

F.J. Noguera · J. Capel · J.I. Alvarez · R. Lozano

Development and mapping of a codominant SCAR marker linked to the *andromonoecious* gene of melon

Received: 7 September 2004 / Accepted: 28 November 2004 / Published online: 14 January 2005
© Springer-Verlag 2005

Abstract Monoecy is an important goal for melon breeding because of the agronomic advantages it provides to parental lines in that they do not require hand emasculation to develop monoecious F_1 hybrids, the latter producing fruits of higher quality. Monoecious phenotype is conferred by the dominant allele of the *andromonoecious* (*a*) gene, whereas recessive homozygous plants are andromonoecious. A bulked segregant analysis (BSA) approach performed in a set of 38 double-haploid lines has allowed us to identify an AFLP marker linked to the *a* gene at 3.3 cM. Following cloning and sequencing of the AFLP fragment, specific PCR primers were designed and used in the amplification of a codominant SCAR marker. Using a backcrossed mapping population of 530 plants, the SCAR marker could be mapped near the *a* locus (5.5 cM). Size difference between the two allelic SCAR fragments is 42 bp and might be due to a deletion/insertion. The SCAR marker is closest to the *a* gene identified to date, and can be useful in breeding programs, using marker-assisted selection procedures to screen for sexual types in melon.

Introduction

Although most flowering plants are hermaphrodite (bisexual flowers develop on each plant), a significant

number of species also produce unisexual flowers, leading to a diversity of sex phenotypes. The genetic and molecular studies performed on different sexual types of these species provided valuable information regarding sex determination mechanisms in plants (Westergaard 1958; Charlesworth 1991; Grant 1999; Ainsworth 2000; Filatov et al. 2000; Liu et al. 2004). Melon (*Cucumis melo* L., $2n = 2x = 24$) is cultivated throughout the world. Breeding selections are generally based of fruit quality and resistance to pests and diseases (Whitaker and Davis 1962; Stepansky et al. 1999). Like other members of the Cucurbitaceae family, melon is remarkable for its diversity of sexual types, and sex expression is most challenging for melon breeders (Rosa 1928; Poole and Grimball 1939; Kenigsbuch and Cohen 1990). Although many traditional melon cultivars are andromonoecious (separate hermaphrodite and male flowers develop on each plant), they are being replaced by new hybrid varieties that tend to be monoecious (plants bearing female and male flowers). Monoecy is of agronomic interest both for breeding practices and for the seed industry due to three main reasons: (1) emasculation of the flowers is not required to avoid inbred lines; (2) the facility to produce hybrid seeds ensures confidentiality and preserves breeder rights; and (3) fruits produced by monoecious hybrids develop a smaller bottom scar (Fig. 1), resulting in a reduced risk of pathogen infections and better fruit quality (Périn et al. 2002b).

The genetic control of sex determination in melon depends on three genes, *andromonoecious* (*a*), *gynomonoecious* (*g*), and *maleness* (*M*), which combine to generate the different sexual types (Poole and Grimball 1939; Kenigsbuch and Cohen 1990). Among these, monoecious (*A-GG*) and andromonoecious (*aaGG*) types are determined by the dominant and recessive allele of the *a* gene in combination with the gynoecious dominant allele (*G*-). Hermaphrodite type is homozygous for *a* and *g* recessive alleles (*aagg*), while *mm* genotype seems to stabilize gynoecy of *AAgg* plants (see Kenigsbuch and Cohen 1990). For breeding purposes, monoecious and andromonoecious types are the ones commonly used as

Communicated by I. Paran

F. J. Noguera · J. Capel · R. Lozano (✉)
Departamento de Biología Aplicada (Genética),
Edificio CITE II-B, Universidad de Almería,
La Cañada de San Urbano s/n, Almería, 04120, Spain
E-mail: rlozano@ual.es
Tel.: +34-950-015111
Fax: +34-950-015476

