Mol Breeding (2007) 20:375–388
DOI 10.1007/s11032-007-9098-6

A consensus map for *Cucurbita pepo*

Amine Zraidi · Gertraud Stift · Martin Pachner · Abdolali Shojaeiyan · Li Gong · Tamas Lelley

Received: 2 October 2006 / Accepted: 5 April 2007 / Published online: 29 April 2007
© Springer Science+Business Media B.V. 2007

Abstract Using random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), and morphological traits, the first genetic maps for *Cucurbita pepo* (2n=2x=40) were constructed and compared. The two mapping populations consisted of 92 F_2 individuals each. One map was developed from a cross between an oil-seed pumpkin breeding line and a zucchini accession, into which genes for resistance to Zucchini Yellow Mosaic Virus (ZYMV) from a related species, *C. moschata*, had been introgressed. The other map was developed from a cross between an oil-seed pumpkin and a crookneck variety. A total of 332 and 323 markers were mapped in the two populations. Markers were distributed in each map over 21 linkage groups and covered an average of 2,200 cM of the *C. pepo* genome. The two maps had 62 loci in common, which enabled identification of 14 homologous linkage groups. Polyacrylamide gel analyses allowed detection of a high number of markers suitable for mapping, 10% of which were co-dominant RAPD loci. In the Pumpkin-Zucchini population, bulked segregant analysis (BSA) identified seven markers less than 7 cM distant from the locus *n*, affecting lignification of the seed coat. One of these markers, linked to the recessive hull-less allele (AW11-420), was also found in the Pumpkin-Crookneck population, 4 cM from *n*. In the Pumpkin-Zucchini population, 24 RAPD markers, previously introduced into *C. pepo* from *C. moschata*, were mapped in two linkage groups (13 and 11 markers in LGpz1 and LGpz2, respectively), together with two sequence characterized amplified region (SCAR) markers linked to genes for resistance to ZYMV.

Keywords DNA markers · *Cucurbita moschata* · BSA · Seed coat · ZYMV · SCAR-marker

A. Zraidi · G. Stift · M. Pachner · A. Shojaeiyan · L. Gong · T. Lelley (✉)
Institute of Biotechnology in Plant Production, Department for Agrobiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Konrad Lorenz Straße 20, 3430 Tulln, Austria
e-mail: tamas.lelley@boku.ac.at

Present Address:
A. Shojaeiyan
Department of Horticulture, Faculty of Agriculture, Ilam University, P.O. Box 516-69315, Ilam, Iran Islamic Republic

Introduction

The genus *Cucurbita* includes some of the oldest domesticated plant species. Three of them, *Cucurbita pepo* L., *Cucurbita maxima* Duchesne, and *Cucurbita moschata* Duchesne, are economically important crop species worldwide (Robinson and Decker-Walters 1997), with *C. pepo* exhibiting the widest variation, especially with respect to fruit characteristics. Duchesne (1786) and Naudin (1856) concluded that

C. pepo L. could be the most polymorphic species in the plant kingdom. Botanical classification based on allozyme variation recognized three subspecies: *C. pepo* subsp. *pepo*, *C. pepo* subsp. *ovifera* (L.) D.S. Decker [syn. *C. pepo* subsp. *texana* (Scheele) Filov], and *C. pepo* subsp. *fraterna* (Bailey) Lira, Andres and Nee (Andres 1987; Decker 1988; H. S. Paris personal communication). Using mainly fruit shape, Paris (1986) classified edible-fruited *C. pepo* into eight cultivar-groups: Pumpkin, Zucchini, Cocozelle, Vegetable Marrow, Acorn, Crookneck, Scallop, and Straightneck. The first four of these cultivar-groups named above belong to subsp. *pepo* and the latter four to subsp. *ovifera* (Paris 2001). Polymorphisms of DNA markers, including RFLP, RAPD, ISSR, AFLP, and SRAPD, have been used to study genetic relationships within *C. pepo* (Torres-Ruiz and Hemleben 1991; Katzir et al. 2000; Paris et al. 2003; Ferriol et al. 2003) and the results support these classifications. Also, the results obtained, using AFLP, ISSR, and SSR were very highly correlated ($P < 0.000001$).

Genetic maps have been constructed from interspecific crosses in the genus *Cucurbita* (Lee et al. 1995; Brown and Myers 2002), and we recently reported the initiation of a map for *C. pepo* (Zraidi and Lelley 2004). Compared to other economically important *Cucurbitaceae*, genetic mapping of *Cucurbita* ($2n = 2\times = 40$) is in its infancy. Mapping efforts in melon, *Cucumis melo* L., resulted in a large number of DNA-based maps (e.g., Baudracco-Arnas and Pitrat 1996; Wang et al. 1997; Périn et al. 1998; Oliver et al. 2001; Danin-Poleg et al. 2002). Similarly, in cucumber, *Cucumis sativus* L., several maps have been developed (e.g., Serquen et al. 1997; Park et al. 2000; Fazio et al. 2002). Efforts to anchor, align, and merge maps with SSR, RFLP or STS markers are underway for cucumber (Bradeen et al. 2001), and melon (Gonzalo et al. 2005).

In Austria, *C. pepo* has been cultivated for centuries primarily for seed oil production (Teppner 2000). Pumpkin seed-oil is a delicious salad oil (Murkovic et al. 1996). It is highly valued for its excellent nutritional quality and medicinal value, especially for preventing and curing benign prostate hyperplasia in its early stage (Blumenthal et al. 1998; Kreuter 2000; Schmidlin and Kreuter 2003), although the chemical agent responsible for this property of the seeds has not been identified yet (Murkovic et al. 2004). A special feature of Austrian oil-pumpkin varieties is their completely hull-less (non-lignified) seed coat (Teppner 2000; Zraidi et al. 2003), which is conferred largely by a single recessive gene, *n* (naked seed) (Paris and Brown 2005).

The principal objective of the present study was to construct a genetic map for *C. pepo*, using RAPD, AFLP, SSR, and morphological markers. Co-linear common markers segregating in two F_2 populations that mapped to the same linkage group were considered to be locus specific, and were used to align and merge the linkage maps that were constructed. Another objective was the identification of markers tightly linked to the seed coat locus *n*, believed to be controlled by a single major gene (Grebenščikov 1954; Prym von Becherer 1955; Zraidi et al. 2003). For this bulked segregant analysis (BSA) (Michelmore et al.1991) was used.

Materials and methods

Plant material

Two *C. pepo* F_2 populations, each derived from a single F_1 fruit, were used for mapping. The first population, which consisted of 92 plants, is here designated as the Pumpkin–Zucchini population, following the horticultural classification of Paris (1986). This population was derived from an intra-subspecific (*C. pepo* subsp. *pepo*) cross between an oil-pumpkin breeding line (SZG1), developed by ''Saatzucht Gleisdorf'' (Gleisdorf, Austria), and a zucchini accession, ''True French Resistant'', a near-isogenic line of the UK zucchini ''True French'' carrying three complementary dominant genes for resistance to Zucchini Yellow Mosaic Virus (ZYMV). These were introgressed from the Portuguese *C. moschata* ''Menina'' (Paris and Cohen 2000). Seeds of ''True French Resistant'' were obtained from H. S. Paris of the A. R. O. Newe Ya'ar Research Center, Ramat Yishay, Israel. The second population, which also consisted of 92 plants, is here designated as Pumpkin–Crookneck population. This population was derived from an inter-subspecific cross (*C. pepo* subsp. *pepo* × *C. pepo* subsp. *ovifera*) between the US oil-pumpkin variety ''Lady Godiva'' and the Italian crookneck variety ''Bianco Friulano.''

Ten seeds from each of the 92 selfed F_2 plants of the Pumpkin–Zucchini population were sown to produce a segregating F_3 progeny. This was used to identify homozygous hull-less (*n/n*) and homozygous hulled (*N/N*) F_2 genotypes to create contrasting DNA pools for BSA (Michelmore et al. 1991).

Morphological traits

Three qualitative gene loci, M (Mottled leaves), n (naked seeds), and Bu (Bush habit) (Paris and Brown 2005), showed clear segregation in the F_2 populations and were scored. The leaf-mottle trait was scored as the presence (M/–) or absence (m/m) of silver patches in the axils of the leaf veins, without regard to the extent or intensity of silver. The seed coat was scored as hulled (N/–) or hull-less (n/n), irrespective of an occasional residual lignification observed in the hull-less seeds (Teppner 2000). The growth habit was scored as plants being either bush (Bu/–) (length of the vine maximum 1.5 m) or vining (bu/bu) (length of vine 2–6 m) types.

DNA extraction

DNA was isolated from fresh young leaves using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA). DNA concentration was determined by the GenQuant RNA/DNA Calculator (Amersham Biosciences Europe, Freiburg, Germany) according to the manufacturer's instructions.

