Theor Appl Genet (1993) 86:205–212

THEORETICAL AND APPLIED GENETICS

Identification and classification of celery cultivars with RAPD markers

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Received September 1, 1992; Accepted September 19, 1992
Communicated by H. F. Linskens

Summary. Twenty-one celery (*Apium graveolens L.* var. *dulce*) cultivars, one celeriac (var. *rapaceum*) and one annual smallage (var. *secalinum*) cultivar were screened for polymorphic RAPD (Random Amplified Polymorphic DNA) markers with 28 arbitrary 10-mer primers. Among a total of 309 bands observed, 29 (9.3%) were polymorphic in the 23 cultivars screened, but only 19 (6.1%) markers were polymorphic within the 21 type dulce cultivars. These markers were sufficient to distinguish each of the cultivars used. The average marker difference was 6.4 between two celery cultivars, 16.7 between celery and annual smallage, 14.7 between celery and celeriac, and 12.0 between annual smallage and celeriac. The celery cultivars surveyed were classified into three groups based on the marker differences. The relationship among the dulce-type cultivars concluded from this research is basically consistent with the known lineage of the cultivars and the previous study using stem protein and isozyme markers. RAPD technology provides a new alternative for cultivar identification and classification in celery.

Key words: Celery – RAPDs – Cultivar identification – Cultivar classification – *Apium graveolens*

Introduction

Development of cultivar-specific genetic markers is desirable for cultivar identification and protection, and seed purity determination. Traditional cultivar identification based on morphological traits requires extensive observations of mature plants and, in many situations, it lacks definition and objectivity (Wrigley et al. 1987). Furthermore, morphological traits cannot serve as unambiguous markers because of environmental influences. Molecular markers have been successfully developed in the last two decades to solve this problem.

Protein and isozyme electrophoresis has been used in many crops (Tanksley and Orton 1983) including celery (Quiros et al. 1987a, b). The major limitation of these techniques is insufficient polymorphism among closely related cultivars. Because proteins are the products of gene expression, they may vary in different tissues, developmental stages, and environments (Beckman and Soller 1983). On the other hand, DNA markers, such as RFLPs (restriction fragment length polymorphism), give a much higher degree of polymorphism and stability. The disadvantages of RFLPs are the laborious procedure involved, the relatively high cost, and the use of radioisotopes. As an alternative, the RAPD (randomly amplified polymorphic DNA) technique developed by Williams et al. (1990) and Welsh and McClelland (1990) provides a faster and easier approach for exploring genetic polymorphism, and also requires very small amounts of DNA. RAPD markers have been successfully used in cultivar analysis in other crops (Hu and Quiros 1991a).

Apium graveolens L. comprises three cultivated forms: celery (var. *dulce*), celeriac (var. *rapaceum*), and smallage (var. *secalinum*). In celery, several morphologic, isozyme and RFLP markers have been developed (Quiros et al. 1987b; Huestis et al. 1992). Four isozyme and six stem protein markers were used to classify 17 celery cultivars into 11 subgroups by Quiros et al. (1987a). We report here a survey of RAPD markers in celery cultivars and their application to cultivar identification and classification.

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Materials and methods

Plant material

Twenty-one celery cultivars, one annual smallage and one celeriac cultivar were used in this study (Table 1). Five to ten individuals of 10-week old were bulked in each cultivar for DNA extraction, except that 20 individuals were used for 'UC1', 'A112', and 'A143'. An F_2 population derived for the cross 'A112' (celeriac) × 'A143' (annual smallage) was screened for Mendelian segregation of the RAPD markers.

Determination of optimum sample size

In order to get a general estimation of the minimum number of plants for each cultivar that should be used to sample its genetic variability, three cultivars ('Tall Utah 52–75', 'Starlet', and 'UC1') were screened for the presence of specific RAPD markers with 14 primers among bulks generated from 5, 10, 20, 30, 40 and 50 individuals.

DNA extraction

Leaf tissue from 5 to 20 individual plants were bulked for genomic DNA extraction using the method described by Hu and Quiros (1991 b). DNA concentration was measured by a Beckman spectrophotometer at a wave length at 260 nm.