J. I. Alvarez
Syngenta Seeds, S.A. Autovía E15, km.417,5,
El Ejido, Almería, 04700, Spain

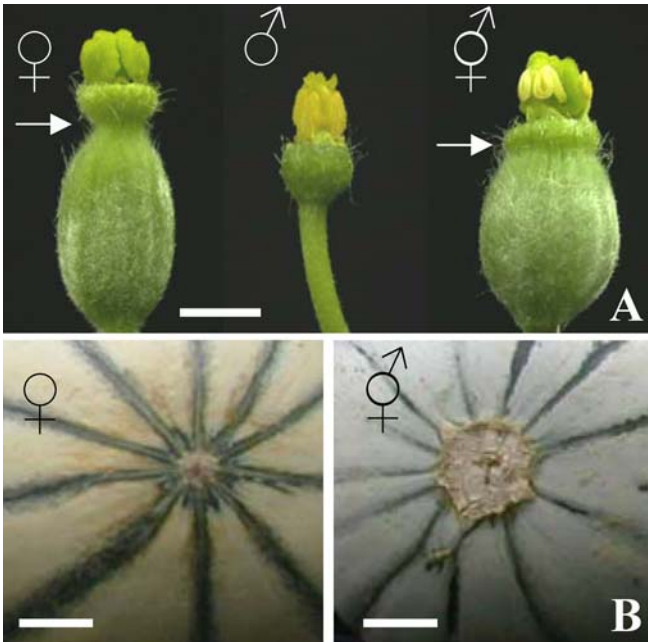


Fig. 1 **A** The *andromonoecious* (*a*) gene has major effect on sexual type of melon. Monoecious plants (bearing the *A* allele) develop separate female (*left*) and male (*center*) flowers, while andromonoecious plants (homozygous *aa*) develop male and hermaphrodite (*right*) flowers. Moreover, the *a* gene has pleiotropic effects on the abscission zone of the flower which is smaller in hermaphrodite than in female flowers (*arrows*). **B** The scar bottom, which is the shutting kept on the fruit after the flower abscission, is also smaller in fruits developed from female (*left*) than from hermaphrodite (*right*) flowers. Scale represents 0.5 cm

plant material, while other sexual types segregating for *G* and *M* genes are not usually considered by melon breeders. Recent mapping results published by Danin-Poleg et al. (2002), Périn et al. (2002a) and Silberstein et al. (2003) have located the *a* gene in two different linkage groups, although in all cases the monoecious US PI 414723 was a donor parent in the corresponding mapping populations. However, both linkage groups contain a resistance gene (*Zym*) and a microsatellite marker (CmGA36), indicating they correspond to the same genomic region. The partial map of Danin-Poleg et al. (2002) included an RAPD marker at 16.2 cM to the *a* gene, while Silberstein et al. (2003) described an RFLP marker linked at 7 cM to the same locus. The genetic map constructed by Périn et al. (2002a) placed the *a* gene on a 25.2-cM linkage region containing two QTLs for ovary and fruit shape. This linkage region is likely to contain genes controlling sex and fruit shape.

The combination of bulked segregant analysis [(BSA) Michelmore et al. 1991] and highly polymorphic PCR-based markers permits the identification and mapping of useful molecular markers for breeding purposes. Markers linked to sex-controlling genes were identified in several dioecious species, including asparagus (*Asparagus officinalis*, Reamon-Bütter et al. 1998; Reamon-Bütter and Jung 2000), hop (*Humulus lupulus*, Polley et al. 1997), *Pistacia vera* (Hormaza et al. 1994), and papaya (*Carica papaya*, Urasaki et al. 2002; Deputy