RAPD markers

A total of 495 10-mer RAPD primers (Operon Technology Inc., Huntsville, USA) were screened for polymorphisms, using DNA-pools of ten plants from each parental genotype. PCR conditions, fragment separation and visualization were conducted as described by Stift et al. (2003), using 10% polyacrylamide gel. Primers producing clear bands, showing polymorphism in both populations, were selected for mapping. Co-dominant RAPD markers were identified by the clear 1:2:1 segregation of the two corresponding fragments and by the complete lack of a recessive allele (Fig. 1).

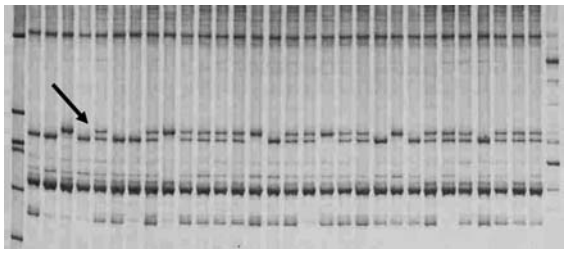


Fig. 1 Part of a 96-well polyacrylamide gel, stained with silver nitrate, showing a co-dominant RAPD marker (*arrow*)

AFLP markers

The AFLP analysis (Vos et al. 1995) was done as described by Hartl et al. (1999) and Buerstmayr et al. (2002), using *Mse*I and *Eco*RI enzymes. Selective amplification was performed using 28 *Eco*RI and *Mse*I primer combinations with two or three randomly chosen selective nucleotides. RAPD and AFLP gels from the two populations were compared and bands, showing identical molecular weights in the two populations, were considered to be common markers.

SSR markers

A screen for potential SSR polymorphisms between the four parental genotypes used in the two crosses was done by J. E. Staub, University of Wisconsin, Madison, USA, using 102 *Cucumis*-SSR primers (Fazio et al. 2002). Eighteen *C. pepo* SSR primer pairs were developed by our laboratory, and were tested on the parents of the Pumpkin–Zucchini population.

Bulked segregant analysis (BSA)

Analysis of F_3 progenies allowed the identification of *N/N* homozygotes from the F_2 in the Pumpkin–Zucchini population. Two DNA pools were made, each containing a mixture of 12 DNA samples from homozygous dominant (hulled) and homozygous recessive (hull-less) plants for the *n* gene (Zraidi et al. 2003). A total of 1,068 RAPD markers were screened for polymorphism using the two DNA-bulks, and markers showing association with the *n* locus were tested on DNA from single plants of the

two pools. Ultimately, markers associated with the trait, found through BSA, were used on the entire F_2 population to calculate the degree of linkage.

Linkage analysis

Marker segregation was tested for fit to 3:1 (dominant loci) and 1:2:1 (SSR and co-dominant RAPD and AFLP markers) ratios at $P = 0.05$ probability. Linkage maps were constructed independently for each population using MAPMAKER/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992). Markers were associated with the *group* command with a likelihood of LOD $\geq$5 and with a maximum distance of 30. Marker-ordering within linkage groups was initially performed using the *order* command with a likelihood of LOD $\geq$3. Linkage groups containing more than six markers, which could not be ordered with this likelihood level, were ordered manually, using the *compare* command. The remaining markers were then integrated into these linkage groups, using the *try* command. Distances were calculated with the Kosambi function (Kosambi 1944). The order of the markers within linkage groups was double checked, using the *ripple* command. Linkage groups in the Pumpkin–Zucchini map are named LGpz, and those of the Pumpkin–Crookneck map LGpc.

Results

Polymorphism

Of the 495 tested RAPD primers, 227 (46%) and 296 (60%) showed polymorphism between the parents of the Pumpkin–Zucchini and the Pumpkin–Crookneck populations, respectively. Finally, 101 primers were selected for mapping because they showed polymorphism in both populations. These primers produced an average of 15 bands, each ranging in size from 150 to 1,900 bp. The average number of polymorphic loci per primer was 2.6 and 2.7 in the Pumpkin–Zucchini and Pumpkin–Crookneck populations, respectively.

AFLP primer combinations generated up to 60 bands, ranging in size from 50 to 700 bp. The 28 AFLP *Eco*RI/*Mse*I primer combinations produced 118 polymorphic loci, with an average of 4.2 loci per primer combination, in the Pumpkin–Zucchini and 180 polymorphic loci, with an average of 6.4 loci per primer combination in the Pumpkin–Crookneck population.

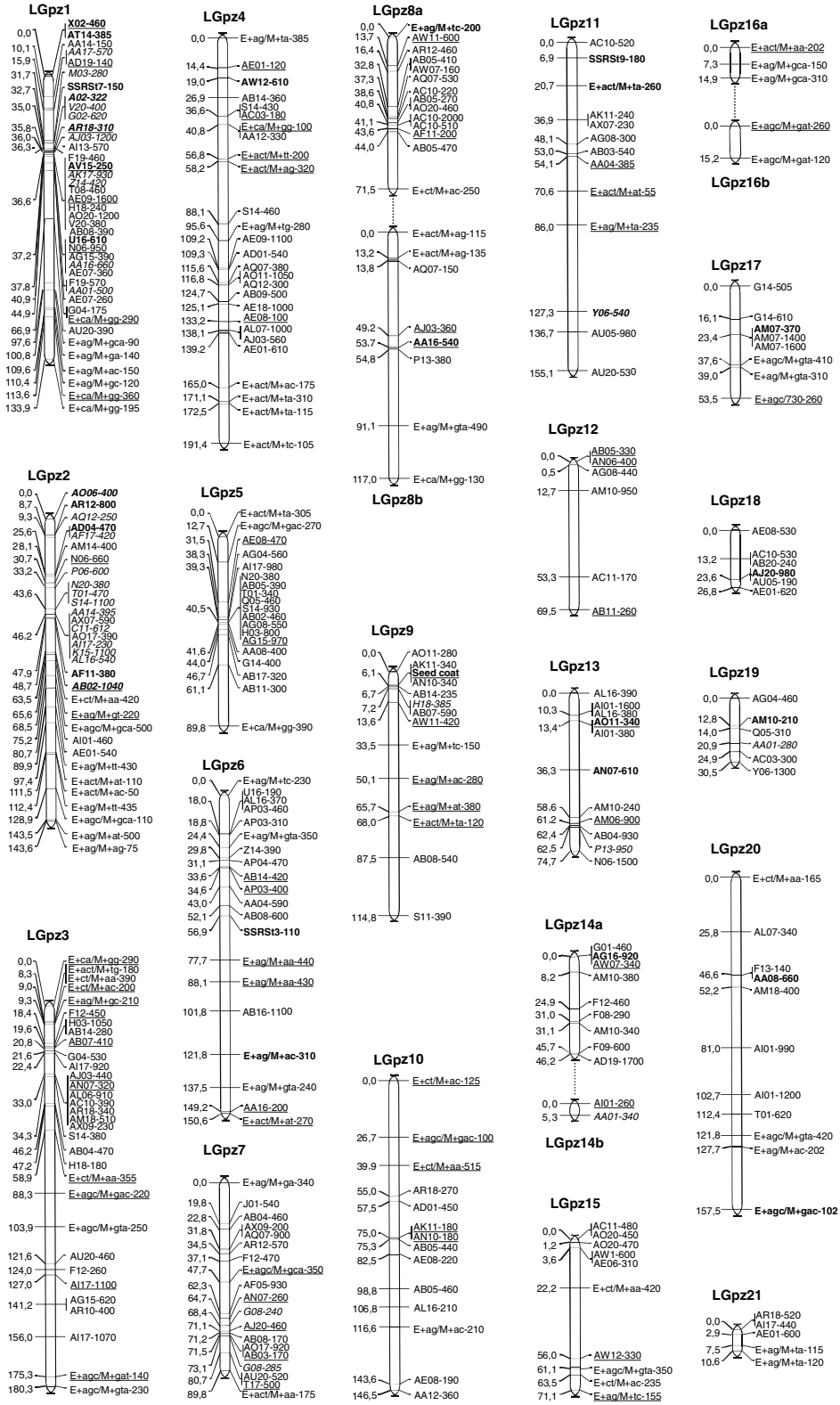
None of the 102 *Cucumis*-SSR markers tested, were polymorphic between the parental genotypes. Of the 18 *Cucurbita*-SSR markers, five (27.7%) were polymorphic between the mapping parents of the Pumpkin–Zucchini population and three could be mapped in the Pumpkin–Zucchini map in linkage groups LGpz1, LGpz6, and LGpz11 (Fig. 2).