DNA amplification

Twenty-eight arbitrary 10-mer primers (20 primers of Set A, and primers B02, B08, B10, B11, B12, B13, B17 and B18 from Operon Technologies, Alameda, California) were used for the polymerase chain reaction (PCR) based on the protocol of Williams et al. (1990) with minor modifications. The reaction condition was: 1 × reaction buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0), and 0.01% triton X-100], 0.2 μM dNTP, 1.9 mM $MgCl_2$, 0.2 μM primer, 0.6 units of *Taq* polymerase, and 15 ng of genomic DNA. The final volume per reaction was 15 μl. DNA was amplified by using the following program: 94 °C for 2 min, 1 cycle; 92 °C for 1 min, 35 °C for 1 min, 72 °C for 2 min, 50 cycles; 72 °C for 5 min, 1 cycle, then followed by soaking at 10 °C. Amplified samples were loaded in 2.0% agarose gels which were then run at 4 V/cm. After staining in 0.5 μg/ml of ethidium bromide for 30 min, the gels were photographed under UV with Polaroid 667 films. All the reactions were repeated three times, and only the reproducible bands were considered in this study.

Nomenclature

Each amplified band was named by the primer used and its size in bp. For example, A10-1550 refers to the 1,550 bp band amplified by primer A10.

Data analysis

The microcomputer package PAUP (Phylogenetic Analysis Using Parsimony, version 2.4) developed by Swofford (Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Ill., USA) was used to calculate the pairwise difference matrix and the phylogram tree among the cultivars.

Table 1. Cultivars used in this study

Cultivar #	UCD accession #	Cultivar	Source
1	A33	Golden Self Blanching	Burpee Seed, Kenya
2	A35	Transgreen	Ferry Morse
3	A36	Tenderscrip	Ferry Morse
4	A37	Surepack	Ferry Morse
5	A38	Florida 2-14	Keystone
6	A39	Florida 683K	Keystone
7	A40	Tall Utah 52-70R	Keystone
8	A41	Tall Utah 52-70HK	Keystone
9	A43	Florimart	Keystone
10	A74	Summit	Ferry Morse
11	A76	Deacon	Moran Seeds
12	A77	Bishop	Moran Seeds
13	A93	Tall Golden Self Blanching	Royal Sluis, Holland
14	A203	UC1	U.C., Davis
15	A233	Tall Utah 52-75	Sunseeds
16	A255	Calmario	Harris Moran (Brendler)
17	A256	Ventura	Ferry Morse (Brendler)
18	A287	Conquistador	Pybas Seeds
19	A286	Matador	Pybas Seeds
20	A285	Picador	Pybas Seeds
21	A288	Starlet	Royal Sluis
22	A143	landrace (annual smallage)	Thailand
23	A112	landrace (celeriac)	Thailand

Results

Marker identification

A total of approximately 309 bands were generated by 28 decamer primers. The number of bands for each primer varied from 4 (A08) to 19 (B08), with an average of 11 bands per primer. The size of the amplified fragments ranged from 200 bp (A03) to 3,800 bp (A12). Among the 309 bands, 213 (68%) were constant among all the cultivars tested. Of the remaining 96 variable bands, 29 (9.3%) contributed by 14 (50%) primers were reproducible and selected as useful polymorphic markers. The remaining 67 bands were unstable and therefore were excluded. Nineteen out of the twenty-nine markers were polymorphic within the 21 celery cultivars. The other ten markers were monomorphic among celery cultivars, and polymorphic when celeriac ('A112') and annual smallage ('A143') were considered in the analysis (Table 2).

All the markers were scored by presence vs absence of a specific amplification product, except for B10-1200 which was scored by band intensity (Fig. 1). In this case, each lane showed either a weak or a very strong band (the strongest or one of the strongest within the lane) at the 1,200 bp position, with a highly reproducible pattern. For statistical purpose, strong bands were considered as present, and weak bands were scored as absent.

