Short Communication

Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers

Leah Silberstein[a], Irina Kovalski[a], Ruguo Huang[1,a],
Kostas Anagnostou[b], Molly M. Kyle Jahn[b], Rafael Perl-Treves[a,*]

[a] *Department of Life Science, Bar-Ilan University, Ramat-Gan 52900, Israel*
[b] *Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, USA*

Abstract

DNA polymorphism among *Cucumis melo* accessions was assessed using RFLPs and RAPDs. Thirteen varieties that represent diverse melon-types were surveyed using 18 RAPD primers. Cluster analysis indicated that the largest divergence among melon-types occurred between *C. melo* var. *momordica* from India and the North American and European muskmelon cultivars. The latter were also well-diverged from vars. *conomon*, *chito* and *dudaim* from the Far East and var. *agrestis* from Africa. Dessert melon varieties belonging to var. *inodorus* and *cantalupensis*, respectively, were not differentiated in this analysis, indicating that most of the genetic variation in the melon germplasm should be sought among land-races and wild accessions. As many as 61% of the Random Amplified Polymorphic DNA (RAPD) primers produced polymorphic patterns between the two varieties Topmark (var. *cantalupensis*) and P.I. 414723 (var. *momordica*), indicating that RAPDs reveal abundant polymorphism in melons. A subset of eight varieties was assayed with RFLP probes, either genomic *Pst*I-clones, or cucumber floral bud-cDNAs. Of the 56 probes surveyed with six restriction enzymes, about 80% detected polymorphism among the eight accessions. It turned out that the melon genome contains abundant *Pst*I-digested repetitive sequences, and that *Eco*RI was the most productive restriction enzyme in detecting polymorphism. The efficient use of cucumber cDNAs for melon genome analysis suggests that the comparative mapping of these two important cucurbit crops may be possible. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *Cucumis melo*; Germplasm; Molecular markers; Cucurbitaceae; Genetic relationship

---

* Corresponding author. Tel.: +972-3-5318096; fax: +972-3-5351824; e-mail: perl@brosh.cc.biu.ac.il
[1] Present address: Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, Canada N0R 1G0.

1. Introduction

Melon (*Cucumis melo* L., family Cucurbitaceae) is an economically important crop, including wild-types and numerous varieties, either consumed as a dessert fruit or a vegetable in Asia and Africa (Whitaker and Davis, 1962). It is regarded as the most morphologically diverse species in the genus *Cucumis* (Kirkbride, 1993). Varieties differ widely in fruit size, morphology and taste, as well as vegetative traits and climatic adaptation. Intra-specific classification of such variability has been quite difficult and confusing. Most taxonomists rely on Naudin's work from 1859 (Pangalo, 1929; Grebenscikov, 1953; Hammer et al., 1986; Munger and Robinson, 1991). In this study we analyzed a small set of melon varieties that represent the major intra-specific groups. Previous variability studies (Shattuck-Eidens et al., 1990; Neuhausen, 1992) failed to include some of the most morphologically diverged varieties (e.g. var. *agrestis*, var. *conomon*, var. *flexuosus*) in their surveys. An attempt to include a wider choice of accessions is found in a recent study by Staub et al. (1997), although, some of the 'exotic' melon-types were still not included. By carefully selecting such material, we can estimate better the real extent of molecular variation in the melon germplasm, and determine whether the impressive diversity in botanical and agronomic traits displayed by melons is reflected at the DNA level. In the present study, the levels of polymorphism in melon DNA was evaluated using two marker techniques, namely, RAPD and restriction fragment length polymorphism (RFLP).

2. Materials and methods

2.1. Plant material

Thirteen *Cucumis melo* and one *Cucumis metuliferus* accessions were obtained from various sources (Table 1; Fig. 1). *C. metuliferus* was included as an out-group in the analysis. Plants for DNA extraction and morphological observations were grown in the greenhouse following standard horticultural practice.