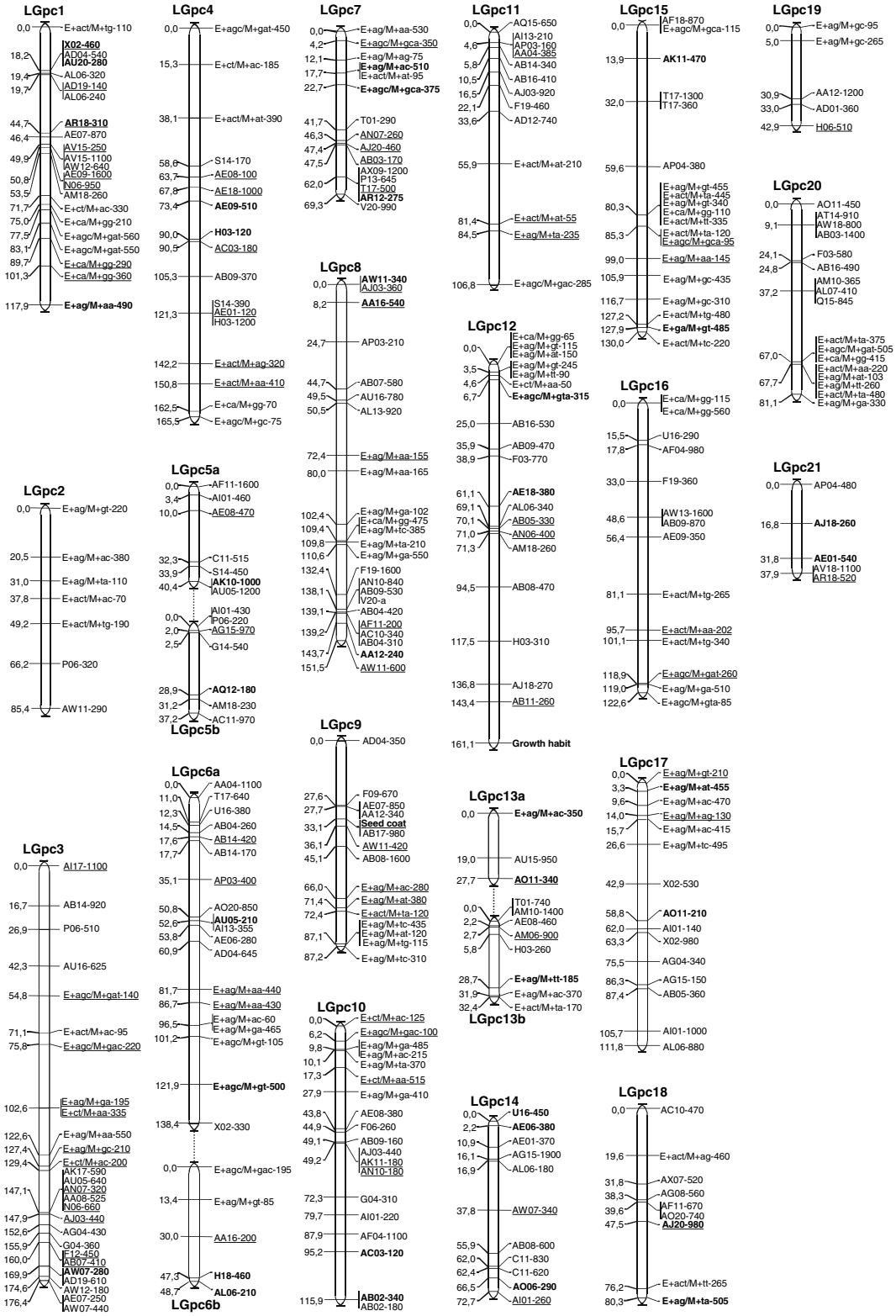
Fig. 2 Linkage map of the Pumpkin–Zucchini population. The loci and their size are listed to the right of the *bars*. To the left are the distances between markers in centiMorgans. RAPD and AFLP are represented by their universal codes. *Underlining* indicates common markers. *Bold* font indicates **co-dominant markers** and *italic* font indicates that the *markers originate from the source of resistance to ZYMV* (*C. moschata* genotype)

Construction and characteristics of the maps

Only linkage groups (major linkage groups) containing more than four markers and including at least one common or one co-dominant marker (Fig. 1) were used in the construction of the two maps (Figs. 2, 3). A number of linkage groups containing four or less markers (minor groups) were at first excluded from the maps. These were eight doublets in the Pumpkin–Zucchini and ten doublets in the Pumpkin–Crookneck map, and two quadruplets and six triplets in each of the two maps. However, some of these linkage groups were later incorporated in the final maps based on the presence of common markers and after the alignment of the two maps. These were two doublets (LGpz14b and LGpz16b) in the Pumpkin–Zucchini map (Fig. 2) and one triplet in each of the two maps (LGpz16a in the Pumpkin–Zucchini map and LGpc13a in the Pumpkin–Crookneck map). Forty-one zucchini markers and 49 crookneck markers were unlinked.

A total of 333 markers (247 RAPD, 82 AFLP, 3 SSR, and the *n* locus), 29 of which were co-dominant (21 RAPDs, 5 AFLPs, and 3 SSRs), were integrated in the Pumpkin–Zucchini map covering 2,140 cM of the genome and distributed over 21 major and 3 minor linkage groups (Fig. 2). The average size of the linkage groups was 90 cM, ranging from 10 cM in LGpz21 to 191 cM in LGpz4. The average number of markers per linkage group was 16 and the average distance between markers was 6.4 cM. Except


