

## Inheritance of RAPD Markers in Melon (*Cucumis melo* L.)

J. E. Staub

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

**Introduction:** Genetic markers have been employed in diversity analysis (2,3,4,7,8) and the construction of maps in melon (1,9). The use of random amplified polymorphic DNA (RAPD) has allowed for discrimination of elite (2,7) and unadapted germplasm (3,4,8). Because of their relative low cost and low technological attributes they have been valuable for diversity analysis (2,7). However, except for the mapping of several RAPD marker loci by Baudracco-Arnas and Pitrat (1996), the genetics RAPD markers in melon has not been widely characterized. This is likely due to the relatively low level of RAPD polymorphisms (~15-20%) in melon. The bands used for diversity analysis are repeatable (4,7), but their genetic attributes have not been characterized. This report details the genetics of RAPD markers assessed in F<sub>2</sub> progeny segregating in four melon populations.

**Materials and Methods:** Crosses were made between an experimental inbred line SA 200 ('Chargyne') x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5. These crosses were made to produce segregating populations that would be useful in mapping disease resistance and sex expression in melon. SA 200 is a gynoeocious Charentais market type received from Clause Seed Company (Bretigny-Sur-Orge Cedex, France), Charentais-1 is a gynoeocious Charentais market type inbred line received from Petoseed Company (now associated with Seminis, Woodland Calif., USA), AR5 and "Top Mark" are disease resistant U.S. Western Shipper market types released by the USDA, ARS, and WI 998 is a gynoeocious line released by the USDA, ARS. Each F<sub>1</sub> was self-pollinated to produce four F<sub>2</sub> populations (i.e., SA 200 x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5) segregation analyses.

DNA was extracted and subjected to PCR using RAPD primers from Operon (Alameda, Calif., USA) and University of British Columbia (UVBC; designated BC; Vancouver, BC, Canada) and electrophoresis was carried out according to Staub et

al. (7). Initially, a survey of all parents was made using about 1,500 primers to identify polymorphism specific to the contrasting parents in a particular cross. Primer products were identified by their primer designation (e.g., B12 = Operon primer and BC541 = UVBC primer) (Table 1). When a primer produced more than one product that was useable for analysis, it was given a lower case letter designation from cathodal to anodal migration position (e.g., BC541a).

Data were obtained for dominant RAPD loci from F<sub>2</sub> families and analyzed by chi-square analysis for conformity to expected 3:1 (df = 1) Mendelian single-factor segregation ratios.

**Results and Discussion:** The primers examined yielded between 25 to 40% polymorphisms (band differences between parents) depending on the cross (data not presented). Potentially useful bands were characterized as having a mobility between 200 to 2,500 bp. Putative loci were then identified as those possessing bands that were reproducible and bright and used for segregation analysis (Table 1). Initially, examination of parents of SA 200 x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5 produced 264, 241, 244, and 251 reproducible band differences between respective parents (putative loci) (data not presented). On average, this represents 17% recovery of potentially useable bands (loci) from the survey of 1,500 primers.

After this initial assessment, 41, 27, 90, and 38, bands (loci) segregated in a predictable manner in progeny from SA 200 x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5 matings, respectively (Table 1). This represents, on average, 3.3% recovery of useable loci from the initial survey of 1,500 primers.

Given the fact that individuals are sometimes misclassified even when scoring loci that historically have proven to be reproducible bright RAPD bands, we are attempting RAPD to SCAR conversion at some

Table 1. F<sub>2</sub> single factor segregation for RAPD primer products<sup>z</sup> in melon (*Cucumis melo* L.).