et al. 2002). In the Cucurbitaceae family, markers linked to sex expression were identified in cucumber (*Cucumis sativus*, Trebitsh et al. 1997; Kahana et al. 1999), in which sex expression is also under the control of three genes (see review of Robinson and Decker-Walters 1997). One of these genes, *F*, promotes female flower production and determines the gynoeceious phenotype when combined with the *MM* genotype, whereas cucumber plants of *ffMM* genotype are monoecious. Moreover, genetic analyses performed on ethylene-related genes suggest that the *F* gene could affect ethylene biosynthesis and sex expression in cucumber plants (Trebitsh et al. 1997; Kahana et al. 1999). Association between sex-related and ethylene-related genes has not been reported in melon. The identification and mapping of molecular markers linked to sex controlling genes would be valuable for breeding and for elucidating the sex-determining mechanisms. In this paper, we report the development of a codominant SCAR marker linked to monoecious trait by using a BSA methodology (Michelmore et al. 1991) with a double-haploid (DH) population of Charentais type melon.

Material and methods

Plant material

Three homozygous breeding lines of melon (*C. melo* L.), all belonging to the Charentais varietal type, were used in this study. A monoecious line (CM) and an andromonoecious line (CA1) were the parents of a DH population composed of 38 individuals used in the BSA analysis. DH lines were generated by pollination of female flowers with Co^{60} gamma-irradiated pollen, followed by in vitro rescue of parthenogenic embryos. In vitro culture conditions and colchicine treatments for chromosome doubling were performed following the protocol described by Lotfi et al. (2003), with minor changes. The third line (CA2) is an andromonoecious line that was crossed with CM to generate a backcross (BC_1) population [(CM×CA2)× CA2] composed of 530 individuals, which was used for linkage analysis. Both CA1 and CA2 were used as female parents and produced round fruits, whereas CM produced oblong fruits. In order to know the genotype and the stability of sexual type in every DH line, 12 offspring plants were grown in the greenhouse and their phenotype scored at three different developmental stages: at the initiation of flowering, in the middle of the reproductive cycle when the plants set the first two full-sized fruits, and at the end of the crop cycle. All plants were grown in a plastic greenhouse located in Almeria (Spain) under natural sunlight conditions.

DNA isolation

Young leaves either from 12 plants of every DH line or from individual BC_1 plants were harvested, quickly

frozen in liquid nitrogen, and stored at -80°C . DNA was extracted using the DNeasy Plant Mini kit (Qiagen), according to the recommendations of the supplier. The total DNA was dissolved in water, visualized after electrophoresis in 1% agarose gels 1× TBE (Tris-borate-EDTA), and quantified by comparison with DNA standards (GIBCO-BRL). Then it was diluted to 100 ng/ μl and kept frozen at -20°C until used in PCR reactions.

Marker analysis

The AFLP analysis was carried out as described by Vos et al. (1995), using *MseI* (New England Biolabs) and *EcoRI* (Roche) as restriction enzymes. Digestion was performed with 5 U each enzyme in a final volume of 40 μl for 2 h at 37°C , and it was followed by the addition of 1 U T4-DNA Ligase (Roche) and incubation for 1 h at 37°C . Pre-amplification reaction were done in a volume of 20 μl , using A as the selective nucleotide for the *EcoRI* primer (*EcoRI*+A) and three different *MseI* primers (*MseI*+C, *MseI*+G, and *MseI*+T). The second PCR amplification was performed with primers that included three selective bases in their sequences being the *EcoRI* primer radioactively labeled with $\gamma[^{33}\text{P}]\text{-ATP}$ (3,000 Ci/mmol). AFLP products were separated on 6% denaturing polyacrylamide gels, using a Sequi-Gen Cell sequencing equipment (Bio-Rad). Electrophoresis was carried out for 2 h in 1× TBE; gels were then dried for 2 h at 80°C and exposed to radiographic Kodak film over 3 days at room temperature.