Determination of optimal sample size and homogeneity of celery cultivars

Three representative cultivars were selected for this purpose: 'Tall Utah 52-75', an old and uniform cultivar,

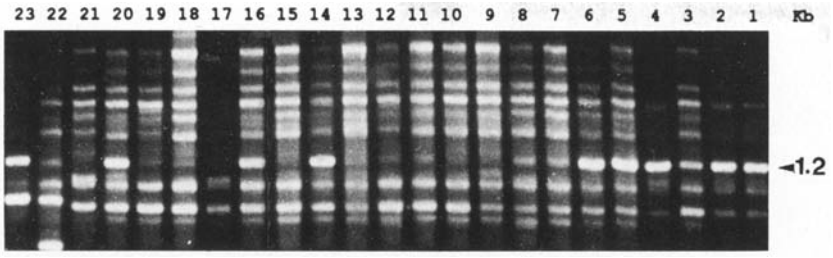

Fig. 1. RAPD profiles grenerated by primer B10. Numbers on top refer to the cultivars listed in Table 1. The marker B10-1200 (*arrow*) was scored as a strong ("+", *lanes 1–6, 14, 16, 20, and 23*) or weak ("−") band. This pattern was highly reproducible

Table 2. Survey of 29 RAPD markers in 21 celery (1–21) cultivars, one annual smallage (22), and one celeriac (23) cultivar. Refer the cultivar numbers to Table 1. "+" mans presence, "−" means absence

RAPD marker	Cultivar #																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A02-700	−	−	+	−	−	−	+	+	+	+	+	+	−	+	+	−	−	+	+	−	+	−	+
A05-2000	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	+	+	−	+	−	−
A07-830	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
A10-2100	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
A10-950	+	+	+	+	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	+	+	−
A10-510	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
A10-420	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	+
A13-1400	+	+	+	−	+	+	+	−	−	−	−	+	+	+	−	+	−	+	+	+	+	+	−
A18-2200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
A18-1700	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	+	−	−
A19-2500	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+
A19-1400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
A19-1000	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
A19-600	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
B02-2200	−	+	−	+	+	+	+	+	+	−	+	−	+	+	−	+	−	−	−	+	−	+	+
B02-1200	+	+	+	+	−	+	+	+	−	−	+	+	−	+	−	+	+	−	+	+	+	−	−
B08-1650	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	−	+	−
B08-1600	−	+	+	+	+	+	+	+	+	−	−	+	+	+	−	+	−	−	+	+	−	+	+
B08-1550	+	−	−	−	−	−	−	−	−	+	+	−	−	−	+	−	+	+	−	−	+	−	−
B08-830	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B08-800	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+
B10-1200	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−	+	−	−	−	+	−	−	+
B10-980	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
B10-950	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
B10-700	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
B12-430	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
B13-950	+	−	+	−	−	−	−	−	+	+	−	+	+	−	−	+	+	+	+	−	+	−	−
B17-650	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
B18-600	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−

'Starlet', a recently developed *Fusarium*-resistant cultivar, and 'UC1', a variable *Fusarium*-resistant breeding line. DNA samples bulked as indicated in Materials and methods were screened for variation with all 14 primers yielding polymorphic markers. Identical marker profiles were detected in 5–50 plant bulks in 'Tall Utah 52-75' and 'Starlet'. In 'UC1', variation was observed for A19-2500, which was absent in bulks of five and ten individuals and present in bulks of 20–50 individuals (data not shown). Based on these results, we used 5–20 plants to sample each cultivar.

Cultivar homogeneity was also surveyed by testing single individuals of 'Starlet' and 'UC1'. Twelve individual plants of each cultivar were scored with ten markers; as expected, 'UC1' had a much higher heterogeneity than 'Starlet' (Table 3).

Monogenic segregation of markers

All five markers followed in the 'A112' × 'A143' F_2 population segregated and fit the expected monogenic 3:1 Mendelian ratio (Table 4). The segregation of the marker A07-830 is shown in Fig. 2.

Cultivar-specific markers

Cultivar-specific markers are listed in Table 5. Only three celery cultivars could be distinguished from the rest by

Table 3. Number of 'UC1' and 'Starlet' individuals not showing the following markers. A total of 12 plants per cultivar were used

'UC1'	A02-700	A10-420	A18-2200	B02-2200	B02-1200	B08-1650	B08-1600	B10-1200	B10-950	B17-650	Average
	4	0	1	0	2	5	0	0	3	0	1.5
'Starlet'	A02-700	A05-2000	A10-950	A13-1400	A18-2200	A18-1700	A19-1400	B08-1550	B10-950	B17-650	Average
	0	0	0	1	1	0	0	0	0	0	0.2