LGpz1
0,0 X02-460
AT14-385
10,1 AA14-150
AA17-570
15,9 AD19-140
31,7 M03-280
32,7 SSRSt7-150
A02-322
35,0 V20-400
G02-620
35,8 AR18-310
36,0 AJ03-1200
36,3 AI13-570
F19-460
AV15-250
AK17-300
Z14-420
T08-460
36,6 AE09-1600
H18-240
AO20-1200
V20-380
AB08-390
37,2 U16-610
N06-950
AG15-390
AA16-660
AE07-360
37,8 F19-570
40,9 AA01-500
44,9 AE07-260
IG04-175
E+ca/M+gg-290
66,9 AU20-390
97,6 E+ag/M+gca-90
100,8 E+ag/M+ga-140
109,6 E+ag/M+ac-150
110,4 E+ag/M+gc-120
113,6 E+ca/M+gg-360
133,9 E+ca/M+gg-195
LGpz2
0,0 AO06-400
8,7 AR12-800
9,3 AQ12-250
25,6 AD04-470
AF17-420
28,1 AM14-400
30,7 N06-660
33,2 P06-600
N20-380
43,6 T01-470
S14-1100
AA14-395
AX07-590
C11-612
46,2 AO17-390
AI17-230
K15-1100
AL16-540
47,9 AF11-380
48,7 AB02-1040
63,5 E+ct/M+aa-420
65,6 E+ag/M+gt-220
68,5 E+agc/M+gca-500
75,2 AI01-460
80,7 AE01-540
89,9 E+ag/M+tt-430
97,4 E+act/M+at-110
111,5 E+act/M+ac-50
112,4 E+ag/M+tt-435
128,9 E+agc/M+gca-110
143,5 E+ag/M+at-500
143,6 E+ag/M+ag-75
LGpz3
0,0 E+ca/M+gg-290
8,3 E+act/M+tg-180
9,0 E+ct/M+aa-390
9,3 E+ct/M+ac-200
18,4 E+ag/M+gc-210
19,6 F12-450
20,8 H03-1050
21,6 AB14-280
22,4 AB07-410
G04-530
AI17-920
33,0 AJ03-440
AN07-320
AU06-910
AC10-390
AR18-340
AM18-510
AX09-230
34,3 S14-380
46,2 AB04-470
47,2 H18-180
58,9 E+ct/M+aa-355
88,3 E+agc/M+gac-220
103,9 E+agc/M+gta-250
121,6 AU20-460
124,0 F12-260
127,0 AI17-1100
141,2 AG15-620
AR10-400
156,0 AI17-1070
175,3 E+agc/M+gat-140
180,3 E+agc/M+gta-230
LGpz4
0,0 E+ag/M+ta-385
14,4 AE01-120
19,0 AW12-610
26,9 AB14-360
36,6 S14-430
AC03-180
40,8 E+ca/M+gg-100
AA12-330
56,8 E+act/M+tt-200
58,2 E+act/M+ag-320
88,1 S14-460
95,6 E+ag/M+tg-280
109,2 AE09-1100
109,3 AD01-540
115,6 AQ07-380
116,8 AO11-1050
AQ12-300
124,7 AB09-500
125,1 AE18-1000
133,2 AE08-100
138,1 AL07-1000
AJ03-560
139,2 AE01-610
165,0 E+act/M+ac-175
171,1 E+act/M+ta-310
172,5 E+act/M+ta-115
191,4 E+act/M+tc-105
LGpz5
0,0 E+act/M+ta-305
12,7 E+agc/M+gac-270
31,5 AE08-470
38,3 AG04-560
39,3 AI17-980
N20-380
AB05-390
D01-340
40,5 Q05-460
S14-930
AB02-460
AG08-550
H03-800
AG15-970
41,6 AA08-400
44,0 G14-400
46,7 AB17-320
61,1 AB11-300
89,8 E+ca/M+gg-390
LGpz6
0,0 E+ag/M+tc-230
18,0 U16-190
AL16-370
AP03-460
18,8 AP03-310
24,4 E+ag/M+gta-350
29,8 Z14-390
31,1 AP04-470
33,6 AB14-420
34,6 AP03-400
43,0 AA04-590
52,1 AB08-600
56,9 SSRSt3-110
77,7 E+ag/M+aa-440
88,1 E+ag/M+aa-430
101,8 AB16-1100
121,8 E+ag/M+ac-310
137,5 E+ag/M+gta-240
149,2 AA16-200
150,6 E+act/M+at-270
LGpz7
0,0 E+ag/M+ga-340
19,8 J01-540
22,8 AB04-460
31,8 AX09-200
AQ07-900
34,5 AR12-570
37,1 F12-470
47,7 E+agc/M+gca-350
62,3 AF05-930
64,7 AN07-260
68,4 G08-240
71,1 AJ20-460
71,2 AB08-170
71,5 AO17-920
AB03-170
73,1 G08-285
80,7 AU20-520
T17-500
89,8 E+act/M+aa-175
LGpz8a
0,0 E+ag/M+tc-200
13,7 AW11-600
16,4 AR12-460
32,8 AB05-410
AW07-160
37,3 AQ07-530
38,6 AC10-220
40,8 AB05-270
AO20-460
41,1 AC10-2000
43,6 AC10-510
AF11-200
44,0 AB05-470
71,5 E+ct/M+ac-250
0,0 E+act/M+ag-115
13,2 E+act/M+ag-135
13,8 AQ07-150
49,2 AJ03-360
53,7 AA16-540
54,8 P13-380
91,1 E+ag/M+gta-490
117,0 E+ca/M+gg-130
LGpz8b
LGpz9
0,0 AO11-280
6,1 AK11-340
Seed coat
AN10-340
6,7 AB14-235
7,2 H18-385
13,6 AB07-590
AW11-420
33,5 E+ag/M+tc-150
50,1 E+ag/M+ac-280
65,7 E+ag/M+at-380
68,0 E+act/M+ta-120
87,5 AB08-540
114,8 S11-390
LGpz10
0,0 E+ct/M+ac-125
26,7 E+agc/M+gac-100
39,9 E+ct/M+aa-515
55,0 AR18-270
57,5 AD01-450
75,0 AK11-180
75,3 AN10-180
82,5 AB05-440
AE08-220
98,8 AB05-460
106,8 AL16-210
116,6 E+ag/M+ac-210
143,6 AE08-190
146,5 AA12-360
LGpz11
0,0 AC10-520
6,9 SSRSt9-180
20,7 E+act/M+ta-260
36,9 AK11-240
AX07-230
48,1 AG08-300
53,0 AB03-540
54,1 AA04-385
70,6 E+act/M+at-55
86,0 E+ag/M+ta-235
127,3 Y06-540
136,7 AU05-980
155,1 AU20-530
LGpz12
0,0 AB05-330
AN06-400
0,5 AG08-440
12,7 AM10-950
53,3 AC11-170
69,5 AB11-260
LGpz13
0,0 AL16-390
10,3 AI01-1600
AL16-380
13,4 AO11-340
AI01-380
36,3 AN07-610
58,6 AM10-240
61,2 AM06-900
62,4 AB04-930
62,5 P13-950
74,7 N06-1500
LGpz14a
G01-460
0,0 AG16-920
AW07-340
8,2 AM10-380
24,9 F12-460
31,0 F08-290
31,1 AM10-340
45,7 F09-600
46,2 AD19-1700
0,0 AI01-260
5,3 AA01-340
LGpz14b
LGpz15
0,0 AC11-480
AO20-450
1,2 AO20-470
3,6 AW1-600
AE06-310
22,2 E+ct/M+aa-420
56,0 AW12-330
61,1 E+agc/M+gta-350
63,5 E+ct/M+ac-235
71,1 E+ag/M+tc-155
LGpz16a
0,0 E+act/M+aa-202
7,3 E+ag/M+gca-150
14,9 E+ag/M+gca-310
0,0 E+agc/M+gat-260
15,2 E+agc/M+gat-120
LGpz16b
LGpz17
0,0 G14-505
16,1 G14-610
23,4 AM07-370
AM07-1400
AM07-1600
37,6 E+agc/M+gta-410
39,0 E+ag/M+gta-310
53,5 E+agc/730-260
LGpz18
0,0 AE08-530
13,2 AC10-530
AB20-240
23,6 AJ20-980
26,8 AU05-190
AE01-620
LGpz19
0,0 AG04-460
12,8 AM10-210
14,0 Q05-310
20,9 AA01-280
24,9 AC03-300
30,5 Y06-1300
LGpz20
0,0 E+ct/M+aa-165
25,8 AL07-340
46,6 F13-140
AA08-660
52,2 AM18-400
81,0 AI01-990
102,7 AI01-1200
112,4 T01-620
121,8 E+agc/M+gta-420
127,7 E+ag/M+ac-202
157,5 E+agc/M+gac-102
LGpz21
0,0 AR18-520
2,9 AI17-440
AE01-600
7,5 E+ag/M+ta-115
10,6 E+ag/M+ta-120