In order to increase the probability of identifying some marker linked to the *a* gene, we generated four bulks from the DH population, composed of eight plants each—two comprising monoecious plants and another two with andromonoecious plants. DNA from the bulks and the two parental lines was simultaneously screened with 79 AFLP primer combinations (Table 1). AFLP markers were named according to the standard list for AFLP primer nomenclature (Keygene, The Netherlands, <http://wheat.pw.usda.gov/ggpages/keygene/AFLPs.html>). Polymorphic bands among the contrasting DNA bulks, which were also displayed by the parents, were further analyzed both in DH and BC₁ individual plants.

Conversion of AFLP markers

The DNA fragment corresponding to the AFLP marker closest to the *a* gene was excised from the gel and purified according to Qu et al. (1998). Briefly, the piece of gel containing the amplified fragment was hydrated and incubated at 37°C overnight; then it was centrifuged to pellet solid debris, and the upper phase was 100-fold-diluted. The DNA isolated was re-amplified by PCR, following the same AFLP selective amplification described above but using non-labeled primers. The

Table 1 AFLP primer combinations used to find markers linked to the *a* gene

Pre-amplification ^a			
E01M02	E01M03	E01M04	
Amplification			
E32M59	E35M89	E36M82	E46M80
E32M60	E36M58	E36M93	E46M85
E32M61	E36M81	E42M79	E32M93
E32M62	E36M89	E42M84	E33M51
E32M63	E42M62	E46M79	E33M59
E32M65	E42M82	E46M84	E33M63
E32M70	E42M93	E32M89	E33M79
E32M79	E46M82	E33M50	E33M85
E32M80	E46M93	E33M62	E35M80
E32M81	E32M85	E33M70	E35M85
E32M82	E33M49	E33M82	E36M55
E32M84	E33M55	E33M79	E36M59
E33M48	E33M61	E35M84	E36M80
E33M54	E33M66	E36M79	E36M85
E33M60	E33M81	E36M61	E42M60
E33M65	E33M93	E36M79	E42M81
E33M80	E33M82	E36M84	E42M89
E33M89	E35M93	E42M58	E46M81
E35M81	E36M60	E42M80	E46M89
	E36M62	E42M85	

^aCodes are according to the standard list for AFLP primer nomenclature

resulting PCR products were purified by size-exclusion chromatography, using the GenElute PCR Clean-up kit (Sigma), visualized, and quantified by 1% agarose gel electrophoresis. The appropriate DNA fragment was cloned in the T/A-cloning vector pGEM-T Easy (Promega) and transformed into *Escherichia coli* strain DH5 α , according to the manufacturer's instructions. Clones of the expected insert size were selected and sequenced using the T7 and SP6 primers. Nucleotide sequences were obtained from an ABIPRISM 377 Sequencer (Applied Biosystem) and used to design SCAR primers by synthesizing two specific oligonucleotides from both ends of the AFLP fragment. Amplification of the SCAR marker was performed in 50 μl , using 10 ng total DNA, 50 ng each SCAR primer, 2.5 mM dNTP, 4 mM MgCl₂, and 1 U RED-*Taq* DNA polymerase (Sigma) in 1× *Taq* buffer. Cycling condition were as follows: 94°C , 60 s; and then 35 cycle of 94°C , 30 s, 56°C 60 s, and 72°C , 60 s; and an extension of 5 min at 72°C . Products from the SCAR experiments were analyzed in 2% agarose gels in 1× TBE.

Analysis of data

A χ^2 test was used to test the goodness-of-fit of the Mendelian segregation of the *a* gene classes. The recombination frequencies between the marker and the *a* gene were calculated according to the method of Allard (1956), whereas map distances were based on Kosambi's mapping function (Kosambi 1944).