Table 4. Segregation of five RAPD markers in the F_2 population of the cross 'A112' × 'A143' and the probability of a 3:1 ratio

Marker	Present	Absent	Probability
A02-700	64	23	0.76
A07-830	57	26	0.18
A10-420	66	23	0.85
A19-600	68	21	0.76
B18-600	66	23	0.85

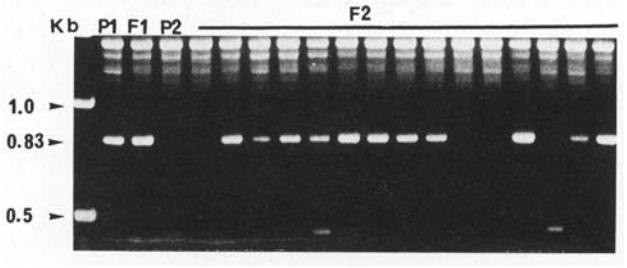

Fig. 2. Segregation of marker A07-830 in the F_2 family 'A112' (celeriac) × 'A143' (annual smallage). *P1* = 'A112', *P2* = 'A143'

Table 5. Cultivar-specific markers

Cultivar	Marker
'Florimart'	A19-1000
'Transgreen'	B08-830
'Tall Golden self-Blanching	A10-510
Celery cultivars	A18-2200, B10-950, B17-650
'A143'	A10-2100, A19-600, B10-700, B18-700
'A112'	B12-430
'A112' and 'A143'	B10-980
'A112' 'A143' and 'UC1'	A19-2500
'A112' and celery cultivars	A19-1400

specific markers: 'Florimart' by A19-1000 (Fig. 3), 'Transgreen' by B08-830, and 'Tall Golden Self Blanching' by A10-510. The markers, A18-2200, B10-950, and B17-650 were celery specific, being present in all of the celery cultivars but absent in celeriac 'A112' and annual smallage 'A143'. Four markers were 'A143'-specific: A10-2100, A19-600 (Fig. 3), B10-700, and B18-600, and only one (B12-430) was 'A112'-specific. A19-2500 was present only in 'A112', 'A143' and 'UC1', while A19-1400 was absent only in 'A143' and present in the rest of the cultivars (Fig. 3).

Cultivar relationship

The pairwise distances among all the cultivars were calculated by PAUP (Table 6). These data were based on the number of markers which were different between each pair of cultivars. The average marker difference was 6.4 between two celery cultivars, 16.7 between celery and annual smallage, 14.7 between celery and celeriac, and 12.0 between annual smallage and celeriac. Thus, RAPDs clearly separated celery, celeriac and annual smallage cultivars. Even though very few cultivar-specific markers were found, any two celery cultivars could be distinguished by at least one marker difference. Three pairs of celery cultivars were closely related and separated by only one marker difference: 'Florida 2-14' and 'Florida 638', 'Tall Utah 52-70R' and 'Tall Utah 52-70HK', and 'Florida-638' and 'Picador'. On the other hand, the less-related celery cultivars were 'UC1' and 'Summit', with 13 marker differences. 'UC1' differed from celeriac by ten marker differences. This line was the closest tested celery cultivar to celeriac. No celery cultivars were closer than 14 marker differences to annual smallage.

A phylogram tree generated by PAUP is shown in Fig. 4. The three cultivated types were readily separated. Among the 21 celery cultivars, three clusters were resolved. The largest cluster, which was assigned as group A, consisted of ten cultivars. Group B and group C contained three and four cultivars, respectively. The other four cultivars ('Golden Self Blanching', 'Ventura', 'Tall Golden Self Blanching', and 'Florimart') could not be assigned to any group. When pairs of celery cultivars with three or less marker differences were pooled (Fig. 5), three cultivar groups, corresponding to the three clusters in Fig. 4, were disclosed. The four unassigned cultivars in Fig. 4, showing more than three marker differences with all the other cultivars, were excluded in Fig. 5. Group B fell in the middle of groups A and C which were remotely related to each other.