LGpc1
0,0 E+act/M+tg-110
18,2 X02-460
AD04-540
AU20-280
19,4 AL06-320
19,7 AD19-140
AL06-240
44,7 AR18-310
46,4 AE07-870
AV15-250
49,9 AV15-1100
AW12-640
AE09-1800
50,8 N06-950
53,5 AM18-260
71,7 E+ct/M+ac-330
75,0 E+ca/M+gg-210
77,5 E+agc/M+gat-560
83,1 E+agc/M+gat-550
89,7 E+ca/M+gg-290
101,3 E+ca/M+gg-360
117,9 E+ag/M+aa-490
LGpc2
0,0 E+ag/M+gt-220
20,5 E+ag/M+ac-380
31,0 E+ag/M+ta-110
37,8 E+act/M+ac-70
49,2 E+act/M+tg-190
66,2 P06-320
85,4 AW11-290
LGpc3
0,0 AI17-1100
16,7 AB14-920
26,9 P06-510
42,3 AU16-625
54,8 E+agc/M+gat-140
71,1 E+act/M+ac-95
75,8 E+agc/M+gac-220
102,6 E+ag/M+ga-195
E+ct/M+aa-335
122,6 E+ag/M+aa-550
127,4 E+ag/M+gc-210
129,4 E+ct/M+ac-200
AK17-590
147,1 AU05-640
AN07-320
AA08-525
N06-660
147,9 AJ03-440
152,6 AG04-430
155,9 G04-360
160,0 F12-450
AB07-410
169,9 AW07-280
AD19-610
174,6 AW12-180
176,4 AE07-250
AW07-440
LGpc4
0,0 E+agc/M+gat-450
15,3 E+ct/M+ac-185
38,1 E+act/M+at-390
58,6 S14-170
63,7 AE08-100
67,8 AE18-1000
73,4 AE09-510
90,0 H03-120
90,5 AC03-180
105,3 AB09-370
S14-390
121,3 AE01-120
H03-1200
142,2 E+act/M+ag-320
150,8 E+act/M+aa-410
162,5 E+ca/M+gg-70
165,5 E+agc/M+gc-75
LGpc5a
0,0 AF11-1600
3,4 AI01-460
10,0 AE08-470
32,3 C11-515
33,9 S14-450
40,4 AK10-1000
AU05-1200
AI01-430
0,0 P06-220
2,0 AG15-970
2,5 G14-540
28,9 AQ12-180
31,2 AM18-230
37,2 AC11-970
LGpc5b
LGpc6a
0,0 AA04-1100
11,0 T17-640
12,3 U16-380
14,5 AB04-260
17,6 AB14-420
17,7 AB14-170
35,1 AP03-400
50,8 AO20-850
52,6 AU05-210
53,8 AI13-355
60,9 AE06-280
AD04-645
81,7 E+ag/M+aa-440
86,7 E+ag/M+aa-430
96,5 E+ag/M+ac-60
E+ag/M+ga-465
101,2 E+agc/M+gt-105
121,9 E+agc/M+gt-500
138,4 X02-330
0,0 E+agc/M+gac-195
13,4 E+ag/M+gt-85
30,0 AA16-200
47,3 H18-460
48,7 AL06-210
LGpc6b
LGpc7
0,0 E+ag/M+aa-530
4,2 E+agc/M+gca-350
12,1 E+ag/M+ag-75
17,7 E+ag/M+ac-510
E+act/M+at-95
22,7 E+agc/M+gca-375
41,7 T01-290
46,3 AN07-260
47,4 AJ20-460
47,5 AB03-170
AX09-1200
P13-645
62,0 T17-500
69,3 AR12-275
V20-990
LGpc8
0,0 AW11-340
AJ03-360
8,2 AA16-540
24,7 AP03-210
44,7 AB07-580
49,5 AU16-780
50,5 AL13-920
72,4 E+ag/M+aa-155
80,0 E+ag/M+aa-165
102,4 E+ag/M+ga-102
109,4 E+ca/M+gg-475
109,8 E+ag/M+tc-385
110,6 E+ag/M+ta-210
E+ag/M+ga-550
132,4 F19-1600
138,1 AN10-840
AB09-530
V20-a
139,1 AB04-420
139,2 AF11-200
AC10-340
AB04-310
143,7 AA12-240
151,5 AW11-600
LGpc9
0,0 AD04-350
27,6 F09-670
27,7 AE07-850
AA12-340
33,1 Seed coat
36,1 AB17-980
45,1 AW11-420
AB08-1600
66,0 E+ag/M+ac-280
71,4 E+ag/M+at-380
72,4 E+act/M+ta-120
87,1 E+ag/M+tc-435
E+ag/M+at-120
E+ag/M+tg-115
87,2 E+ag/M+tc-310
LGpc10
0,0 E+ct/M+ac-125
6,2 E+agc/M+gac-100
9,8 E+ag/M+ga-485
E+ag/M+ac-215
10,1 E+ag/M+ta-370
17,3 E+ct/M+aa-515
27,9 E+ag/M+ga-410
43,8 AE08-380
44,9 F06-260
49,1 AB09-160
AJ03-440
49,2 AK11-180
AN10-180
72,3 G04-310
79,7 AI01-220
87,9 AF04-1100
95,2 AC03-120
115,9 AB02-340
AB02-180
LGpc11
0,0 AQ15-650
AI13-210
4,6 AP03-160
AA04-385
5,8 AB14-340
10,5 AB16-410
16,5 AJ03-920
22,1 F19-460
33,6 AD12-740
55,9 E+act/M+at-210
81,4 E+act/M+at-55
84,5 E+ag/M+ta-235
106,8 E+agc/M+gac-285
LGpc12
0,0 E+ca/M+gg-65
E+ag/M+gt-115
E+ag/M+at-150
3,5 E+ag/M+gt-245
4,6 E+ag/M+tt-90
6,7 E+ct/M+aa-50
E+agc/M+gta-315
25,0 AB16-530
35,9 AB09-470
38,9 F03-770
61,1 AE18-380
69,1 AL06-340
70,1 AB05-330
71,0 AN06-400
71,3 AM18-260
94,5 AB08-470
117,5 H03-310
136,8 AJ18-270
143,4 AB11-260
161,1 Growth habit
LGpc13a
0,0 E+ag/M+ac-350
19,0 AU15-950
27,7 AO11-340
0,0 T01-740
AM10-1400
2,2 AE08-460
2,7 AM06-900
5,8 H03-260
28,7 E+ag/M+tt-185
31,9 E+ag/M+ac-370
32,4 E+act/M+ta-170
LGpc13b
LGpc14
0,0 U16-450
2,2 AE06-380
10,9 AE01-370
16,1 AG15-1900
16,9 AL06-180
37,8 AW07-340
55,9 AB08-600
62,0 C11-830
62,4 C11-620
66,5 AO06-290
72,7 AI01-260
LGpc15
0,0 AF18-870
E+agc/M+gca-115
13,9 AK11-470
32,0 T17-1300
T17-360
59,6 AP04-380
E+ag/M+gt-455
E+act/M+ta-445
80,3 E+ag/M+gt-340
E+ca/M+gg-110
E+act/M+tt-335
85,3 E+act/M+ta-120
E+agc/M+gca-95
99,0 E+ag/M+aa-145
105,9 E+ag/M+gc-435
116,7 E+ag/M+gc-310
127,2 E+act/M+tg-480
127,9 E+ga/M+gt-485
130,0 E+act/M+tc-220
LGpc16
0,0 E+ca/M+gg-115
E+ca/M+gg-560
15,5 U16-290
17,8 AF04-980
33,0 F19-360
48,6 AW13-1600
AB09-870
56,4 AE09-350
81,1 E+act/M+tg-265
95,7 E+act/M+aa-202
101,1 E+act/M+tg-340
118,9 E+agc/M+gat-260
119,0 E+ag/M+ga-510
122,6 E+agc/M+gta-85
LGpc17
0,0 E+ag/M+gt-210
3,3 E+ag/M+at-455
9,6 E+ag/M+ac-470
14,0 E+ag/M+ag-130
15,7 E+ag/M+ac-415
26,6 E+ag/M+tc-495
42,9 X02-530
58,8 AO11-210
62,0 AI01-140
63,3 X02-980
75,5 AG04-340
86,3 AG15-150
87,4 AB05-360
105,7 AI01-1000
111,8 AL06-880
LGpc18
0,0 AC10-470
19,6 E+act/M+ag-460
31,8 AX07-520
38,3 AG08-560
39,6 AF11-670
AO20-740
47,5 AJ20-980
76,2 E+act/M+tt-265
80,3 E+ag/M+ta-505
LGpc19
0,0 E+ag/M+gc-95
5,0 E+ag/M+gc-265
30,9 AA12-1200
33,0 AD01-360
42,9 H06-510
LGpc20
0,0 AO11-450
AT14-910
9,1 AW18-800
AB03-1400
24,1 F03-580
24,8 AB16-490
AM10-365
37,2 AL07-410
Q15-845
67,0 E+act/M+ta-375
E+agc/M+gat-505
E+ca/M+gg-415
67,7 E+act/M+aa-220
E+ag/M+at-103
E+ag/M+ht-260
81,1 E+act/M+ta-480
E+ag/M+ga-330
LGpc21
0,0 AP04-480
16,8 AJ18-260
31,8 AE01-540
37,9 AV18-1100
AR18-520

◀**Fig. 3** Linkage map of the Pumpkin–Crookneck population. The loci and their size are listed to the right of the *bars*. To the left are the distances between markers in centiMorgans. RAPD and AFLP are represented by their universal codes. *Underlining* indicates common markers. *Bold* font indicates **co-dominant markers**

linkage group LGpz21, all linkage groups contained at least one co-dominant marker, with a maximum of six in LGpz1, or one common marker, with a maximum of 11 in LGpz3 (Fig. 2).

The Pumpkin–Crookneck map contained 323 markers consisting of 196 RAPD markers, of which 20 were co-dominant, 125 AFLP with 12 co-dominant markers, and two gene loci, *n* on LGpc9 and Bu on LGpc12. This map covers 2,234 cM, distributed over 21 major and 3 minor linkage groups ranging in size from 38 cM in LGpc21 to 176 cM in LGpc3, with an average of 93 cM. Except LGpc20 which, however, contained 17 markers, all other linkage groups contained at least one co-dominant and/or one common marker (Fig. 3). The average number of markers was 15.5 per linkage group, and the distance between markers was 6.9 cM (Fig. 3). The distribution of RAPD and AFLP markers throughout the genome was markedly different (Figs. 2, 3). While the distribution of RAPD markers was relatively even in both maps, AFLP markers tended to cluster in most cases.

Segregation distortions

Chi-square tests ($P = 0.05$) revealed that the segregation of 20 (4.7%) loci in the Pumpkin–Zucchini and 26 (5.6%) loci in the Pumpkin–Crookneck map were distorted. Markers having distorted segregation were at first excluded from grouping and ordering analyses. They were mapped after creating linkage groups using only markers without segregation distortion. Ten distorted markers in the Pumpkin–Zucchini and 15 in the Pumpkin–Crookneck map were grouped in doublets or triplets and were not included in the final maps. Eleven distorted markers, distributed over five linkage groups, were included in the Pumpkin–Zucchini map, and ten, distributed over four linkage groups, in the Pumpkin–Crookneck map. Some of these markers were grouped together in the same linkage group in both maps, namely LGpz/LGpc5 and LGpz/LGpc8. The Pumpkin–Zucchini map contains 31 RAPD markers originating from the source of ZYMV resistance, *C. moschata*. None of these 31 markers showed segregation distortions.

Markers for ZYMV resistance and hull-less seed coat

About 24 of the 31 *C. moschata* specific markers were mapped onto two linkage groups in clusters of the Pumpkin–Zucchini map. LGpz1 contains 11 and LGpz2 contains 13 of these markers, two of which are closely linked to the ZYMV-resistance loci. These two markers have been converted to SCARs (unpublished results). The remaining seven markers were distributed over six linkage groups, namely two in LGpz7, and one in each of the following linkage groups: LGpz9, LGpz11, LGpz13, LGpz14b, and LGpz19 (see *italic* font in Fig. 2).

The BSA identified 148 of the 1,068 primers which were polymorphic between the two bulks, i.e., two pools of 12 DNA samples from homozygous plants for hulled and hull-less seeds, respectively. After testing these primers on the 12 individual DNA samples composing each of the two bulks, only seven showed association with the seed coat characteristic. Upon genotyping the 92 F_2 plants of the Pumpkin–Zucchini population with these markers, five (AK11-340, AN10-340, AB14-235, H18-385, and AB07-590) were found, flanking the *n* locus in linkage group LGpz9 with a distance <1.5 cM (Fig. 2). Two markers (AO11-280 and AW11-420) were <6.5 cM away from the *n* locus. In five of the seven cases, the RAPD band was linked to the *N* allele, while two, AB14-235 and AW11-420, were linked to the *n* allele. While mapping the Pumpkin–Crookneck population, four markers (F09-670, AE07-850, AA12-340, and AB17-980) were found to be tightly linked to the *N* allele at a distance <7 cM. The fifth marker (AW11-420), linked to the *n* allele, only 3 cM distant, was present in both populations (Fig. 4).

