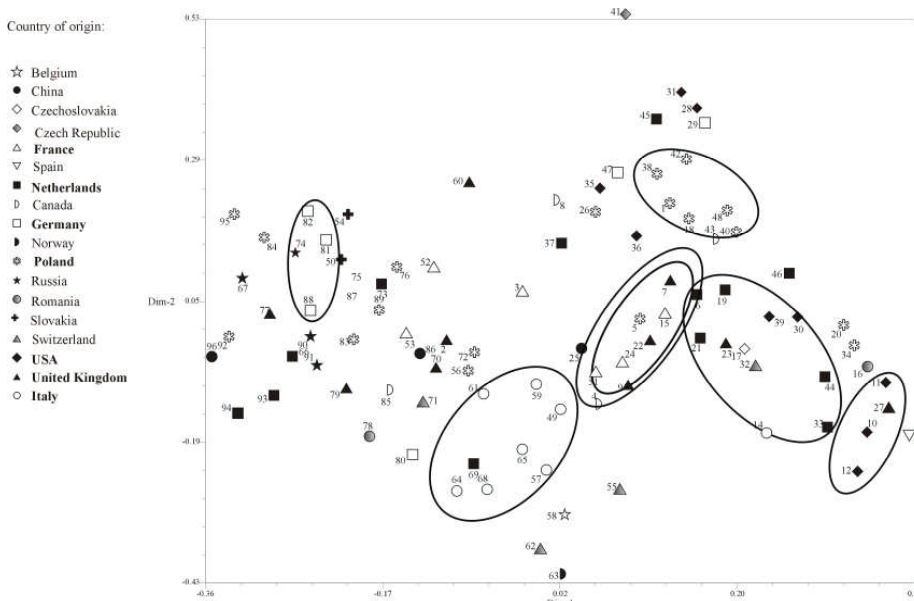

Figure 2. Relatedness of 96 analyzed cultivars estimated with the PCo-A referred to available information about country of their origin

'Settler', 2nd cluster: 'Elsanta', 'Emily', 'Gerida', 'Eros'). No direct correlations were found between PCo-A-based position of other analyzed cultivars and their pedigree characterized by breeders (Fig. 1, Tab. 1) what could

be a result of not fully recognized DNA polymorphism in available tests or it could be caused by breeder's mistake in the determination of parental forms (out crossing).

Summarizing, our results demonstrate that contemporary breeding uses genetic resources in a very narrow range. The majority of obtained PCo-A clusters, groups the derivatives of cultivars 'Gorella', 'Senga Sengana', 'Holiday' and 'Camarosa' representing old Dutch, German and American breedings (Darrow, 1966; Khanizadeh and Cousineau, 2005). As a consequence, the majority of analyzed cultivars bred in each country are closely related and create no more than two similarity clusters. Groups of these very closely related cultivars originated *inter alia*, from Italian ('Madelaine', 'Marmolada', 'Maya', 'Miss', 'Onda' and 'Patty'), Dutch ('Bogota', 'Elista', 'Elsanta', 'Lambada', 'Gorella' and 'Kimberly') and Polish breedings ('Aga', 'Dukat', 'Kama', 'Karel', 'Kaster' and 'Luna') (Tab.1, Fig. 2).

The presented results indicate some unexplored opportunities of genetic resource global transfer. Simultaneously, despite well-known obstacles in distant hybridization (Bringhurst and Voth, 1976, 1984; Hancock et al., 2008), it seems that world-wide strawberry breeding needs an extension of the genetic pool, which would also include valuable wild species (Klaus Olbricht, pers. com.).

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ANALIZA ODMIAN TRUSKAWKI UŻYWANYCH W EUROPEJSKICH PROGRAMACH HODOWLANYCH METODĄ PCo-A, NA PODSTAWIE OCENY POLIMORFIZMU DNA BADANYCH ROŚLIN

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STRESZCZENIE

Stopień pokrewieństwa 96 odmian truskawki, wytworzonych w różnych ośrodkach hodowlanych w 17 państwach, oceniono na podstawie wyników badań polimorfizmu analizowanych roślin. W reakcjach amplifikacji na matrycy DNA z analizowanych genotypów wygenerowano 558 polimorficznych amplikonów o wielkości od 80 do 2600 pz. W reakcjach RAPD polimorficzne fragmenty DNA stanowiły 58% łącznej liczby produktów PCR, w reakcjach ISSR – 75%, SSR – 70%, a w reakcjach selektywnych AFLP – 67%. Dane dotyczące polimorfizmu DNA zgrupowano, używając metody PCo-A (analiza składników głównych) i odniesiono do dostępnych informacji na temat rodowodu i kraju pochodzenia odmiany. Uzyskane wyniki wskazują, że generalnie współczesna hodowla korzysta z bardzo wąskiej puli materiałów wyjściowych. W konsekwencji, analizowane odmiany wykazały bardzo bliskie pokrewieństwo i w przypadku poszczególnych ośrodków hodowlanych były zgromadzone w pojedynczych, a maksymalnie w dwóch skupieniach.

Słowa kluczowe: pokrewieństwo genetyczne, *F.* x *ananassa*, odmiana, markery RAPD, SSR, ISSR, AFLP