Results

F₁ plants from crosses between a Charentais monoecious line, CM, and two Charentais andromonoecious lines, CA1 and CA2, were all monoecious, indicating that the development of unisexual flowers is a dominant character. To further characterize the genetic control of monoecy, 38 DH lines were obtained from the hybrid CM × CA1. A segregation analysis of the *a* gene was carried out in 12 descendants of each DH plant as well as in a BC₁ population composed of 530 descendants from the backcross (CM × CA2) × CA2. Both segregating populations (DH and BC₁) were generated from the same monoecious donor parent, and they were found to fit a segregation ratio 1:1 for either sexual type (monoecious:andromonoecious). χ^2 test value was highly significant in the DH population (0.24, $P=0.62$), but showed a lower level of significance in the BC₁ population (5.7, $P=0.02$). Nevertheless, all these results confirmed the monogenic inheritance of the monoecy/andromonoecy in melon as was previously described by Kenigsbuch and Cohen (1990).

Genetic linkage of AFLP markers to the *andromonoecious* gene

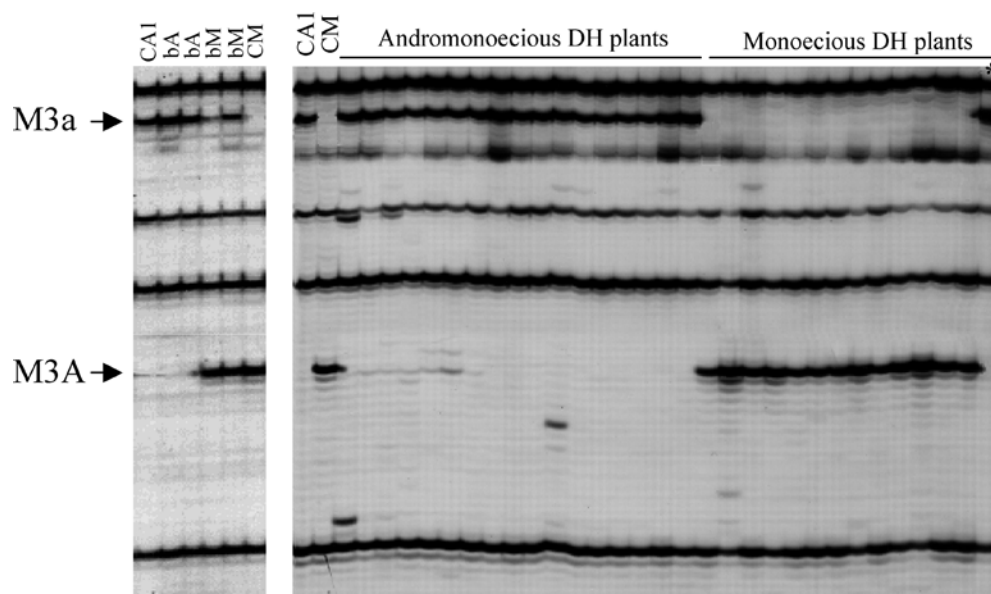
A total 79 AFLP primer combinations were used to screen DNA from the two parental lines (CM and CA1) and four DNA bulks constructed for the BSA analysis, two of them from monoecious and two from andromonoecious DH plants. PCR analyses resulted in the amplification of 5,119 AFLP fragments, of which 1,893 (37%) were polymorphic among the CM and CA1 parents. As expected, most of the primer combinations used in the BSA analysis did not detect any polymor-

phism among the four DNA bulks. However, one AFLP primer combination (E33M85) exhibited a polymorphic banding pattern between parents that was also maintained when compared DNA profiles from monoecious and andromonoecious bulks (Fig. 2). This primer combination produced a DNA fragment in the monoecious parental line, named M3A, which was also present in both monoecious DNA bulks but not in the andromonoecious plants, either parental CA1 line or bulks (Fig. 2). Interestingly, the same primer combination amplified another DNA fragment, M3a, with a complementary pattern to M3A. The M3a marker was shown by the andromonoecious plants (CA1 and bulks) but was absent in the monoecious parent and in one of the monoecious DNA bulks (Fig. 2). This result indicated that this primer combination amplified molecular markers putatively linked to the *A/a* gene. In order to confirm this hypothesis, both markers were mapped in the DH population (Fig. 2). This preliminary linkage analysis revealed a single recombination event between the marker and the gene, indicating a genetic distance of 3.3 cM between the marker M3 and the *a* gene (Kosambi mapping function). Polymorphism detected by this AFLP primer combination behaved as a codominant marker (M3) with two alleles, M3A and M3a, linked to the monoecious (*A*) and andromonoecious (*a*) alleles, respectively.