Discussion

RAPD markers and their polymorphisms

Three cultivated forms (celery, celeriac, and smallage) of *A. graveolens* shared a large proportion (68%) of RAPD

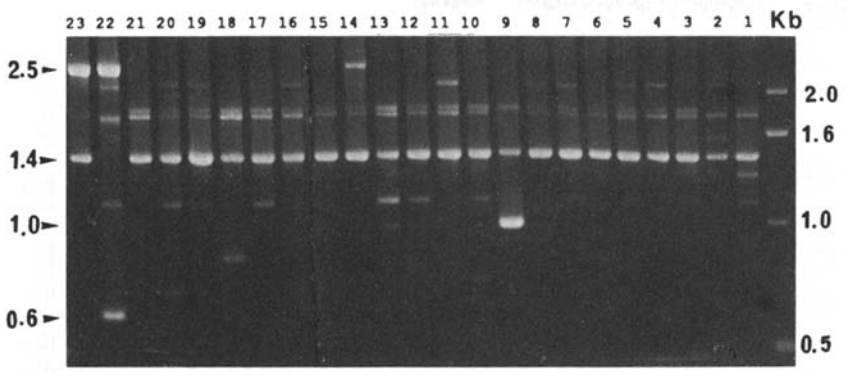

Fig. 3. Cultivar-specific markers generated by primer A19. Numbers on top refer to the cultivars listed in Table 1. On the right side is the DNA size standard (1 kb ladder). The *arrows* indicate the specific markers. A19-2500 is specific to celeriac 'A112', annual smallage 'A143', and 'UC1' (*lanes 23, 22, and 14*, respectively); A19-1400 is absent only in 'A143' (*lane 22*); A19-1000 is specific to 'Florimart' (*lane 9*); A19-600 is specific to 'A143' (*lane 22*)

Table 6. Pairwise marker difference among 21 celery (1–21) cultivars, one annual smallage (22), and one celeriac (23) cultivar

Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 Golden Self Blanching	–																						
2 Transgreen	8	–																					
3 Tenderscrip	5	5	–																				
4 Surepack	7	3	4	–																			
5 Florida 2-14	8	4	5	3	–																		
6 Florida 683K	7	3	4	2	1	–																	
7 Tall Utah 52-70R	9	5	4	4	3	2	–																
8 Tall Utah 52-70HK	10	6	5	3	4	3	1	–															
9 Florimark	13	9	8	8	7	8	6	5	–														
10 Summit	7	11	8	10	11	12	10	9	8	–													
11 Deacon	8	8	7	5	6	5	3	2	7	7	–												
12 Bishop	7	7	2	6	5	4	2	3	6	8	5	–											
13 Tall Gld. Self Blanch.	9	7	6	6	3	4	4	5	6	10	7	4	–										
14 UC1	8	6	5	5	4	3	3	4	9	13	6	5	7	–									
15 Tall Uta 52-57	7	11	8	8	9	10	8	7	10	2	5	8	10	11	–								
16 Calmario	5	5	4	4	3	2	4	5	8	12	7	4	4	3	12	–							
17 Ventura	6	10	7	7	8	7	7	6	9	5	4	5	7	10	5	7	–						
18 Conquistador	5	11	6	10	9	10	8	9	10	2	7	6	8	11	2	10	5	–					
19 Matador	6	8	3	7	8	7	5	6	9	5	8	3	7	8	5	7	6	3	–				
20 Picador	8	4	5	3	2	1	3	4	9	13	6	5	5	4	11	3	8	11	8	–			
21 Starlet	5	9	6	10	11	10	8	9	10	2	7	6	10	11	4	10	5	2	3	11	–		
22 A143	18	16	17	15	14	15	15	16	17	21	18	17	15	14	19	15	20	19	18	16	21	–	
23 A112	18	14	15	13	12	13	13	12	11	17	14	15	15	10	17	13	18	19	18	12	19	12	–

markers from the 28 primers used in our survey, which implies a high degree of homology among these cultivated types. Useful polymorphic markers comprised only 9.3% of the total 309 bands. The high proportion of spurious bands (22.7%) shows the importance of using multiple runs and strict control of the experimental conditions. Only marker B10-1200 was scored in terms of weak or strong bands. This may reflect differences of the copy number of this sequence among the cultivars. The other markers were dominant and scored as presence or absence of specific bands. Reliability of the markers as monogenic trait was confirmed by the Mendelian segregation observed in the five markers tested (Table 4).