Cross	Primer	No.		Chi.		P		Cross	Primer	No.		Chi.		P		
		bp	F2	Obs	Exp	sq	value>			bp	F2	Obs	Exp.	Sq.	value >	
SA 200 x TM	M7	675	76	57	57	NA	NA	WI 998 x TM	AK5	800	93	69	69.75	0.01	0.70	
	U13	831	76	57	57	NA	NA		M7-2	750	87	66	65.25	0.01	0.70	
	AT7a	1275	76	57	57	NA	NA		AO8	1600	84	64	63	0.02	0.70	
	AV11	815	79	59	59.25	0.00	0.95		A11-1	960	76	56	57	0.02	0.70	
	D16	1850	77	58	57.75	0.00	0.95		AI8-1	800	89	68	66.75	0.02	0.70	
	AB17	831	78	59	58.5	0.00	0.95		AT7-1	860	87	64	65.25	0.02	0.70	
	AC7a	450	78	58	58.5	0.00	0.95		AD12	1100	86	66	64.5	0.03	0.70	
	AG15	975	78	58	58.5	0.00	0.95		AB16-1	1375	78	57	58.5	0.04	0.70	
	R11	1100	78	59	58.5	0.00	0.95		AJ20	800	92	71	69	0.06	0.70	
	U10	870	78	59	58.5	0.00	0.95		J7	1600	92	67	69	0.06	0.70	
	AV11	1900	74	55	55.5	0.00	0.95		AG13	975	88	68	66	0.06	0.70	
	Y13	610	74	56	55.5	0.00	0.95		AI9	520	93	72	69.75	0.07	0.70	
	AV1	1375	70	53	52.5	0.00	0.95		J7	700	93	72	69.75	0.07	0.70	
	K4	564	50	37	37.5	0.01	0.70		AB16	1400	87	63	65.25	0.08	0.70	
	I4b	831	71	54	53.25	0.01	0.70		AH3-2	1700	87	63	65.25	0.08	0.70	
	E8	1300	76	58	57	0.02	0.70		AT7-1	950	85	66	63.75	0.08	0.70	
	M7	650	76	56	57	0.02	0.70		V1-1	564	82	59	61.5	0.10	0.70	
	W3a	1000	75	55	56.25	0.03	0.70		Charent. x AR5	Z3a	700	92	69	69	NA	NA
	R19b	564	75	55	56.25	0.03	0.70		D16	2200	93	70	69.75	0.00	0.95	
	C13	1275	73	56	54.75	0.03	0.70		F1	1100	93	70	69.75	0.00	0.95	
Y15	1050	71	52	53.25	0.03	0.70	O19a	1200	93	70	69.75	0.00	0.95			
AM14a	1500	78	60	58.5	0.04	0.70	AB4b	1350	93	70	69.75	0.00	0.95			
Y13	575	74	54	55.5	0.04	0.70	AT2b	1000	93	70	69.75	0.00	0.95			
P6	1200	79	61	59.25	0.05	0.70	AU19	580	93	70	69.75	0.00	0.95			
AE3a	1250	75	58	56.25	0.05	0.70	BC299	700	93	70	69.75	0.00	0.95			
AL9	1650	75	58	56.25	0.05	0.70	AU2a	1400	90	67	67.5	0.00	0.95			
E8	675	76	59	57	0.07	0.70	BC628	830	90	68	67.5	0.00	0.95			
AH2b	500	76	55	57	0.07	0.70	I4	970	93	69	69.75	0.01	0.95			
C20	700	76	55	57	0.07	0.70	AB8	1350	93	69	69.75	0.01	0.95			
AB4b	1325	45	32	33.75	0.09	0.70	AT15b	1890	91	69	68.25	0.01	0.95			
AI11	600	78	56	58.5	0.11	0.70	H2	600	89	66	66.75	0.01	0.95			
W10c	830	78	56	58.5	0.11	0.70	C20	1000	93	71	69.75	0.02	0.70			
AA14	1375	74	53	55.5	0.11	0.70	D9a	800	93	71	69.75	0.02	0.70			
AG2	1375	74	53	55.5	0.11	0.70	E6	700	93	71	69.75	0.02	0.70			
B11	1400	80	63	60	0.15	0.10	U13	1000	93	71	69.75	0.02	0.70			
AK5b	1500	78	62	58.5	0.21	0.10	AA12	830	93	71	69.75	0.02	0.70			
G6	1890	78	55	58.5	0.21	0.10	R5a	1000	91	67	68.25	0.02	0.70			
AF7	1370	70	49	52.5	0.23	0.10	AV4	2027	91	67	68.25	0.02	0.70			
G8	1375	79	63	59.25	0.24	0.10	AB17	1000	44	32	33	0.03	0.70			
L1	564	79	63	59.25	0.24	0.10	R11	1300	90	69	67.5	0.03	0.70			
AJ12	750	75	60	56.25	0.25	0.10	S4	1910	93	68	69.75	0.04	0.70			
WI 998 x TM	A16	1600	92	69	69	NA	NA	W10	575	93	68	69.75	0.04	0.70		
	AT2	800	84	63	63	NA	NA	AF12b	800	93	68	69.75	0.04	0.70		
	BC226	1400	92	69	69	NA	NA	AL9a	1890	93	68	69.75	0.04	0.70		
	O2-1	1100	88	66	66	NA	NA	C10	900	92	71	69	0.06	0.70		
	AF7	947	91	68	68.25	0.00	0.95	AU2b	700	92	67	69	0.06	0.70		
	AL8-1	820	75	56	56.25	0.00	0.95	AB1	831	93	72	69.75	0.07	0.70		
	E6-1	1100	86	64	64.5	0.00	0.95	AD12	1000	93	72	69.75	0.07	0.70		
	AH9-1	1050	78	58	58.5	0.00	0.95	AM1	950	93	72	69.75	0.07	0.70		
	Z9	2050	78	59	58.5	0.00	0.95	AP2	1375	93	72	69.75	0.07	0.70		
	AB4-2	1300	93	69	69.75	0.01	0.70	BC541a	2100	93	72	69.75	0.07	0.70		