2.2. RAPD analysis

DNA for RAPD analysis was extracted from melon leaves according to Dellaporta et al. (1983). RAPD reactions were performed using random decamers (Operon Technologies, Alameda – A, B, C, D, L and R primer-sets), according to Williams et al. (1990), using an Appligene thermocycler (Crocodile II model). Reactions were replicated at least twice to control reproducibility of patterns.

Table 1
Plant accessions used in this study

Code	Name	Origin (source)	Description
C.MET	*Cucumis metuliferus*	African origin (Tzofar, Israel)	Wild species; viny, 'horned gerkhin' spiky fruits
AGR	*C. melo* var. *agrestis*	Africa (Den Nijs)	Wild, weedy, African variety. Small inedible fruit; monoecious
FLX	*C. melo* var. *flexuosus*	Middle East (S. Niego)	'Snake melon', 'Facus'; cucumber-like, non-sweet fruit; monoecious
CON	*C. melo* var. *conomon*	Far East (H. Munger)	Pickling melon; medium-size non-sweet fruit, andromonoecious
CHI	*C. melo* var. *chito*	Far East (H. Munger)	'Mango melon'; Viny, small fruits, monoecious
DUD	*C. melo* var. *dudaim*	Far East (H. Munger)	Looks similar to chito; monoecious
MOM	*C. melo* var. *momordica* P.I. 414723	Breeding line derived from Indian variety (H. Munger)	Virus, mildew tolerance; large non-sweet fruit splitting at maturity, monoecious
TM	*C. melo* var. *cantalupensis*, Cv. Topmark	USA (H. Munger)	American netted dessert-melon, andromonoecious
END	*C. melo* var. *cantalupensis*, Cv. Ein-Dor	Israel(R. Hermann)	Large, sweet, netted Israeli melon ('Ananas type'); andromonoecious
DHA	*C. melo* var. *cantalupensis*, Cv. Dvash-Haogen	Israel(R. Hermann)	Netted Israeli dessert melon, 'Ha'Ogen type'; andromonoecious
YC	*C. melo* P.I. 124111F	Breeding line derived from Indian P.I. (Y. Cohen)	Flattened, mildly sweet fruit; several fungal disease resistances; monoecious
CHA	*C. melo* var. *cantalupensis*, Cv. Charentais	France (Y. Cohen)	French dessert melons, ribbed, aromatic; andro-monoecious
CAS	*C. melo* var. *inodorus*, Cv. Rochet-Pamal	Spain (R. Hermann)	Spanish dessert melons, odorless, long keeping, 'Cassaba type'; andromonoecious
HON	*C. melo* var. *inodorus*, Cv. Honeydew	USA (H. Munger)	American 'winter melons', sweet, odorless, andro-monoecious

Codes used in the text, varietal names, geographical origin and source of seeds are indicated, as well as a few descriptors.

Fig. 1. *Cucumis* accessions used in this study. Mature fruits of 12 of the accessions are shown; accession codes are according to Table 1. Scale-bar indicates size in cm.

2.3. RFLP analysis

A melon genomic fragment-library was generated according to the standard protocols (Kovalski et al., 1995). Cucumber cDNA libraries from floral buds were prepared in our laboratory (Rosenman et al., 1996) in the λZAPII vector

(Stratagene). DNA for RFLP analysis was prepared according to Baudracco-Arnas (1995). For Southern blots, 3–5 µg restriction-digested DNA samples were run on 0.8% agarose gels and blotted to charged nylon membranes (Du Pont) according to the manufacturer's instructions. Hybridization was performed at 65°C for the melon genomic clones, and at 55°C for the cucumber cDNA clones, in 6% PEG, 5% SDS, 5 × sSSPE and 50 µg/ml denatured salmon DNA.

2.4. Cluster analysis

Genetic distances between taxa were computed by the PAUP program (Swofford, 1993). Cluster analysis using parsimony methods was performed using the PAUP program (Swofford, 1993) with different Heuristic search-options. For distance-based construction of shortest trees, we used the PHYLIP programs NEIGHBOR and KITSCH (Felsenstein, 1993).