Among the 28 primers scored, only 12 showed 19 polymorphic markers within the 21 celery cultivars used. This polymorphism was much lower than that detected in *Brassica* by Hu and Quiros (1991a). In their study, four primers showed as many as 16 polymorphic markers among 14 broccoli cultivars and 18 polymorphic markers among 12 cauliflower cultivars. Our result was consistent with the observation that most of the celery cultivars in North America have a narrow genetic base (Quiros et al. 1987a) being derived from only a few old European cultivars (Guzman et al. 1973). In spite of the low level of polymorphism within the celery cultivars, the RAPD technique could still detect enough polymorphism to distinguish each cultivar from all others by at least one marker difference. The polymorphism was much more apparent among different cultivated forms. From our RAPD data, celeriac and annual smallage were closer to each other than either of them to celery cultivars. This conclusion agrees with the celery RFLP data (Huestis

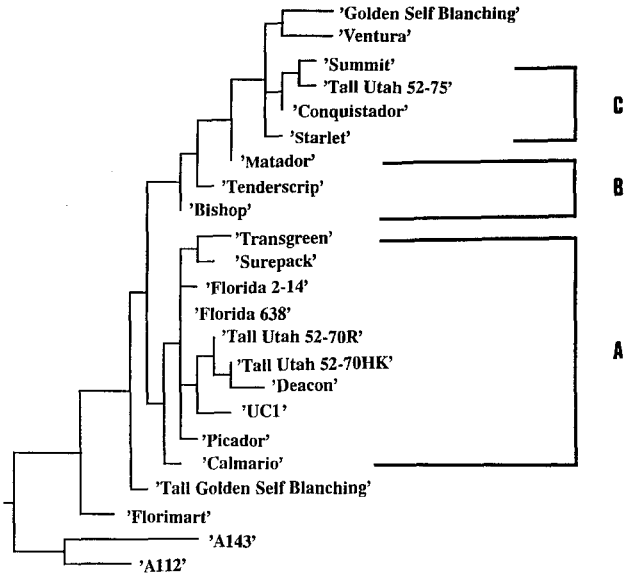

Fig. 4. The phylogram tree generated by the PAUP program. Celeriac ('A112'), annual smallage ('A143'), and celery cultivars were clearly separated. Among the celery cultivars, three clusters *A, B, and C* could be discerned. Four celery cultivars ('Golden Self Blanching', 'Ventura', 'Tall Golden Self Blanching', and 'Florimart') outside of these clusters remained unassigned

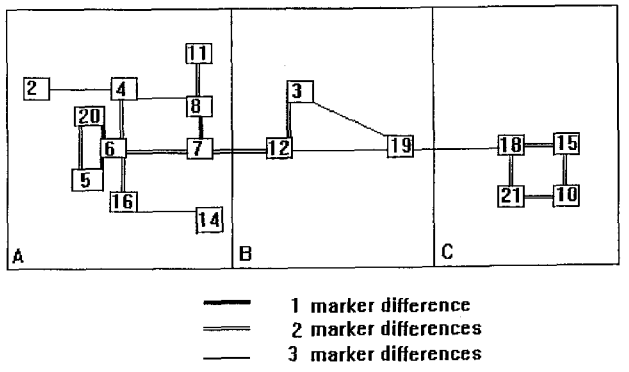

Fig. 5. Relationship of celery cultivars represented by their marker differences. Numbers refer to the cultivars in Table 1. Only the pairs of cultivars with marker differences of three or less are shown. The cultivars can be classified into three groups *A, B, and C*, which are the same as those indicated in Fig. 4

et al. 1992). Celery was closer to celeriac than to annual smallage. This may be partly due to the introduction of *Fusarium* Yellows resistance from celeriac into several celery cultivars such as 'UC1', 'Picador', 'Matador', and 'Starlet'.