Alignment of the two maps

Gels of both populations, containing either RAPD or AFLP fragments, were compared to identify common markers based on their identical fragment size. Fifty-two RAPD (19.4%) and 40 AFLP loci (33.8%), were detected. Of the 92 common markers scored, 62 were

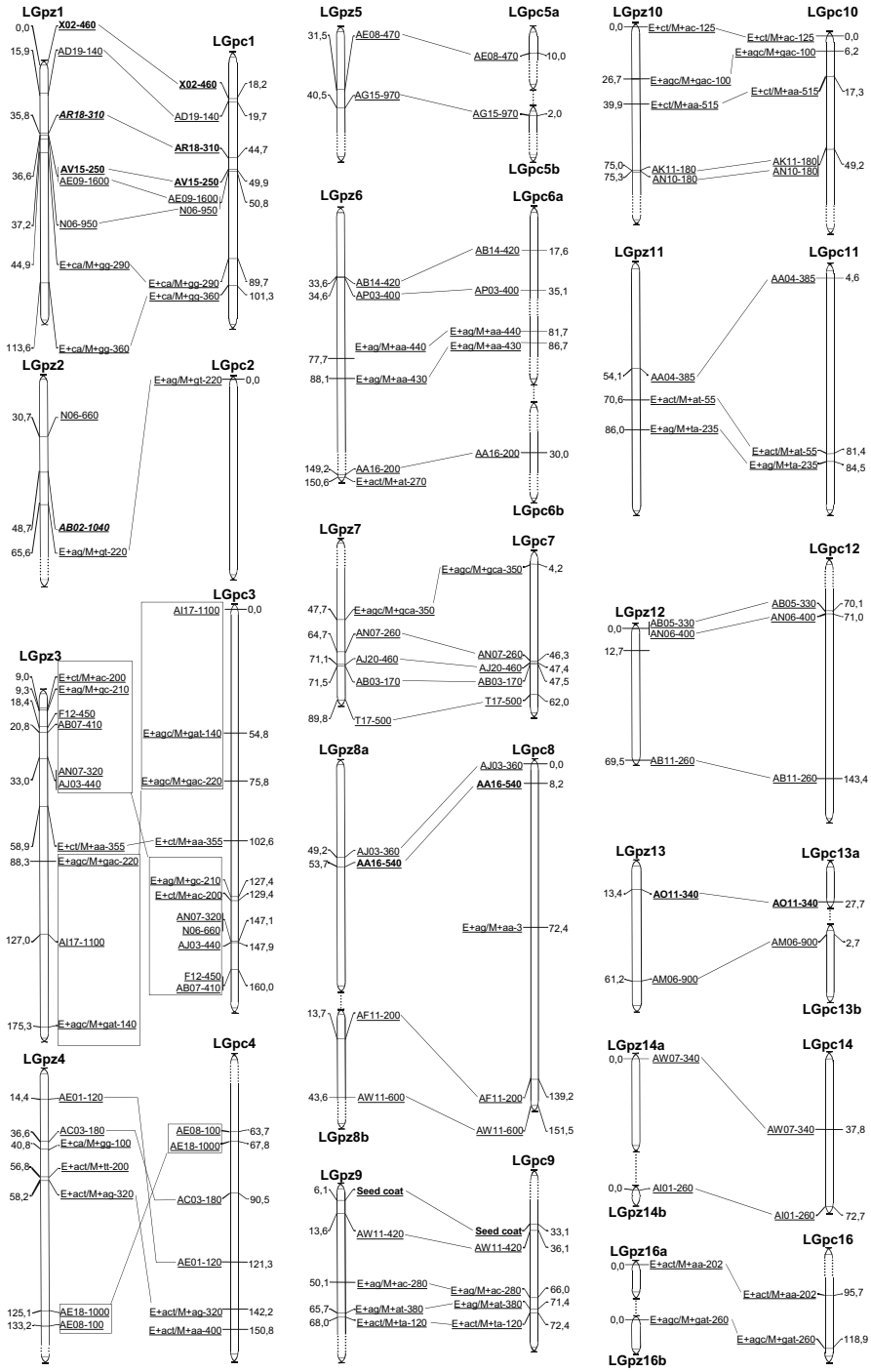


Fig. 4 Alignment of the two maps. The linkage groups include only the common markers, which are shown with the same code numbers as in the original maps. Linkage groups on the left-hand side of the aligned maps are from the Pumpkin–Zucchini map, those on the right from the Pumpkin–Crookneck map

used in mapping to recognize identical linkage groups (Fig. 4). We initially identified ten linkage groups that contained at least three common markers. When using relaxed grouping, i.e., LOD $\geq$3 and recombination frequency of higher than 35, the two linkage groups LGpz8a and LGpz8b were joined in the Pumpkin–Zucchini map (Fig. 2). In the same way the two linkage groups LGpc6a and LGpc6b were joined in the Pumpkin–Crookneck map (Fig. 3). Based on the presence of common markers, LGpz14 and

LGpz16 in the Pumpkin–Zucchini map and LGpc5 and LGpc13 in the Pumpkin–Crookneck map were connected (Fig. 4). Based on the presence of at least two common markers in each of the two maps, 14 linkage groups were aligned with an average of four common markers and a maximum of ten (LG3 in Fig. 4). LG2 contained three common markers in the Pumpkin–Zucchini map, yet only one of these markers could be mapped in the Pumpkin–Crookneck population. Therefore the alignment of this linkage group is based on only one marker. Although the order of common markers was, in most cases, conserved, markers in LG3 and LG4 had to be rearranged (Fig. 4). The average distances between common markers in the two populations were, in most cases, similar.

Discussion

By virtue of their marker coverage, density and consistency, the maps presented herein are suggested as reference maps for *C. pepo*. We have used mainly RAPD and AFLP markers as they are universal in their application and require no sequence information for their development. The combination of these markers allowed the construction of moderately saturated maps in both populations. Although they are, in general, dominant markers which limits their use in comparative mapping, they were successfully used here and in other studies for comparison of linkage groups and to align genetic maps (Plomion et al. 1995; Krutovskii et al. 1998; Laucou et al. 1998; Costa et al. 2000). Map alignment and map merging have generally been done on different populations, derived from the same two parental genotypes, or on two populations having one parental genotype in common. In our case, we used four different parental genotypes to create the two mapping populations, but two of the four parents are closely related, both being oil pumpkins. The number of common markers (92 in total, i.e., almost 30%) was relatively high. They allowed the establishment of a skeletal map for *C. pepo*, in which 62 mapped common loci were used to align 15 linkage groups. We consider these 15 linkage groups to represent different chromosomes, that is three quarters of the haploid number of chromosomes of *C. pepo*. The 62 common loci, represented by fragments of identical size in the two populations and therefore considered to be orthologous, were contributed in almost equal numbers by the four parents. This suggests an even distribution of alleles in the four parents. The order of these markers was conserved in most cases. Only in two cases rearrangements were observed, which could be due to the size of the mapping population, and/or to the algorithm used for arranging the markers (Périn et al. 2000). Other reasons could be genotyping errors, missing values and mapping distorted markers (Hackett and Broadfoot 2003).

Polymorphism

The significantly higher level of polymorphism, as revealed by both RAPD and AFLP markers, between the parents of the pumpkin/crookneck population than between the parents of the pumpkin/zucchini population clearly reflects the taxonomical position of the three cultivar-groups within the species. Pumpkin and zucchini belong to the same subspecies, *C. pepo* subsp. *pepo*. They were most probably domesticated in Mexico. The crookneck variety, on the other hand, belongs to *C. pepo* subsp. *ovifera* and was domesticated most likely in eastern US. The genetic relationship among these cultivar groups is solidly established by isozyme and DNA-markers (Decker 1988, Sanjur et al. 2002, Paris et al. 2003).

The level of polymorphism of RAPD in our mapping populations was higher than that found by Brown and Myers (2002), even though they used an inter-specific (*C. pepo* × *C. moschata*) mapping population. The greater amount of polymorphism detected in our populations may partly be due to the use of polyacrylamide instead of agarose for fragment separation (Stift et al. 2003).

Results of mapping using AFLP markers have not been previously published for *Cucurbita* and therefore comparison of the polymorphism level is not possible. Nevertheless, this polymorphism level in our study was similar to that found in *C. melo* (Wang et al. 1997), which is probably in part due to the similar genome size of the two species (Bennett and Leitch 2004). The AFLP technique produced twice as many polymorphic bands as the RAPD marker system (average polymorphism of the two populations: 5.4 vs. 2.7). Similar results have been reported

in other species (Powell et al. 1996; Milbourne et al. 1997; Costa et al. 2000; Haussmann et al. 2002; Zhong et al. 2004).