3. Results

3.1. Survey of RFLP polymorphism

We have constructed a probe library of melon genomic DNA. Plasmid inserts were Southern-hybridized with radiolabeled total genomic DNA as probe. Those yielding strong signals (38% of the clones) were discarded as repetitive, but the remaining ones still contained about 10% repetitive clones. A second source of probes was a cDNAs library from cucumber early-stage floral buds. RFLP probes were assayed on Southern blots containing genomic DNA from eight *C. melo* accessions (No. 2–9 in Table 1), digested with six restriction enzymes: *Eco*RI, *Eco*RV, *Hind*III, *Dra*I, *Xba*I and *Mva*I. Table 2 summarizes the results obtained with 25 genomic clones and 34 cDNA clones.

Of the 23 different genomic clones (the other two were copies of a repetitive element), 19 (83%) were polymorphic (i.e. at least one variety could be differentiated from the rest). About 48% of the probes detected polymorphism between the two accessions, TM, a netted dessert melon, (Cv. Topmark) and an Indian melon of var. *momordica*, MOM (P.I. 414723). These two were shown by Neuhausen (1992) to be well diverged, and we have used them to construct a mapping population segregating for WMV, ZYMV and powdery mildew resistances. Of the 33 different cDNA probes, 82% were polymorphic among the 8 varieties and 64% between the two parents. However, only a fraction of the polymorphic probe–enzyme combinations produced a pattern with two easily distinguished parental bands. The others only detected presence/ absence of a band, or consisted of rather small differences in the migration of allelic bands. Genomic *Pst*I probes revealed a smaller proportion of fully useful polymorphism

Table 2
Extent of polymorphism detected with different types of RFLP probes between melon varieties

	Cucumber cDNA probes			Melon genomic probes		
A. RFLPs	No. assayed	No. different	No. polymorphic	No. assayed	No. different	No. polymorphic
Two-parent survey	34	33	21 (64%)	25	23	11 (48%)
8-varieties survey	34	33	27 (82%)	25	23	19 (83%)

Each probe was reacted with DNA from eight melon accessions digested with six restriction enzymes.
Number of non-redundant probes out of the total number is shown.
Number (and percentage) of probes detecting an RFLP between any of the eight varieties, as well as polymorphism between the two accessions used as mapping parents, Cv. Topmark and P.I. 414723, are shown.

than did the floral bud cDNAs. In addition, repetitive clones were quite frequent among the *Pst*I genomic clones, reducing the efficiency of sampling new markers. Only 1 out of 34 cDNA clones turned out to be redundant. At moderate stringency, all the cucumber clones produced clear and strong patterns on melon genomic blots.

Restriction enzymes differed in the amount of polymorphism detected. *Eco*RI was the most polymorphic enzyme (55.4% of probes revealed polymorphism among eight accessions), while *Dra*I was the least polymorphic (28.5%). An additional endonuclease, *Bsu*15I, often detected polymorphism even when the above six enzymes did not. Most of the polymorphism was detected with a single restriction enzyme, and only in one case the hybridization pattern strongly suggested a deletion/insertion event as the basis of the polymorphism.

3.2. RAPD variation among melon accessions

For a preliminary evaluation of RAPD primers, the two accessions, TM and MOM, were analyzed. One hundred and fifteen decamer-primers were reacted

Table 3
Levels of RAPD polymorphism[a]

	Cucumber cDNA probes		Melon genomic probes	
B. RAPDS	No. primers assayed	No. primers polymorphic	No. bands	No. bands polymorphic
Two-parent survey	115	70 (61%)	616	148 (24%)
13-varieties survey	18	18 (100%)	176	96 (54.5%)