Relationship between 'UC1' and celeriac

Among all the celery cultivars surveyed in this study, 'UC1' was the closest to celeriac, with ten marker differences. This is consistent with the fact that 'UC1' was developed as a breeding line resistant to *Fusarium* Yellows by crossing celeriac with celery cultivars (Orton et al. 1984). This relationship was manifested by marker A19-2500, which is only present in celeriac 'A112', annual smallage 'A143' and 'UC1', and absent in all the rest of the celery cultivars. The A19-2500 marker in 'UC1' was most likely transferred from celeriac plants. A19-2500 was very strong in 'A112' and 'A143' and much weaker in 'UC1' (Fig. 3). One or both of the following possibilities may account for this pattern: (1) A19-2500 is segregating in 'UC1', (2) A19-2500 is a high-copy locus and only a few copies were transferred to 'UC1'.

Table 7. Comparison of classification of celery cultivars by Quiros et al. (1987) and the present study. Cultivars surveyed in this study but not by Quiros et al. (1987) are not included. Stars indicate the discrepancies between the two classifications

Cultivar	Group by Quiros et al.	Group in current study
'Deacon'	A	A
'Tall Utah 52-70R'	A	A
'Tall Utah 52-70HK'	A	A
'Calmario'	A	A
'Florida 2-14'	A	A
'Florida 683K'	A	A
'Transgreen'	A′	A
'Ventura'*	A′	Unassigned
'Bishop'	B	B
'Tenderscrip'	B	B
'Florimart'*	C	Unassigned
'Surepack'*	C	A
'Tall Uta 52-75'	D	C
'Summit'	D	C
'Golden self-Blanching'	E	Unassigned
'Tall Golden self-Blanching'	F	Unassigned

Optimum sample size for RAPD survey

The number of individual plants that should be bulked for sampling the variabilities of the cultivars is a function of marker frequency in the population and the method of sampling. The estimation of marker frequency may involve a laborious survey of a large number of individuals and markers. As a feasible alternative we scored bulks made up of 5–50 individuals with the primers showing polymorphic markers. Bulks of five individuals in 'Tall Utah 52-75' and 'Starlet', which represent old and new cultivars respectively, showed the same number of bands as those of 10, 20, 30, 40, and 50 plants. This result indicates that a substantial homogeneity of the markers used exists in most of the celery cultivars, and that DNA samples bulked from five plants could be good representatives of those cultivars. The lower homogeneity level of 'UC1', further confirmed by screening 12 individual plants with ten primers, as well as those of 'A112' and 'A143' (data not shown), required larger samples. Bulks of more than 20 plants may not necessarily increase the possibility of discovering more segregating markers, because the sensitivity of RAPD is limited. Michelmore et al. (1991) reported that allele products present in the DNA bulk below 10% were barely detectable or undetectable. Our conclu-

sion is that DNA bulks of 5–20 plants are sufficient for RAPDs in most of the celery cultivars, depending on the level of homogeneity.

Grouping of celery cultivars

Since groups A and C were apart with group B in the middle, it is likely that the group B cultivars represent hybrids of the cultivars or of the ancestors of groups A and C. Interpretation of these data should be taken with caution because the results may depend on the sampling method (harvesting from a single or very few plants vs bulking from a large number of individuals) and the breeding method (single plant selection vs bulking). Even though the data did not provide direct information about the lineage relationship of these cultivars, it could be very informative in revealing the genetic relatedness among the cultivars and possible sources of cultivar development.

Group A. Out of the ten group-A members, according to Guzman et al. (1973) and Quiros (1992), six ('Tall Utah 52-70R', 'Tall Utah 52-70HK', 'Florida 2-14', 'Florida 683', 'Deacon' and 'Calmario') were derived from 'Tall Utah 52-70', a cultivar developed by Ferry Morse Seed Company. 'Transgreen' was developed from 'Tall Utah 52-70R' and 'Tall Utah 52-70HK'. 'Surepack' was developed from 'Florimart' and an unknown source. From our results, it is likely that this unknown source came from group A. In the development of 'UC1' as a *Fusarium* Yellows-resistant breeding line, 'Tall Utah 52-70R' was extensively used in crossing and backcrossing (Orton et al. 1984). Thus the location of 'UC1' in group A was expected. 'UC1' was used to breed 'Picardor', a group A cultivar which resembles 'UC1' (Greathead, personal communication). In conclusion, group A cultivars are basically 'Tall Utah 52-70' related.

