

## SNP Discovery and Mapping in Melon (*Cucumis melo* L.)

**Maya Lotan-Pompan, Galil Tzuri, Vitaly Portnoy, Rotem Beja and Nurit Katzir**

*Agricultural Research Organization, Department of Genetics and Vegetable Crops, Newe Ya'ar Research Center, P. O. Box 1021, Ramat Yishay 30-095, Israel*  
[katzirn@volcani.agri.gov.il](mailto:katzirn@volcani.agri.gov.il)

**Amir Sherman, Shahar Cohen, Nir Dai and Arthur A. Schaffer**

*Agricultural Research Organization, Dept. of Genetics and Vegetable Crops, P. O. Box 6, Bet Dagan, 50-250, Israel*

**Jim Giovannoni**

*Boyce Thompson Institute for Plant Research, Tower Road, Cornell University, Ithaca, NY 14853, U.S.A.*

**Introduction:** Several studies have applied various marker types (including RFLP, RAPD, AFLP and SSR) for mapping and assessing genetic variation within *C. melo* (1, 3, 6, 7). A partial linkage map of melon from a cross between PI 414723 (*C. melo* Group *Acidulus*) and 'Dulce' (*C. melo* Group *Reticulatus*) was previously described by us (2). Single nucleotide polymorphisms (SNPs) are an abundant form of genetic variation in the genome of various species that are extremely useful for gene mapping and phylogenetic studies. In recent years, SNPs have become important as genomic markers with numerous technical methods developed for their detection (4). In this study, SNP markers for genes belonging to major fruit metabolic pathways were developed. In order to link between these markers and fruit quality traits, these SNPs are currently being located on the PI 414723 × 'Dulce' map.

**Materials and Methods:** *Plant material.* The F<sub>2</sub> mapping population included 112 individuals derived from a cross between 'Dulce' and PI 414723 (2). The 'Dulce' fruit is aromatic, sweet, of high pH, with orange-colored flesh and a netted rind. The PI 414723 fruit is non-aromatic, non-sweet, and acidic, with salmon-colored flesh and no net.

*SNP discovery.* From the melon EST database (<http://melon.bti.cornell.edu>), three different methods were used for SNP detection: (i) comparative analysis of sequences of different genotypes, (ii) primers designed for the end sequences of interesting ESTs were used to amplify the genomic DNA of both parental lines ('Dulce' and PI 414723); this was followed by screening of the amplicons using either DHPLC (Denaturing High Performance Liquid Chromatography) or SSCP (Single Strand Conformational Polymorphisms), in order to find polymorphism between the parental amplicons. The polymorphic amplicons were re-sequenced in order to validate the SNPs, (iii) direct sequencing of parental amplicons.

*Genotyping.* SNP genotyping was conducted using the Sequenom MassARRAY® platform, by the high-throughput genotyping assays: hME and iPLEX. These assays allowed multiple PCR reactions in a single well (5). JoinMap 3.0 (Kyazma, Holland) was used for linkage analysis and map calculations.

**Results:** SNP Markers for ca. 100 genes belonging to major fruit metabolic pathways were developed (Table 1). These include genes encoding for key enzymatic and transport steps in carbohydrate, acid, volatile and carotenoid metabolism in melon and

other cucurbits. SNP frequency was found to vary between one in 5000 bp to ten in 300 bp. A search for linkages between SNPs and traits is underway using a RIL population derived from the F<sub>2</sub> population.

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Table 1. Genes associated with carbohydrate, carotenoid and organic acid metabolism. SNPs were developed for all the listed genes. Stars mark the genes located on the melon map.

Carbohydrate genes		Carotenoid genes	Organic acid genes		
Alkaline alpha galactosidase 1	Hexose transporter 1	* Geranylgeranyl Pyrophosphate reductase	Phosphoenol pyruvate carboxykinase	NAD malate dehydrogenase (mitochondria)	* Vacuolar pump V0-a subunit
* Alkaline alpha galactosidase 2	* Hexose transporter 6	Geranylgeranyl pyrophosphate synthase	* Phosphoenol pyruvate carboxylase (a)	NAD malate dehydrogenase (glyoxysome)	* Vacuolar pump V0-c' subunit
* Acid alpha galactosidase 1	* Acid invertase 1	* Phytoene synthase 1	* Phosphoenol pyruvate carboxylase (b)	* NADP malic enzyme (chloroplast)	* Vacuolar pump V0-c subunit
* Putative galactose kinase	* Acid invertase 2	* Phytoene synthase 2	* Citrate synthase (glyoxysome)	* NAD malate dehydrogenase (chloroplast)	* Vacuolar pump V0-d subunit
Galactose-1-phosphate uridylyltransferase	*Alkaline/neutral invertase 1	* Phytoene desaturase	* Citrate synthase (mitochondria)	* Malate synthase (glyoxysome)	* Vacuolar pump V0-e subunit
* UDP-glucose 4-epimerase 1	*Alkaline/neutral invertase 2	* Zeta carotene desaturase	* Aconitase	* NADP Isocitrate lyase	* Vacuolar pump V1-a subunit
UDP-glucose 4-epimerase 2	* Sucrose synthase	* Lycopene beta-cyclase	* NAD Isocitrate dehydrogenase ( $\beta$ )	ATP citrate lyase (b)	* Vacuolar pump V1-b subunit
UDP-glucose 4-epimerase 3	Sucrose synthase 1	* Beta-carotene hydroxylase	* $\alpha$ keto glutarate dehydrogenase (E2)	NADP malic enzyme (cytosol)	Vacuolar pump V1-C subunit
UDP-glucose pyrophosphorylase	* Sucrose cleavage protein-like	* Carotenoid isomerase	* Succinyl CoA synthetase ( $\alpha$ )	* NAD Isocitrate dehydrogenase	* Vacuolar pump V1-d subunit
*Phosphoglucomutase (cytosol)	Hexokinase 2		* Succinyl CoA synthetase ( $\beta$ )	* Citrate transporter (mitochondria)	* Vacuolar pump V1-e subunit
* Sucrose-phosphate synthase 1	Fructokinase 3		* Succinate dehydrogenase ( $\alpha$ )	*Citrate transporter (glyoxysome)	* Vacuolar pump V1-f subunit
Putative sucrose transporter			* Succinate dehydrogenase ( $\beta$ )		Vacuolar pump V1-G subunit
Sugar transporter superfamily			* Fumarase		Vacuolar H <sup>+</sup> -translocating inorganic pyrophosphatase