SSRs are versatile, highly informative, co-dominant markers for many eukaryotic genomes (Wang et al. 1994). They are locus specific, abundant and evenly distributed throughout the genome, and were recommended as standard markers to be used in the construction of highly saturated genetic maps (Beckmann and Soller 1990). Unfortunately, they showed poor transferability among cucurbit genera, as predicted by Katzir et al. (1996). The four parents of the two maps were genotyped for more than 100 melon SSR markers. Only a few of the markers generated bands, and none of them were polymorphic between the parents used to create the mapping populations. This was not the case when using *Cucurbita* SSR markers. Five of the available 18 *C. pepo* specific SSR markers showed polymorphism, and three of them could be mapped.

Genome coverage

The genome coverage in the Pumpkin–Zucchini map (2,140 cM) and in the Pumpkin–Crookneck map (2,234 cM) was slightly higher than the genome coverage (1,954 cM) of the inter-specific map reported by Brown and Myers (2002). The average distance between markers they found (13 cM) was, however, twice as large as that of our study (~6.6 cM), and is probably due to the low number of polymorphic markers they could use for mapping (148 vs. 330 markers). This is, however, understandable considering that Brown and Myers (2002) used a backcross population for mapping, which effectively reduces the number of potential polymorphisms to half.

Marker distribution

The difference in distribution among RAPD and AFLP markers described here are in good agreement with several reports in the literature, especially on clustering of AFLP markers in other species (Qi et al. 1998; Bert et al. 1999; Boivin et al. 1999; Haanstra et al. 1999; Vuylsteke et al. 1999; Young et al. 1999; Haussmann et al. 2002). This can be attributed to the use of *Eco*RI that cuts in AT-rich domains mainly located in the centromeric and telomeric regions. In contrast, however, De la Rosa et al. (2003) reported even distribution of both marker types, AFLP (using *Eco*RI) and RAPD in olive.

Co-dominant markers

Polyacrylamide gel electrophoresis, due to its higher resolution, resulted in additional scorable RAPD fragments and identification of co-dominant RAPD markers (Fig. 1). In the two populations RAPD primers produced approximately 10% co-dominant loci, which was twice that found in the AFLP marker system. The number of RAPD-based co-dominant loci was considerably greater than that reported for other species (Grattapaglia and Sederoff 1994; Laucou et al. 1998; Krutovskii et al. 1998), likely due to the use of polyacrylamide instead of agarose gel for fragment separation. Two co-dominant RAPD markers, linked to ZYMV resistance, were isolated, sequenced, and successfully converted into SCAR markers. One allele pair differed in length by six nucleotides, the other pair differed in three base substitutions (SNPs). It is well known, that electrophoretic mobility of DNA in polyacrylamide is also affected by its base composition causing structural changes of the molecules. Thus, molecules of the same size can differ in mobility and can be distinguished using polyacrylamide gel electrophoresis.

Distorted segregation

In comparison to other crop species (Laucou et al. 1998; Haanstra et al. 1999; Bert et al. 1999; Cai et al. 2004), the number of distorted loci found in this study was relatively low (~5%). It is worth mentioning the absence of any segregation distortion among the 31 markers originating from the source of resistance against ZYMV, *C. moschata*. Obviously, the integration of the genetic material from *C. moschata* into *C. pepo*, after backcrossing with the latter, was without any negative consequences. Segregation distortions have been reported in numerous genetic mapping studies, especially in populations derived from inter-specific crosses (e.g., Paterson et al. 1988; Keim et al. 1990; Heun et al. 1991; Echt et al. 1994). The mechanism of segregation distortion is not clearly understood, but it may have a number of different causes, such as chromosomal imbalance (aneuploidy), chromosomal rearrangements (translo-

cations), competition among gametes, or genes affecting the viability of the embryo, as was suggested recently by Gonzalo et al. (2005). This can be the reason for the higher frequency (14%) of distorted markers in the inter-specific hybrid between *C. pepo* and *C. moschata* reported by Brown and Myers (2002).

Segregation distortion in *C. pepo* has been observed by Wilson and Payne (1994) and Paris (2000). In the first study, different pollen mixtures were applied in crosses between cultivated and free-living genotypes of *C. pepo* to study the competition of microgametophytes in fertilization. In the latter publication inter-subspecific crosses between two *C. pepo* genotypes were studied. Results of both experiments suggested an interaction between microgametophyte and gynoecium, which could, however, also be partly caused by the structure of gynoecium (Wilson and Payne 1994). Paris (2000) put forward the idea that interaction of microgametophyte with gynoecium and consequently gametophytic selection could partly be responsible for the continuous existence of groups of particular fruit shapes (cultivar-groups) throughout the centuries (Paris 2000). In our case, the average of 5% distorted markers in both populations was relatively low. Some of these markers were grouped in the same linkage groups in both maps, e.g., two in LGpz/LGpc5 and three in LGpz/LGpc8. The remaining were scattered individually in different linkage groups. No discernible difference between the intra- and inter-subspecific cross, with respect to segregation distortion, was observed.

Tagging the seed coat character

Of the seven markers, which were found linked to the *n* locus in the Pumpkin–Zucchini map, only two were in coupling phase with the hull-less allele. The marker AW11-420, which was 7 cM away from the hull-less allele in the Pumpkin–Zucchini map, was also found linked, at a distance of 3 cM, to the *n/n* (hull-less) allele in the Pumpkin–Crookneck map. This marker could play an important role in map-based cloning experiments, studying the genetics of the lignification of the seed-coat in *C. pepo*. The usefulness of this marker in selecting for hull-less genotypes has to be validated. Using BSA, Haley et al. (1994) identified RAPD markers in common bean, linked in both, coupling and repulsion, to a resistance allele against Bean Common Mosaic Virus (BCMV). In a case study they demonstrated that repulsion-phase linkages provided greater selection efficiency than coupling-phase linkage, even when the former had a greater linkage distance from the pest resistance allele. Repulsion-phase markers greatly improved selection efficiency for homozygous resistant genotypes. This we shall test too for the repulsion-phase markers in hull-less pumpkin.

Tagging ZYMV resistance

Three independent complementary genes confer resistance to ZYMV in *C. pepo* (Paris and Cohen 2000). About 24 of 31 bands introduced into *C. pepo* from the source of resistance, *C. moschata*, were mapped to LGpz1 and LGpz2. The remaining seven markers were distributed over six other linkage groups, and conceivably one of them is linked to the third putative resistance gene. Increasing map density may join some linkage groups into one, carrying the third independent resistance gene.

Acknowledgments This research was financially supported by the Austrian Science Fund (FWF Project, No. P15773) and by the State of Lower Austria. Our thanks to J. E. Staub, USDA–ARS and University of Wisconsin, Madison, for testing the *Cucumis*-SSRs on the four parents used in our crosses. We are grateful to J. E. Staub and H. S. Paris for critically reading the manuscript.

References

Andres TC (1987) *Cucurbita fraterna*, the closest wild relative and progenitor of *C. pepo*. Cucurbit Genet Coop Rep 10:69–71

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93:57–64

Beckmann JS, Soller M (1990) Towards a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite site. Biotechnology 8:30–32

Bennett MD, Leitch IJ (2004) Plant DNA C-values database (release 3.0, Dec. 2004) http://www.rbgkew.org.uk/cval/homepage.html

Bert PF, Charmet G, Sourdille P, Hayward MD, Balfourier F (1999) A high-density molecular map for ryegrass (*Lolium perenne*) using AFLP markers. Theor Appl Genet 99:445–452

Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW, Rister RS (eds), Klein S, Rister RS (trans) (1998) The complete German commission E monographs.

S. 193. American Botanical Council; Boston; Integrative Medicine Communications, Austin

Boivin K, Deu M, Rami J-F, Trouche G, Hamon P (1999) Towards a saturated sorghum map using RFLP and AFLP markers. Theor Appl Genet 98:320–328

Bradeen JM, Staub JE, Wye C, Antonise R, Peleman J (2001) Towards an expanded and integrated linkage map of cucumber (*Cucumis sativus* L). Genome 44:111–119

Brown RN, Myers JR (2002) A genetic map of squash (*Cucurbita* ssp.) with randomly amplified polymorphic DNA markers and morphological markers. J Am Soc Hortic Sci 127:568–575

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Genet 104:84–91

Cai H, Inoue M, Yuyama N, Nakayama S (2004) An AFLP-based linkage map of Zoysiagrass (*Zoysia japonica*). Plant Breed 123:543–548

Costa P, Pot D, Dubos C, Frigerio J-M, Pionneau C, Bodénès C, Bertocchi E, Cervera M-T, Remington DL, Plomion C (2000) A genetic map of maritime pine based on AFLP, RAPD and Protein markers. Theor Appl Genet 100:39–48

Danin-Poleg Y, Tadmor Y, Tzuri G, Reis N, Hirschberg J, Katzir N (2002) Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. Euphytica 125:373–384

Decker DS (1988) Origin(s), evolution, and systematics in *Cucurbita pepo* (Cucurbitaceae). Econ Bot 42:4–15

De la Rosa R, Angiolillo A, Guerriero C, Pellegrini M, Rallo L, Besnard G, Berville A, Martin A, Baldoni L (2003) A first linkage map of olive (*Olea europaea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. Theor Appl Genet 106:1273–1282