[a] Expressed as the number and percentage of primers and bands that detect differences between cultivar Topmark and P.I. 414723 ('two-parent survey') and among 13 *C. melo* varieties ('13-varieties-survey')

with the two DNA samples and 61% detected polymorphism (Table 3). The proportion of polymorphic bands was 24%, indicating that RAPDs are an abundant source of polymorphic markers in melon. Using 18 primers that yielded several strong bands/samples, we performed RAPD reactions on a panel of 13 *C. melo* accessions and one *C. metuliferus* accession (Table 1; Fig. 1), including representative accessions of all the main 'botanical varieties' following the classification of Munger and Robinson (1991). A total of 176 bands was scored: 75 bands were polymorphic between the outgroup *C. metuliferus* and the 13 *C. melo* accessions, but not at the intra-specific level. Within *C. melo*, 96 bands (54.5%) were polymorphic (Table 3). A genetic distance matrix (representing the proportion of unshared characters) was computed by the PAUP software (Swofford, 1993) between all pair-wise combinations of accessions, and fed to the distance matrix-program KITSCH (Felsenstein, 1993), to obtain the best tree shown in Fig. 2. We also analyzed the same RAPD data using the PAUP program,
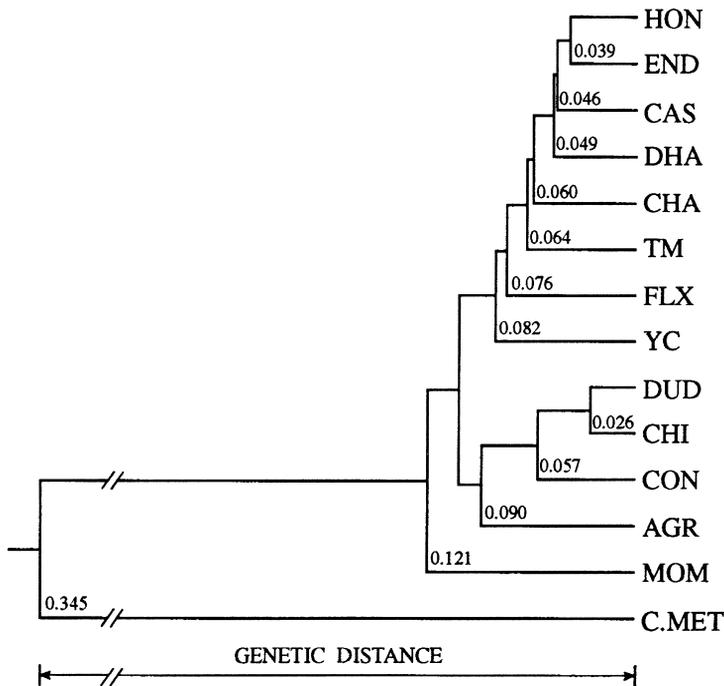

Fig. 2. Phylogenetic tree computed by the KITSCH program of PHYLIP software package, displaying clustering relationships between 13 *C. melo* accessions and a *C. metuliferus* accession, based on a data set of 176 RAPD bands. Length of terminal branches (in genetic distance units) are indicated. Genetic distance values between all pairwise combinations of accessions were computed as the proportion of different characters of the total number of characters (missing values ignored) and these were used as input to the clustering program that searched for the shortest tree under a molecular clock assumption. Varieties are indicated by codes according to Table 1.

that uses informative character-states rather than distance values. Several heuristic searches were performed to obtained trees whose consensus topology was very similar to the distance-matrix-based tree shown here.

According to this analysis, the longest branch separates *C. metuliferus* (the outgroup species) from all the melon accessions. The next node separates MOM (var *momordica*) from the rest. Other well-diverged taxa are AGR (var. *agrestis*, that includes wild small fruited melons of Asia and Africa) and the cluster of non-sweet varieties from the Far-East: CHI (var. *chito*), DUD (var. *dudaim*) and CON (var. *conomon*). Since some accessions of *chito* and *dudaim* are very similar morphologically, Munger and Robinson (1991) suggested to include these types in one botanical group. The relationships among the rest of the taxa were not consistent, although the distance values indicate that var. *flexuosus* (FLX; non-sweet snake melons) and the mildly sweet Indian P.I. 124111 (YC) are more distant from the American and European dessert cultivars.