Development of a codominant SCAR marker

M3 marker was converted to a SCAR marker, which could be useful for genotyping selection. With this objective, it was characterized by cloning and sequencing the two DNA fragments corresponding to M3A and M3a markers. The sequence of the monoecious allele M3A was 83-bp long and exhibited a 42-bp deletion

Fig. 2 AFLP analysis performed in andromonoecious (CA1) and monoecious (CM) parents as well as in double-haploid (DH) lines, the latter grouped in two andromonoecious (*bA*) and two monoecious bulks (*bM*). Bulked segregant analysis is shown in the *left panel*, while the individual analysis performed in DH plants is shown in the *right panel*. The two M3 allelic markers, M3A and M3a (arrows), cosegregate with *A* (monoecious) and *a* (andromonoecious) alleles respectively, except for one monoecious DH plant (*asterisk*). Only a section of the AFLP gel is shown for a better quality of the figure



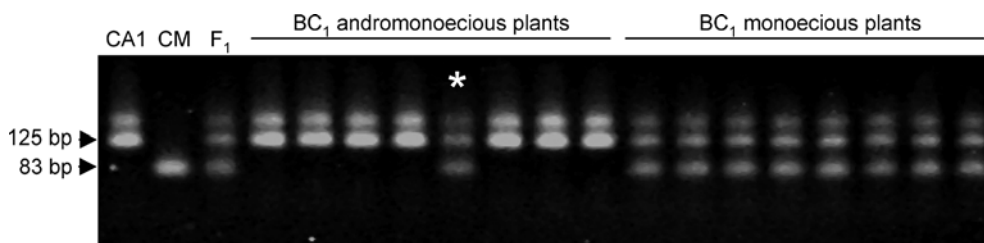


Fig. 4 PCR amplification of the codominant SCAR marker M3 in the parental lines, CA1 and CM, the F_1 progeny, and 16 BC_1 plants. Among the BC_1 plants, eight were andromonoecious and eight monoecious, and a recombinant individual is shown (*asterisk*). Molecular size of PCR products is indicated (*left*)

by Danin-Poleg et al. (2002) located an RAPD marker at a distance of 16.2 cM from the *a* gene in linkage group IV, whereas Périn et al. (2002a) mapped this gene in a region covering 25.2 cM in linkage group II of a reference map. Although the latter is the most saturated map published to date for melon, with an the average genetic distance of 3.24 cM between markers, there is a poor density of molecular markers in the chromosome region flanking the *a* locus. More recently, the genetic map developed by Silberstein et al. (2003) describes an RFLP located 7 cM away from the *a* gene, which was the closest marker until the publication of the present paper. Nevertheless, the genetic distance and the dominant nature of the RAPD marker on the one hand, and the complexity of the RFLP technique on the other, make difficult the extensive and routine use of these markers for breeding purposes. Thus, the M3 SCAR developed may be considered the most reliable and closest molecular marker to the *a* gene identified in melon genome. The codominant nature of M3 and the late expression of the target trait (monoecious/andromonoecious type) make this marker a useful tool for a early genotyping selection of desirable sexual types in this species. Interestingly, M3 was identified from a DH population generated from a cross between two phylogenetically related Charentais lines. This suggests that in a BSA approach, not only the genetic distance between the parental lines but also the homozygosity level in chromosome regions flanking the locus of interest, may contribute to finding markers closely linked to this locus.

