Frequency of RAPD Polymorphisms in Melon (*Cucumis melo* L.) Germplasm in Different Geographic Regions

Ana Isabel López-Sesé and J. E. Staub

USDA-ARS Vegetable Crops Research Unit, University of Wisconsin-Madison Department of Horticulture, 1575 Linden Dr. Madison WI 53706

Introduction: The genetic diversity of melon groups has been characterized using molecular analyses (5, 6, & 8). Random amplified polymorphic DNA markers have been used by García et al. (1) and Staub et al. (7) to assess the genetic diversity of elite germplasm. Likewise, Mliki et al. (3) and Nakata (4) used the same markers employed by Staub et al. (7) to define the diversity among African and Japanese germplasm, respectively. Mliki et al. (3) determined genetic differences among African landraces and between these landraces and elite U.S./European melon market class accessions used by Staub et al. (7). Nakata et al. (3) assessed the genetic variation of major Japanese melon market classes (i.e., House, Earl's and Oriental types), and then defined the genetic relationships between these market classes and a standard germplasm reference array (RA) drawn from accessions (Groups Cantaloupensis, Conomon, Inodorus, and Flexuosus) used by Staub et al. (7) and African landraces studied by Mliki et al. (3). We summarized herein the percentage of RAPD band presence within distinctly different geographic regions.

Materials and Methods: The RADP profiling of melon market classes by Staub et al. (7) included Charentais (7), European (6) and U.S. Western Shipper (3), U.S. Eastern Market (4), Galia (7), Ogen (6), Honeydew (2), Casaba (9), group Conomon (1), and group Flexuosus (1) accessions. These accessions were received from seed companies (5) and the U.S. Department of Agriculture, Agricultural Research Service. Mliki et al. (3) examined 108 exotic melon (*Cucumis melo* L.) accessions, and Nakata et al. (4) used 67 melon (*C. melo* L.) cultivars from five Japanese seed companies [Sakata (59), Yokohama Ueki (2), Nihon Engei Kenkyuukai (1), Kobayashi (4), and Tohoku (1)].

Molecular data were estimated from amplification products obtained by using 57 RAPD primers (Operon and University of British Columbia (BC)] (Table 1). A marker was considered repeatable if PCR yielded a consistent result in all of three (or more) replications (putative loci; see companion paper this issue). Tabulations summarize the percentage of RAPD band presence within a market class or subspecies and among European and U.S. germplasm as an estimation of the polymorphism level and diversity within groups. This was calculated as number of accessions with band presence divided by the total number of accessions examined and then multiplied by 100. This calculation is hereafter referred as percent frequency. When more than one polymorphic product per primer was obtained, the average of percent frequency of all the markers was tabulated. Accessions from the European, U.S., and African germplasm were further selected and used as reference accessions (RA) by Nakata et al. (4).

Results and Discussion: Germplasm examined from Europe, USA, and Japan should be considered elite since it presents either refined inbred lines or commercial hybrids. African landrace accessions are Group Conomon-like by their genetic relationship to the other germplasm as determined by RAPD marker analysis (4).

All primers used herein produced amplifications products that were polymorphic between and among groups (Table 1). Because different accessions were used in some analyses, the most appropriate comparisons are grouped in Table 1.

The majority of RAPD bands produced by any one primer were in a similar percentage in European and U.S. accessions. Notable exceptions were recorded in the comparison of European and U.S. germplasm using B12, AD12, AM2, BC318, BC388, BC551, BC627, and BC654. Relative percent frequency differences between these groups and corresponding RA groups were similar for most of the primers used. In rare cases the average percent frequency increased or decreased slightly when only portions of the accessions were accessed. This was the case when RA accessions were examined using AJ18, BC526, and BC551 in European and U.S. accessions, and when using C1, D7, AT5, and AW10 to examine