The two major groups of dessert melon consumed in Europe and the USA are var. *inodorus* and var. *cantalupensis*. Cassaba and Honeydew melons (HON, CAS; var. *inodorus)* lack aroma and exhibit slow, non-climacteric ripening while var. *cantalupensis*, represented by netted (OGN, END, TM) and Charentais (CHA) types produce aromatic and climacteric fruits. These two groups of sweet melons could not be separated by our present data. Adding more RAPD bands to this data-set resulted in similar clustering with little improvement in resolution (not shown).

4. Discussion

Inter-specific variation in the genus *Cucumis* has been studied previously using isozymes and chloroplast DNA variation (Perl-Treves and Galun, 1986; Perl-Treves et al., 1986; Staub et al., 1992). However, within *Cucumis melo* isozyme variation was much lower (Dane, 1983; Perl-Treves et al., 1986). In a study by Neuhausen (1992), 33% of the RFLPs detected by melon genomic and cDNA probes, distinguished at least one of seven melon accessions. A larger set of accessions was then analyzed, but most of these were muskmelon, Honeydew and Cassaba cultivars (var. *cantalupensis* and var. *inodorus*), with a single 'exotic' accession, the Indian P.I. 414723 (var. *momordica*). Morphologically and geographically diverged material was not included, e.g., var. *agrestis* - wild weedy-types from Asia and Africa; vars. *conomon* and *dudaim* - smooth Far-Eastern pickling types; var. *flexuosus* – elongated Asian/Middle-Eastern types eaten as cucumbers. We included these types in our survey (Table 1) in order to encompass the widest variation that may be found within *C. melo*. A recent study by Staub et al. (1997) analyzed seven melon accessions, including var *flexuosus* and var *conomon*, while the oriental varieties *chito* and *dudaim*, or the variety

agrestis, that includes accessions of wild or semi-wild origin, were not sampled. The latter is certainly one of the most diverged germplasm, with very small (<5 cm), often bitter fruits with no flesh, slender vines and very small seeds.

Our RAPD-based phylogeny places *C. melo* var. *momordica* (MOM) at the outmost intra-specific branch (Fig. 2). This confirms the result of Neuhausen (1992). Other exotic accessions are also well-diverged from dessert-type melons: useful amounts of polymorphism occur between cantaloupes and *conomon* (CON), *dudaim* (DUD), *agrestis* (AGR). On the other hand, clear distinctions between var. *cantalupensis* and var. *inodorus* were not observed. Such lack of resolution was also observed by Staub et al. (1997) and may indicate that these two groups are quite close, despite the important horticultural differences between them, e.g., presence of aroma, climacteric ripening, fruit shelf-life. We intend to try to better resolve melon intra-specific relationships by studying more accessions of each varietal group. We should, however, consider the possibility that the impressive morphological variation used to classify melon into 'varieties' resulted from intensive selection over a relatively short time-span, and may be the result of very few genetic changes in developmental regulator genes, with little 'overall' variation in DNA sequence. Another problem in resolving phylogenies of cultivated plants may be the 'horizontal' gene flow between botanical groups by breeders and travelers. This may explain the inability to resolve cultivated *inodorus* and *cantalupensis* types (and possibly also *flexuosus*) by our molecular data.

This study also compared the efficiency of detecting DNA polymorphism suitable for genetic mapping in melon using RFLPs and RAPDs. Almost half of the genomic clones detected RFLPs between cv. Topmark and P.I. 414723. Higher (64%) rates were achieved using cucumber cDNAs as probes. RAPD polymorphism levels between these two accessions were also high (61% of the primers, 24% of the bands). Comparable levels were observed by Baudracco-Arnas and Pitrat (1996). Our preliminary experience in trying to map them in a segregating population nevertheless indicates that only a sub-set of the RFLPs yields well-resolved alternate alleles, that can be easily scored as co-dominant markers. RAPDs present, in a mapping situation, typical disadvantages as well, and unless the polymorphic band is among the 2–3 brightest ones produced by a given primer, ambiguities in scoring arise and may introduce mistakes into the data (Kesseli et al., 1994). A further reduction in the number of mappable RFLPs and RAPDs results from markers that exhibit strong segregation distortion in the F2. Thus, a significant portion of the markers will be discarded in the mapping process.