Searching analysis of the databases did not reveal homology of the M3 DNA fragment with any known sequence, indicating that M3 is most probably part of a non-coding region of the melon genome. The short length of the M3 allelic fragments does not refute the fact that they could be part of a coding sequence, which would not necessarily be related to sex determination given the genetic distance of M3 from the *a* gene. Whether this is the case, a more detailed characterization of the genomic region containing the M3 marker is needed to probe this hypothesis. Interestingly, a restriction fragment homologous to the ACC-synthase1 gene (*CS-ACSI*) has only been observed in gynoeious plants of cucumber (*C. sativus*), not in monoecious ones (Trebitsh et al. 1997). These results not only suggested a functional role of ethylene in sex determination but also

that *CS-ACSI*, a gene involved in the biosynthesis of this hormone, could correspond to the *Female (F)* gene of cucumber. In melon, on the other hand, none of the few ethylene-related genes mapped to date are located in the same linkage group as the *a* gene (Périn et al. 2002a; Silberstein et al. 2003). Our results on the comparative sequencing analysis between the parental lines of the DH population regarding three ACC-oxidase genes and five ACC-synthase genes did not reveal any molecular polymorphism in the coding regions of these regulatory genes that can be associated with the sex type (data not shown). Exhaustive genetic and physiological studies will be required to determine the functions of ethylene-related genes in the sex differentiation process of plant species, for which melon and other cucurbits are excellent plant models.

Sex of melon flowers appears to be strongly associated to the fruit shape, as all monoecious DH plants produced more oblong fruits compared to the round ones produced by andromonoecious DH lines. These results agree with those obtained by Périn et al. (2002b), who demonstrated a pleiotropic effect of the *a* gene on fruit shape, using genetic and sex-reversion analyses. These results seem to confirm the influence of *a* gene on early developmental patterns of melon fruits. The same authors reported two tightly linked QTLs responsible for the variation of fruit shape located in the same 25.2-cM chromosome region as the *a* gene. Although fruit shape is a polygenic trait, the SCAR marker described in this work is located close to both the *a* gene and the two major QTLs that determine fruit shape. This could facilitate breeding selection not only of sexual types but also of fruit shape in melon.

References

- Ainsworth C (2000) Boys and girls come out to play: the molecular biology of dioecious plants. *Ann Bot* 86:211–221
- Allard W (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia* 24:235–278
- Charlesworth B (1991) The evolution of sex chromosomes. *Science* 251:1030–1033
- Danin-Poleg Y, Reis N, Baudracco-Arnas S, Pitrat M, Staub JE, Oliver M, Arús P, de Vicente CM, Katzir N (2000) Simple sequence repeats in *Cucumis* mapping and map merging. *Genome* 43:963–974

