

The use of RAPD markers for strawberry identification and genetic diversity studies

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Abstract

RAPD markers were used to identify and assess the genetic diversity level among 9 strawberry cultivars differing in reaction to photoperiod. 21 (29%) out of 73 RAPD markers obtained with 6 selected primers were monomorphic; 11 (15%) markers were specific to cultivars: 'Ambryo', 'Cambridge Favourite', 'Rapella', 'Teresa' and 'Senga Sengana'. The number of profiles revealed in the studies ranged from 3 to 9. Banding profiles obtained separately with E20, D6 and D7 primers were sufficient in order to identify all cultivars. The results obtained confirm the usefulness and suitability of RAPD markers for identification of strawberry cultivars. The mean similarity within analyzed strawberry genotypes was 53% and varied from 32% in the case of 'Cambridge Favourite' vs. 'Teresa' to 77% for the combination of 'Elsanta' and 'Redgauntlet'. On the basis of analyses performed, the progeny of a cross between cultivars 'Ambryo' with 'Teresa' or 'Senga Sengana' is expected to yield the highest polymorphism and thus is the most suitable for genetic map construction and identification of markers linked with photoperiod reaction.

Key words: DNA polymorphism, *Fragaria x ananassa* Duch., genetic diversity, RAPD.

Introduction

The diversity level in *Fragaria* spp. was evaluated using various systems such as morphological¹⁻³, chemical⁴, and biochemical⁵⁻⁷ markers. However, external factors such as temperature, humidity, light or the ages of plants can modify classification based on these systems. The polymerase chain reaction (PCR) is reliable tool for assessing of differences between organisms with simultaneous elimination of influence of environment. Randomly Amplified Polymorphic DNA (RAPD) technique⁸ was the most frequently used modification of this method. Strawberries (*Fragaria x ananassa* Duch.) have been extensively analyzed using randomly generated markers for clone identification and diversity studies⁹⁻¹⁴ (Table 1). The aim of the work was to verify the usefulness of the RAPD method for identification and genetic diversity studies in representation of 9 strawberry cultivars with different reactions to photoperiod with intention of selecting parents suitable for creating of mapping population.

Material and Methods

Experiments were carried out with 9 strawberry cultivars: one day-neutral 'Ambryo' and 8 short-day: 'Cambridge Favourite', 'Elsanta', 'Hapil', 'Honeye', 'Redgauntlet', 'Teresa', 'Rapella' and 'Senga Sengana'. DNA was extracted using the method of Doyle and Doyle¹⁵ as modified by Gawel and Jarret¹⁶. A PCR reaction was performed in a 25 mL volume consisting of 200 nM of each dNTP, 2.5 mM MgCl₂, 50 mM KCl, 0.02 U Taq DNA polymerase, 200nM of primer and 50-75ng of template DNA. Thermal profile conditions were as follows: initial denaturation 5 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 35°C, 2 min at 72°C, with a final elongation of 5 min at 72°C. Amplification products were separated on 1.5% agarose with EtBr staining. Photographs of gels were analysed using Scion Image Beta 2 software. The presence or lack of a band were considered as single trait and values 1 or 0 were

assigned respectively. Genetic pairwise similarities between analysed materials were estimated using the formula developed by Dice as in Nei and Li¹⁷: $F = 2N_{XY} / (N_X + N_Y)$ where N_{XY} is the number of bands shared by two compared genotypes X and Y, N_X and N_Y are the total number of bands observed in genotype X and Y, respectively. Based on the matrix of genetic similarity, cluster analysis was performed. The UPGMA method (unweighted pair-group method with arithmetic averages) was used for clustering employing the NTSYS-pc program¹⁸. Confidence values for nodes were obtained following the bootstrap procedure with the Winboot program¹⁹.

Results and Discussion

Cultivar identification: The primers used differed in size of amplified products (Table 2). The highest variation (370-2800 bp) was observed for D6 and the smallest one was for D17. 21 (29%) out of 73 RAPD markers obtained with 6 primers were monomorphic, (i.e. were observed in all cultivars tested). 11 (15%) markers were specific to cvs: 'Ambryo', 'Cambridge Favourite', 'Rapella', 'Teresa' and 'Senga Sengana'. Hancock et al.²⁰ obtained from 2 to 6 unique amplification profiles with each of 10 RAPD primers at the pool of 8 strawberry genotypes. Similarly Levi et al.¹⁰ observed from 3 to 8 DNA patterns with 8 primers tested that agrees with our results. Banding profiles obtained with E20, D6 and D7 primers were separately sufficient to identify all materials studied. These results suggested, that a small number of RAPD primers is sufficient to prepare genotype-specific banding patterns for cultivar identification. The results obtained confirm the usefulness and suitability of RAPD markers for strawberry cultivars identification and authors property rights protection.