Cross	Primer	No.		Chi.		P		Cross	Primer	No.		Chi.		P		
		bp	F2	Obs	Exp	sq	value>			bp	F2	Obs	Exp.	Sq.	value >	
Charent. x AR5	B11	950	93	67	69.75	0.11	0.70	Charent. x AR5	AF7c	600	93	79	69.75	1.23	0.20	
	N11a	1375	93	67	69.75	0.11	0.70		T17a	1910	78	49	58.5	1.54	0.20	
	Y10	775	93	67	69.75	0.11	0.70		AF12a	1000	93	59	69.75	1.66	0.10	
	AF7b	831	93	67	69.75	0.11	0.70		U7	1050	87	52	65.25	2.69	0.10	
	AH20	500	93	67	69.75	0.11	0.70		TM x AR5	K4 c	831	43	32	32.25	0.00	0.95
	AJ17	700	93	67	69.75	0.11	0.70			E6	960	62	47	46.5	0.01	0.95
	AK3	500	92	66	69	0.13	0.70			Z11 b	300	42	31	31.5	0.01	0.95
	L15	800	93	73	69.75	0.15	0.70			X16	575	38	29	28.5	0.01	0.95
	Y15	1000	93	73	69.75	0.15	0.70			AM18	831	22	16	16.5	0.02	0.70
	AF20c	580	93	73	69.75	0.15	0.70			AF7	831	45	33	33.75	0.02	0.70
	AG4a	1400	93	73	69.75	0.15	0.70			AG15	974	45	33	33.75	0.02	0.70
	AT2a	1100	93	73	69.75	0.15	0.70			F1	2027	43	33	32.25	0.02	0.70
	BC388	1100	93	73	69.75	0.15	0.70			Z18	831	43	33	32.25	0.02	0.70
	J4	831	93	66	69.75	0.20	0.50			BC388	1090	76	56	57	0.02	0.70
	AG10a	530	93	66	69.75	0.20	0.50			AX19	300	98	72	73.5	0.03	0.70
	AL8b	400	93	66	69.75	0.20	0.50			W7	831	40	29	30	0.03	0.70
	L1	800	91	72	68.25	0.21	0.50			AX20	900	42	33	31.5	0.07	0.70
	Q10	1580	91	72	68.25	0.21	0.50			BC526	825	39	31	29.25	0.10	0.70
	AK5	800	78	62	58.5	0.21	0.50			AJ20	1375	44	31	33	0.12	0.70
	AV11	1000	89	63	66.75	0.21	0.50			AT3	1570	44	35	33	0.12	0.70
	B14	1400	92	65	69	0.23	0.50			AV11 b	831	40	28	30	0.13	0.70
	F3	400	92	73	69	0.23	0.50			AF20	1400	22	15	16.5	0.13	0.70
	AX16	1100	92	65	69	0.23	0.50			AP2	1100	43	30	32.5	0.15	0.70
	D9b	750	93	74	69.75	0.26	0.50			AV11 a	960	72	57	54	0.16	0.50
	J7c	400	93	74	69.75	0.26	0.50			K4 a	975	42	29	31.5	0.19	0.50
	BC654	1000	93	74	69.75	0.26	0.50			AQ6	947	45	31	33.75	0.22	0.50
	U8	974	93	65	69.75	0.32	0.50			H2	825	43	35	32.25	0.23	0.50
	AE2b	550	93	65	69.75	0.32	0.50			AO18 a	1800	43	35	32.25	0.23	0.50
	AF20a	2300	93	65	69.75	0.32	0.50			BC299	700	40	33	30	0.30	0.50
	AJ12	800	93	65	69.75	0.32	0.50			U5	564	45	37	33.75	0.31	0.50
	AT15a	2000	91	73	68.25	0.33	0.50			U10	835	45	37	33.75	0.31	0.50
	L11	1110	92	64	69	0.36	0.50			O6	625	42	28	31.5	0.39	0.50
	C13	1375	93	75	69.75	0.40	0.50			T1	947	69	57	51.15	0.53	0.30
K4	700	93	75	69.75	0.40	0.50	AX6 a	575		45	29	33.75	0.67	0.30		
N11b	1000	93	75	69.75	0.40	0.50	W10	835		74	50	55.5	0.55	0.30		
U1	1904	93	75	69.75	0.40	0.50	Z11 a	1584		43	37	32.25	0.69	0.30		
AT2c	780	93	75	69.75	0.40	0.50	AD12	1000		73	61	54.75	0.71	0.30		
BC388	1000	93	75	69.75	0.40	0.50	E1	795	76	50	57	0.86	0.30			
O19c	400	93	64	69.75	0.47	0.30	L2	1000	42	37	31.5	0.96	0.30			
AF7a	840	93	64	69.75	0.47	0.30	AB3	835	42	37	31.5	0.96	0.30			
AM19	1900	89	61	66.75	0.50	0.30	Q10 a	1800	73	45	54.75	1.74	0.10			
AL9b	600	93	76	69.75	0.56	0.30	Z8	831	44	41	33	1.94	0.10			
AF20b	1375	93	77	69.75	0.75	0.30										
AG4b	700	93	77	69.75	0.75	0.30										
X17	700	67	44	50.25	0.78	0.30										
X19	1910	93	62	69.75	0.86	0.30										
A17b	900	87	73	65.25	0.92	0.30										
Z3b	600	92	61	69	0.93	0.30										
AI14	700	93	78	69.75	0.98	0.30										
AN1	800	93	78	69.75	0.98	0.30										
J7b	775	93	61	69.75	1.10	0.20										