- 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
- Deputy JC, Ming R, Ma H, Liu Z, Fitch MMM, Wang M, Manshardt R, Stiles JI (2002) Molecular markers for sex determination in papaya (*Carica papaya* L.). *Theor Appl Genet* 106:107–111
- Filatov DA, Monéger F, Negrutiu I, Charlesworth D (2000) Low variability in a Y-linked plant gene and its implications for Y-chromosome evolution. *Nature* 404:388–390
- Grant SR (1999) Genetics of gender dimorphism in higher plants. In: Geber MA, Dawson TE, Delph LF (eds) *Gender and sexual dimorphism in flowering plants*. Springer, Berlin Heidelberg New York, pp 247–274
- Hormaza JI, Dollo L, Polito VS (1994) Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. *Theor Appl Genet* 89:9–13
- Kahana A, Silberstein L, Kessler N, Goldstein RS, Perl-Treves R (1999) Expression of ACC oxidase genes differs among sex genotypes and sex phases in cucumber. *Plant Mol Biol* 41:517–528
- Kenigsbuch D, Cohen Y (1990) The inheritance of gynoecey in muskmelon. *Genome* 33:317–320
- Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugen* 12:172–175
- Liu Z, Moore PH, Ma H, Ackerman CM, Ragiba M, Yu Q, Pearl HM, Kim MS, Charlton JW, Stiles JI, Zee FT, Paterson AH, Ming R (2004) A primitive Y chromosome in papaya marks incipient sex chromosome evolution. *Nature* 427:348–352
- Lotfi M, Alan AR, Henning MJ, Jahn MM, Earle ED (2003) Production of haploid and doubled haploid plants of melon (*Cucumis melo* L.) for use in breeding for multiple virus resistance. *Plant Cell Rep* 21:1121–1128
- Michelmore RW, Paran I, Kesseli V (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA* 88:9828–9832
- Neuhausen SL (1992) Evaluation of restriction fragment length polymorphism in *Cucumis melo*. *Theor Appl Genet* 83:379–384
- Périn C, Hagen LS, De Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002a) A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor Appl Genet* 104:1017–1034
- Périn C, Hagen LS, Giovinazzo N, Besombes D, Dogimont C, Pitrat M (2002b) Genetic control of fruit shape acts prior to anthesis in melon (*Cucumis melo* L.). *Mol Genet Genom* 266:933–941
- Polley A, Seigner E, Ganai MW (1997) Identification of sex in hop (*Humulus lupulus*) using molecular markers. *Genome* 40:357–361
- Poole CF, Grimbail PC (1939) Inheritance of new sex forms in *Cucumis melo* L. *J Hered* 30:21
- Qu LJ, Foote TN, Roberts MA, Money TA, Aragón-Alcaide L, Snape JW, Moore G (1998) A simple PCR-bases method for scoring the *ph1b* deletion in wheat. *Theor Appl Genet* 96:371–375
- Reamon-Bütter SM, Jung C (2000) AFLP-derived STS markers for the identification of sex in *Asparagus officinalis* L. *Theor Appl Genet* 100:432–438
- Reamon-Bütter SM, Schondelmaier J, Jung C (1998) AFLP markers tightly linked to the sex locus in *Asparagus officinalis* L. *Mol Breed* 4:91–98
- Robinson RW, Decker-Walters DS (1997) *Cucurbits*. CAB International, New York
- Rosa JT (1928) The inheritance of flower types in *Cucumis* and *Cytrullus*. *Hilgardia* 3:233–250
- Shattuck-Eidens DS, Bell RN, Neuhausen SL, Helentjaris T (1990) DNA sequence variation within maize and melon: observations from PCR amplification and direct sequencing. *Genetics* 126:207–217
- Silberstein L, Kovalski I, Brotman Y, Périn C, Dogimont C, Pitrat M, Klingler J, Thompson G, Portnoy V, Katzir N, Perl-Treves R (2003) Linkage map of *Cucumis melo* including phenotypic traits and sequence-characterized genes. *Genome* 46:761–773
- Staub JE, Danin-Poleg Y, Fazio G, Horejsi T, Reis N, Katzir N (2000) Comparative analysis of cultivated melon groups (*Cucumis melo* L.) using random amplified polymorphic DNA and simple sequence repeat markers. *Euphytica* 111:225–241
- Stepansky A, Kovalski I, Perl-Treves R (1999) Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. *Plant Syst Evol* 217:313–332
- Trebitsh T, Staub JE, O'Neill S (1997) Identification of a 1-aminocyclopropane-1-carboxylic acid synthase gene linked to the *Female* (*F*) locus that enhances females sex expression in cucumber. *Plant Physiol* 113:987–995
- Urasaki N, Tukumoto M, Tarota K, Ban Y, Kayano T, Tanaka H, Oku H, Chinen I, Terauchi R (2002) A male and hermaphrodite specific RAPD marker for papaya (*Carica papaya* L.). *Theor Appl Genet* 104:281–285
- Vos P, Hogers R, Bleekers 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
- Westergaard M (1958) The mechanism of sex determination in dioecious flowering plants. *Adv Genet* 9:217–281
- Whitaker TW, Davis DW (1962) *Cucurbits: botany, cultivation and utilization*. Interscience, New York