Our successful hybridization of cucumber RFLP probes to melon DNA indicates that comparative maps could be generated for these two *Cucumis* species. Linkage-group conservation between melon and cucumber might enable geneticists to locate agronomically important genes in one crop, based on mapping data obtained in the other crop, and gain important insight to genome structure and evolution of the genus *Cucumis*.

Acknowledgements

References

Baudracco-Arnas, S., 1995. A simple and inexpensive method for DNA extraction from *Cucumis melo* L. Cucurbit Genet. Coop. Reports 18, 50–51.

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.

Dane, F., 1983. Cucurbits, In: Tanksley, S.D., Orton, T.J. (Eds.). Isozymes in Plant Genetics and Breeding, Part B. Elsevier, Amsterdam, The Netherlands.

Dellaporta, S.L., Wood, J., Hicks, J.B., 1983. A plant DNA minipreparation: version II. Plant Mol. Biol. Reporter 1, 19–21.

Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle.

Grebenscikov, I., 1953. Die entwicklung der melonsystematik. Kulturpflanze 1, 121–138.

Hammer, K., Hanelt, P., Perrino, P., 1986. Carosello and the taxonomy of *Cucumis melo* L. especially of its vegetable races. Kulturpflanze 34, 249–259.

Kesseli, R.V., Paran, I., Michelmore, R.W., 1994. Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136, 1435–1446.

Kirkbride, J.H., 1993. Biosystematic Monograph of the Genus *Cucumis* (Cucurbitaceae). Parkway, North Carolina, p. 159.

Kovalski, I., Silberstein, L., Huang, R., Perl-Treves, R., 1995. Development of molecular markers for genome analysis in *Cucumis melo*. In: Lester, G., Dunlap, J. (Eds.), Cucurbitaceae '94, Proc. Cucurbitaceae Conf. South Padre Islands, Texas, 1–4 November 1994.

Munger, H.M., Robinson, R.W., 1991. Nomenclature of *Cucumis melo* L. Cucurbit Genet. Coop. Reports 14, 43–44.

Neuhausen, S.L., 1992. Evaluation of restriction fragment length polymorphism in *Cucumis melo*. Theor. Appl. Genet. 83, 379–384.

Pangalo, K.J., 1929. Critical review of the main literature on the taxonomy, geography and origin of cultivated and partially wild melons. Trudy Prikl. Bot. 23, 397-442 [In Russian, and translated into English for USDA by G. Saad in 1986].

Perl-Treves, R., Galun, E., 1986. The *Cucumis* plastome: physical map, intrageneric variation, and phylogenetic relationships. Theor. Appl. Genet. 71, 417–429.

Perl-Treves, R., Zamir, D., Navot, N., Galun, E., 1986. Phylogeny of *Cucumis* based on isozyme variability and its comparison with plastome phylogeny. Theor. Appl. Genet. 71, 430–436.

Rosenman, N., Kahana, A., Xiang, Y., Perl-Treves, R., 1996. Expression of Agamous-like genes in male and female flowers of cucumber. In: Proc. VI Eucarpia Meeting on Cucurbit Genetics and Breeding, Malaga, Spain 28–30 May 1996.

Shattuck-Eidens, D.M., Bell, R.N., Neuhausen, S.L., Hellentjaris, T., 1990. DNA sequence variation within maize and melon: Observations form polymerase chain reaction amplification and direct sequencing. Genetics 126, 207–217.

Staub, J.E., Knerr, L.D., Holder, D.J., May, B., 1992. Phylogenetic relationships among several African *Cucumis* species. Can. J. Bot. 70, 509–517.

Staub, J.E., Box, J., Meglic, V., Horejsi, T.F., McCreight, J.D., 1997. Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in *Cucumis*. Genet. Res. Crop Evol. 44, 257–269.

Swofford, L., 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, IL.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18, 6531–6535.

Whitaker and Davis, 1962.