Genetic diversity: RAPD markers allow for the estimating of genetic similarities between analyzed genotypes with the elimination of influence of environmental factors. The mean similarity within analyzed strawberry cultivars based on 52

polymorphisms was 53% and varied from 32% in case of 'Cambridge Favourite' vs. 'Teresa' to 77% for combination of 'Elsanta' and 'Redgauntlet'. Pairwise similarities between analysed strawberry cultivars were shown graphically in Figure 1. Two clusters were discernable; the first consisted of 'Teresa', 'Rapella' and 'Senga Sengana', while the rest of cultivars formed the second group with higher genetic diversity. According to Levi et al.¹⁰ the cultivars 'Rapella' and 'Cambridge Favourite' were similar at 77% as opposed to the 44% found in current studies. Graham et al.¹¹ observed an 85.3% average similarity between the cvs. 'Cambridge Favourite', 'Elsanta' and 'Honeye' what was about 30% more than observed in our studies. These discrepancies could be explained by the use of all mono- and polymorphic markers for similarity estimation in previous studies. The addition of monomorphic bands to similarity estimates raises them by 23%, which explains most of the differences observed. In spite of the wide range of available statistical methods and formulas that hinder the comparison of results, the RAPD markers seem to be a sufficient method for the genetic diversity estimation between *Fragaria x ananassa* cultivars.

Conclusions

The results obtained confirm the value of RAPD markers for strawberry cultivars identification, authors property rights protection and selection of parents suitable for creating of mapping population.

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Table 1. The use of DNA markers for identification and diversity studies in strawberry.

No of cultivars tested	Similarity range	No of polymorphisms	Reference
8	not assessed	10	Gidoni et al. ⁹
8	74% do 95%	25	Levi et al. ¹⁰
8	62% do 89%	79	Graham et al. ¹¹
75	not assessed	91	Landry et al. ¹²
41	0% do 90%	15	Degani et al. ¹³
19	53% do 88%	193	Degani et al. ¹⁴

Table 2. Characteristics of banding patterns obtained with selected primers.

Primer	Product			No of profiles	Markersize	
	polymorphic	monomorphic	specific		min	max
E20	10	4	1	9	520	2540
D4	3	2	0	4	850	1600
D5	9	8	3	8	380	2040
D6	12	5	4	9	370	2800
D7	16	1	3	9	460	2600
D17	2	1	0	3	680	1180
sum	52	21	5	9	370	2800

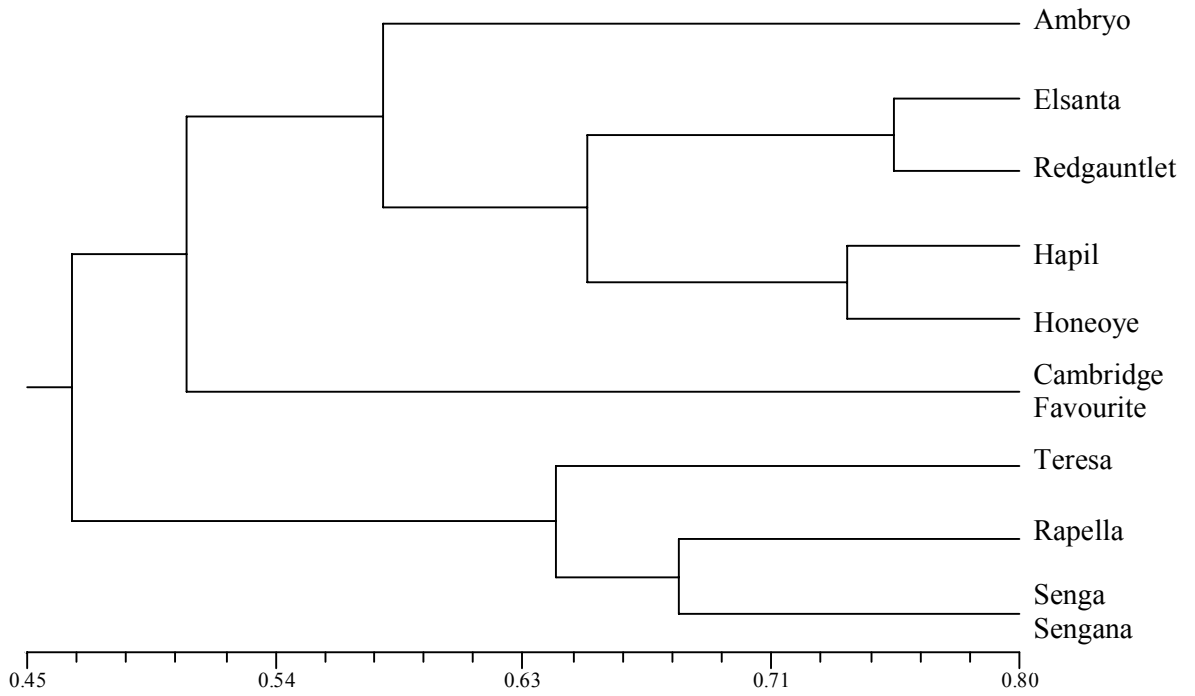


Figure 1: UPGMA dendrogram obtained on the basis of similarity values.