Europe ^{wx}	USA^{w}	Africa ^x	Europe-RA ^y	USA-RA ^y	Africa-RA ^y	Japan ^z
56.3	75.0	33.0				51.2
93.8	85.7	97.0	63.5	54.2	46.4	67.5
71.1	76.8	21.0	71.8	94.4	81.8	56.2
96.9	92.9					
87.5	92.9					
46.9	42.9					
62.5	89.3	26.0	61.5	83.3	40.0	71.6
51.6	60.7	30.0	74.4	73.3	42.2	55.7
55.1	47.6	36.5	73.1	58.3	43.3	52.2
61.5	64.3					
46.9	60.7	83.0			80.0	52.2
65.6	50.0					
71.9	85.7					
40.6	28.6	43.3			37.8	76.6
39.1	50.0					
76.0	69.0					
71.9	71.4	49.7	59.0	62.2	66.7	69.0
64.1	67.9	51.5	60.0	55.6	37.8	66.7
67.2	78.6	38.0	19.2	16.7	43.3	34.3
96.9	85.7	43.0	97.4	88.9	42.2	91.0
37.5	42.9					
65.6	85.7					
68.8	73.8					
43.8	50.0					
71.9		66.7	61.5	61.1	62.2	59.4
77.1						
		0.0	61.5	75.0	20.0	79.1
						84.3
		56.5	53.8	55.6	55.6	41.5
	67.9					
						58.2
						26.9
		71.0	48.7	55.6	75.6	64.7
		32.5	83.3	40.0	66.7	55.2
	57.1	60.0	61.5		80.0	17.9
						87.1
		62.0	76.9	83.3	73.3	44.8
96.9	100.0					
66.7	69.0					
51.0	61.9					
67.7	73.8	57.0	0.0	0.0	58.3	44.8
	$\begin{array}{c} 56.3\\ 93.8\\ 71.1\\ 96.9\\ 87.5\\ 46.9\\ 62.5\\ 51.6\\ 55.1\\ 61.5\\ 46.9\\ 65.6\\ 71.9\\ 40.6\\ 39.1\\ 76.0\\ 71.9\\ 40.6\\ 39.1\\ 76.0\\ 71.9\\ 64.1\\ 67.2\\ 96.9\\ 37.5\\ 65.6\\ 68.8\\ 43.8\\ 82.8\\ 67.2\\ 71.9\\ 77.1\\ 67.2\\ 75.0\\ 68.8\\ 30.2\\ 76.6\\ 65.6\\ 62.5\\ 70.1\\ 85.5\\ 78.1\\ 85.4\\ 96.9\\ 61.3\\ 76.6\\ 53.1\\ 96.9\\ 61.3\\ 76.6\\ 53.1\\ 96.9\\ 66.7\end{array}$	56.3 75.0 93.8 85.7 71.1 76.8 96.9 92.9 87.5 92.9 46.9 42.9 62.5 89.3 51.6 60.7 55.1 47.6 61.5 64.3 46.9 60.7 65.6 50.0 71.9 85.7 40.6 28.6 39.1 50.0 71.9 85.7 40.6 28.6 39.1 50.0 71.9 71.4 64.1 67.9 67.2 78.6 96.9 85.7 37.5 42.9 65.6 85.7 68.8 73.8 43.8 50.0 82.8 85.7 67.2 71.4 75.0 71.4 75.0 71.4 75.0 71.4 75.0 71.4 75.5 82.1 78.6 73.8 62.5 64.3 70.1 78.6 75.5 82.1 78.1 100.0 85.4 100.0 96.9 96.4 61.3 57.1 76.6 46.4 53.1 75.0 96.9 100.0 66.7 69.0	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 1. Percentage of RAPD band presence detected in accessions from different geographic regions.

BC551 BC605 BC617 BC627 BC628	76.6 18.8 100.0 12.5 66.7	92.9 14.3 92.9 42.9 66 7	38.5	20.5	38.9	42.2	37.8
BC628 BC642 BC646 BC652 BC654	66.7 72.5 69.4 75.0 56.3	66.7 81.0 75.0 78.6 39.3					
BC663	96.9	92.9					

^wData taken from Staub et al., (2000) (elite germplasm).

^xData of entire study taken from Mliki et al., (2001) (landraces).

^yData of standard accessions selected to from an array that defined the genetic diversity present in that data set (reference accessions, RA).

^zData taken from Nakata et al., (2001) (elite germplasm).

African RA accessions (Table 1). In other cases, change in the relative difference between RA groups was evident when AW14 and CBC231 were used to describe European and US accessions.

Some primers provided products at relatively low frequency in African germplasm that were evident in elite RA germplasm (e.g., AK16, AT5, AT15, and AW14) (Table 1). Also a lower percent frequency of band presence was observed in Japanese germplasm when compared to European, U.S., and African RA groups (e.g., D7, W7, BC299, and BC388) (Table 1).

This primer set was chosen by Staub et al. (5) for its ability to detect polymorphism in a broad array of melon germplasm. Thus, they are likely to maximize the detection of genetic variation in other arrays of melon germplasm.

Literature Cited

- García, E., M. Jamilena, J.I. Álvarez, T. Arnedo, J.L. Oliver, & R. Lozano. 1998. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. Theor. Appl. Genet. 96: 878-885.
- Katzir, N., T. Danin-Poleg, G. Tzuri, Z. Karchi, U. Lavi, & P.B. Cregan. 1996. Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor. Appl. Genet. 93: 1282-1290.

- Mliki, A., J. E. Staub, Z. Sun, and A. Ghorbel. 2001. Genetic diversity in melon (*Cucumis melo* L.): An evaluation of African germplasm. Genet. Res. Crop Evol. (in press).
- Nakata E., J. E. Staub, and A. López-Sesé. 2001. Genetic diversity in Japanese melon (*Cucumis melo* L.) as assessed by random amplified polymorphic DNA and simple sequence repeat markers. Theor. Appl. Genet. (under review).
- Silberstein, L., I. Kovalski, R.G. Huang, K. Anagnostu, M.M.K. Jahn, & R. Perl-Treves. 1999. Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers. Sci. Hort. 79: 101-111.
- Staub, J.E., J. Box, V. Meglic, T.F. Horejsi, & J.D. McCreight. 1997. Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in *Cucumis*. Gen. Res. Crop Evol. 44: 257-269.
- Staub, J.E., Y. Danin-Poleg, G. Fazio, T. Horejsi, N. Reis, & N. Katzir. 2000. Comparison analysis of cultivated melon groups (*Cucumis melo* L.) using random amplified polymorphic DNA and simple sequence repeat markers. Euphytica 115: 225-241.
- Stepansky, A., I. Kovalski, & R. Perl-Treves. 1999. Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. Plant Syst Evol 217: 313-332.