Group B. This group, on the other hand, is related to the old cultivar 'Summer Pascal Utah'. According to Quiros (1992), 'Tenderscrip' was selected from 'Summer Pascal Utah', and 'Bishop' derived from 'Tenderscrip', 'Matador', resulting from a cross between 'UC1' (group A) and 'Tall Utah 52-75' (group C) (Toth and Lacy 1992), showed approximately the same distance to groups A and C. The classification of 'Matador' in group B was not unexpected because this group, as mentioned above, lies between groups A and C.

Group C. 'Tall Utah 52-75' and 'Summit' are believed to have been developed from an unknown source and from 'Tall Utah 52-70'-related cultivars (Quiros 1992). This unknown source is most likely the founder of group C. 'Conquistador' and 'Starlet' are new cultivars derived from 'UC1', but they showed no close relationship with 'UC1'. This discrepancy could be due to the possibility that they were derived from crosses between 'UC1' and some group C-related cultivars, and also to the fact that 'UC1' is a highly heterogeneous population.

Comparison with previous work

Quiros et al. (1987) classified 17 celery cultivars into seven major groups based on six stem protein markers. The previous and our current groupings were compatible for 13 out of the 16 cultivars compared (Table 7). All the cultivars in the previous groups A and A′, except 'Ventura', were included in our group A. Thus, this group is equivalent to the previous groups A and A′. Groups B in both studies are also equivalent. Group D in the previous study is equivalent to our group C. 'Golden Self-Blanching' and 'Tall Golden Self-Blanching' were the only members in the previous group E and F, respectively. Their isolated positions also showed in the current study by remaining unassigned.

The classifications in both studies are agreeable for most cultivars, implying that RAPDs are at least as reliable as protein markers for cultivar classification and identification in celery. The differences between them may be primarily due to the different type and number of markers used, and the possibility of sampling variation cannot be excluded. This discrepancy could be alleviated by using a larger number and different types of markers with more representative samples.

Acknowledgements. We are indebted to Jinguo Hu and Gordon Huestis for advise, to Vincent D'Antonio for technical assistance, to Pybas Seeds Company and Royal Sluis Company for cultivar samples. This research was supported by the grant from the California Advisory Celery Board.

References

Beckmann JS, Soller M (1983) Restriction fragment length polymorphism in genetic improvement: methodologies, mapping and cost. Theor Appl Genet 67:33–43

Guzman VL, Burdine HW, Harris ED, Orsenigo JR, Showalter RK, Thayer PL, Winchester JA, Wolf EA, Berger RD, Genung WG, Zitter TZ (1973) Celery production on organic soils of South Florida. Bull 757, Florida Agricultural Experiment Station

Heustis G, MacGrath M, Quiros CF (1992) Develoment of gentic markers in celery based on restriction fragment length polymorphisms. Theor Appl Genet (in press)

Hu J, Quiros CF (1991a) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Hu J, Quiros CF (1991b) Molecular and cytological evidence of deletions in alien chromosomes for two monosomic addition lines of *Brassica campestris-oleracea*. Theor Appl Genet 81:221–226

Mitchelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Prot Natl Acad Sci 88:9828–9832

Orton TJ, Hulbert SH, Durgan ME, Quiros CF (1984) UC1, Fusarium Yellow-resistant celery breeding line. Hortiscience 19:594

Quiros CF (1992) Genetic improvement in celery. In: Kalloo, Bergh BO (eds) Genetic improvement of vegetable crops. Pergamon Press, Oxford, UK (in press)

Quiros CF, McGrath M, Stites J (1987a) Use of stem proteins and isozymes for the identification of celery cultivars. Plant Cell Rep 6:114–117

Quiros CF, Douches D, D'Antonio V (1987b) Inheritence of annual habit in celery: cosegregation with isozyme and anthocyanin markers. Theor Appl Genet 74:203–208

Tanksley SD, Orton TJ (1983) Isozymes in plant genetics and breeding. Elsevier, Amsterdam

Toth KF, Lacy ML (1992) Field Evaluation of celery germ plasm for resistant to *fusarium oxysporum* F. Sp. *Apii* Race 2. Res Rep 523, Agricultural Experiment Station, East Lansing, Michigan State University

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AE, Levak KJ, Rafalski JA, Tingey SC (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wrigley CW, Batey IL, Skerritt JH (1987) Complementing traditional methods of identifying cereal varieties with novel procedures. Seed Sci Technol 15:679–688