<sup>z</sup> Products designated as primer and lower case letter (e.g., AC7a) (NA = not applicable).

of the loci (e.g., M7<sub>675</sub>, U13<sub>831</sub>, AT7a<sub>1275</sub>, A16<sub>1600</sub>, AT2<sub>800</sub>, BC226<sub>1400</sub>, 02-1<sub>1100</sub>, and Z3a<sub>700</sub>). Although this type of conversion has proven difficult in cucumber (6), if success is achieved in melon we will make additional conversions. This will allow for the development of a standard array of SCARs markers, and permit their use in diversity analysis and genetic map construction along with previously published codominant markers (5). A standard marker array and the use of reference accessions from previous studies (e.g., 7) will provide powerful set of tools for diversity analysis.

### Literature Cited

1. Baudracco-Arnas, S. and M. Pitrat. 1996. A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. *Theor. Appl. Genet.* 93:57-64.
2. García, E., M. Jamilena, J.I. Álvarez, T. Arnedo, J.L. Oliver, and R. Lozano. 1998. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. *Theor. Appl. Genet.* 96: 878-885.
3. 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.
4. Staub, J.E., J. Box, V. Meglic, T.F. Horejsi, and 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.
5. Staub, J.E., V. Meglic, and J. D. McCreight. 1998. Inheritance and linkage relationships of melon (*Cucumis melo* L.) isozymes. *J. Amer. Soc. Hort. Sci.* 123:264-272.
6. Horejsi, T., J. Box and J. E. Staub. 1999. Efficiency of RAPD to SCAR marker conversion and their comparative PCR sensitivity in cucumber. *J. Amer. Soc. Hort. Sci.* 124:128-135.
7. Staub, J.E., Y. Danin-Poleg, G. Fazio, T. Horejsi, N. Reis, and 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.
8. Stepansky, A., I. Kovalski, and 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.
9. Wang, Y.H., C.E. Thomas, and R.A. Dean. 1997. A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. *Theor. Appl. Genet.* 95:791-798.