Duchesne AN (1786) Essai sur l'histoire naturelle des courges. Pankoucke CJ, Paris, p 46

Echt CS, Kidwell KK, Knapp SJ, Osborn TC, McCoy TJ (1994) Linkage mapping in diploid alfalfa (*Medicago sativa*). Genome 37:61–71

Fazio G, Staub JE, Chung SM (2002) Development and characterization of PCR markers in cucumber (*Cucumis sativus* L.) J Am Soc Hortic Sci 127:545–557

Ferriol M, Picó B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theor Appl Genet 107:271–282

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arus P, Monforte AJ (2005) Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). Theor Appl Genet 110: 802–811

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137:1121–1137

Grebenščikov I (1954) Zur Vererbung der Dünnschaligkeit bei *Cucurbita pepo* L. Züchter 24:162–166

Haanstra JP, Wye W, Verbakel H, Meijer-Dekens F, Van den Berg P, Odinot P, Van Heusden AW, Tanksley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* x *L. pennellii* F_2 populations. Theor Appl Genet 99:254–271

Hackett CA, Broadfoot LB (2003) Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. Heredity 90:33–38

Haley SD, Afanador L, Kelly JD (1994) Selection for monogenic pest resistance traits with coupling- and repulsion-phase RAPD markers. Crop Sci 34:1061–1066

Hartl L, Mohler V, Zeller FJ, Hsam SLK, Schweizer G (1999) Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat. Genome 42:322–329

Haussmann BIG, Hess DE, Seetharama N, Welz HG, Geiger HH (2002) Construction of a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers, and comparison with other sorghum maps. Theor Appl Genet 105:629–637

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Katzir N, Danin-Poleg Y, Tzuri G, Karchi Z, Lavi U, Cregan PB (1996) Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor Appl Genet 93:1282–1290

Katzir N, Tadmor Y, Tzuri G, Leshzeshen E, Mozes-Daube N, Danin-Poleg Y, Paris HS (2000) Further ISSR and preliminary SSR analysis of relationships among accessions of *Cucurbita pepo*. In: Katzir N, Paris HS (eds) Proceedings of the cucurbitaceae 2000. Acta Hortic 510:433–439

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735–742

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kreuter MH (2000) Hormone disbalance and urinary bladder disease. Phyto Novum, Emil Flachsmann AG, Zürich Switzerland, pp 2–24

Krutovskii KV, Vollmer SS, Sorensen FC, Adams WT, Knapp SJ, Strauss SH (1998) RAPD genome maps of Douglas-fir. J Hered 89:197–205

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Laucou V, Haurogné K, Ellis N, Rameau C (1998) Genetic mapping in pea. 1. RAPD linkage map of *Pisum sativum*. Theor Appl Genet 97:905–915

Lee YH, Jeon HJ, Hong KH, Kim BD (1995) Use of random amplified polymorphic DNA for linkage group analysis in an interspecific cross hybrid F_2 generation of *Cucurbita*. J Kor Soc Hortic Sci 36:323–330

Lincoln SE, Daly MJ, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Inst Tech Rpt 3rd Edition

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance gene by bulked segregant analysis: a rapid method to detect markers in spe-

cific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Milbourne D, Meyer R, Bradshaw JE, Baird E, Bonar N, Provan J, Powell W, Waugh R (1997) Comparison of PCR-based marker systems for the analysis of genetic relationship in cultivated potato. Mol Breed 3:127–136

Murkovic M, Hillebrand A, Winkler J, Leitner E, Pfannhauser W (1996) Variability of fatty acid content in pumkin seeds (*Cucurbita pepo* L.) Z. Lebensm Unters Forsch 203:216–219

Murkovic M, Piironen V, Lampi AM, Kraushofer T, Sontag G (2004) Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil (Part 1: non-volatile compounds). Food Chem 84:359–365

Naudin C (1856) Nouvelles recherches sur les caractères spécifiques et les variétés des plantes du genre *Cucurbita*. Ann Sci Nat Bot IV 6:5–73, pl. 1–3

Oliver M, Garcia-Mas J, Cardús M, Pueyo N, López-Sesé AI, Arroyo M, Gómez-Paniagua H, Arús P, de Vicente MC (2001) Construction of a reference linkage map of melon. Genome 44:836–845

Paris HS (1986) A proposed subspecific classification for *Cucurbita pepo*. Phytologia 61:133–138

Paris HS (2000) Segregation distortion in *Cucurbita pepo*. In: Katzir N, Paris HS (eds) Proceedings of the cucurbitaceae 2000. Acta Hortic 510:199–202

Paris HS, Cohen S (2000) Oligogenic inheritance for resistance to zucchini yellow mosaic virus in *Cucurbita pepo*. Ann Appl Biol 136:209–214

Paris HS (2001) History of the cultivar-groups of *Cucurbita pepo*. Hortic Rev 25:71–170

Paris HS, Yonash N, Portnoy V, Mozes-Daube N, Tzuri G, Katzir N (2003) Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. Theor Appl Genet 106:971–978

Paris HS, Brown RN (2005) The genes of pumpkin and squash. HortScience 40:1620–1630

Park YH, Sensoy S, Wye C, Antonise R, Peleman J, Havey MJ (2000) A genetic map of cucumber composed of RAPDs, RFLPs, AFLPs, and loci conditioning resistance to papaya ringspot and zucchini yellow mosaic viruses. Genome 43:1003–1010

Paterson AH, Landers ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Périn C, Hagen L, Dogimont C, de Conto V, Pitrat M (1998) Construction of a genetic map of melon with molecular markers and horticultural traits. In: McCreight JD (ed) Cucurbitaceae 98: evaluation and enhancement of cucurbit germplasm. ASHS Press, Alexandria, VA, pp 370–376

Périn C, Hagen L, Dogimont C, de Conto V, Lecomte L, Pitrat M (2000) Construction of a reference genetic map of melon. In: Katzir N, Paris HS (eds) Proceedings of the cucurbitaceae 2000. Acta Hortic 510:367–374

Plomion C, O'Malley DM, Durel CE (1995) Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. Theor Appl Genet 90:1028–1034

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellites) markers for germplasm analysis. Mol Breed 2:225–238

Prym von Becherer L (1955) Untersuchungen zur Anatomie und zum Erbverhalten der Samenschalen von *Cucurbita maxima* DUCH. und *Cucurbita pepo* L. Züchter 25:1–14

Qi X, Stam P, Lindhout P (1998) Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor Appl Genet 96:376–384

Robinson RW, Decker-Walters DS (1997) Cucurbits. CAB International, New York

Schmidlin CB, Kreuter MH (2003) *Cucurbita pepo*, mögliche Einfluss auf hormonelle Ungleichgewicht bei Inkontinenz. Phytotherapie 3:2–4

Serquen FC, Bacher J, Staub JE (1997) Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativus* L.) using random-amplified polymorphic DNA markers. Mol Breed 3:257–268

Sanjur OI, Piperno DR, Andres ThC, Wessel-Beaver L (2002) Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae) inferred from mitochondrial gene: implications for crop plant evolution and areas of origin. Proc Natl Acad Sci USA 99:535–540

Stift G, Pachner M, Lelley T (2003) Comparison of RAPD fragment separation in agarose and polyacrylamide gel by studying *Cucurbita* species. Cucurbit Genet Coop Rep 26:62–65

Teppner H (2000) *Cucurbita pepo* (Cucurbitaceae)—history, seed coat types, thin coated seeds and their genetics. Phyton 40:1–42

Torres-Ruiz R, Hemleben V (1991) Use of ribosomal DNA spacer probes to distinguish cultivars of *Cucurbita pepo* L. and other Cucurbitaceae. Euphytica 53:11–17

Vos P, Hogers R, Bleeker M, Reijans M, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor Appl Genet 99:921–935

Wang YH, Thomas CE, Dean RA (1997) A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. Theor Appl Genet 95:791–798

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeates. Theor Appl Genet 88:1–6

Wilson HD, Payne JS (1994) Crop/weed microgametophyte competition in *Cucurbita pepo* (Cucurbitaceae). Am J Bot 81:1531–1537

Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution in the soybean genome. Theor Appl Genet 99:785–790

Zhong D, Pai A, Yan G (2004) AFLP-based genetic linkage map for the red flour beetle (*Tribolium castaneum*). J Hered 95:53–61

Zraidi A, Obermayer R, Pachner M, Lelley T (2003) On the genetics and histology of the hull-less character of Styrian Oil-Pumpkin (*Cucurbita pepo* L.). Cucurbit Genet Coop Rep 26:57–61

Zraidi A, Lelley T (2004) Genetic map for pumpkin *Cucurbita pepo* using random amplified polymorphic DNA markers. In: Lebeda A, Paris HS (eds) Progress in cucurbit genetics and breeding research. Proceedings of the cucurbitaceae 2004, 8th EUCARPIA meeting on cucurbit genetics and breeding, Olomouc, pp 507–514