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BARD

FINAL REPORT

PROJECT NO. IS-4223-09

Systems Approach to Understanding Metabolic Diversity of Melon

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Final Scientific Report

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Project Title: Systems approach to understanding metabolic diversity of melon

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Appendix G6a

STATE OF ISRAEL

MINISTRY OF AGRICULTURE

AGRICULTURAL RESEARCH ORGANIZATION

THE VOLCANI CENTER

P O BOX 6, BET DAGAN

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	3	3		6
Submitted, in review, in preparation	3			3
Invited review papers				
Book chapters	1		1	2
Books				
Master theses				
Ph.D. theses			1	1
Abstracts	7		4	11
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	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	3	3	1	7
Longer Visits (Sabbaticals)				

Details of cooperation

Cooperation among the participants was excellent: The participating researchers met several times to discuss and coordinate the project: N. Katzir visited Cornell in May 2010, J. Giovannoni visited Israel in December 2012 and Z. Fei visited Israel in June 2013. The two PIs and several of the Co-PIs met at the Plant and Animal Genome meeting, San Diego, in January 2011, 2012 and 2013 and in the SOL-ICuGI meeting in Kobe, Japan in November 2011. Material (RNA) was transferred from Newe Ya'ar to Cornell where libraries for the RNA-seq were constructed and the sequencing was performed. Plant material was also transferred to the Weizmann Institute for untargeted metabolomics analyses. Transcriptomic and metabolomics data was transferred back and forth and data analysis was discussed throughout the project. The frequent meetings and discussions also affected the further development of the Cucurbit Genomics Network (<http://www.icugi.org/>) with new tools implemented to enhance the usefulness of the data deposited (and see below)

Abstract

Fruit quality is determined by numerous genetic factors that affect taste, aroma, color, texture, nutritional value and shelf life. To unravel the genetic components involved in the metabolic pathways behind these traits, the major goal of the project was to identify novel genes that are involved in, or that regulate, these pathways using correlation analysis between genotype, metabolite and gene expression data.

The original and specific research objectives were: (1) Collection of replicated fruit from a population of 96 RI lines derived from parents distinguished by great diversity in fruit development and quality phenotypes, (2) Phenotypic and metabolic profiling of mature fruit from all 96 RI lines and their parents, (3) 454 pyrosequencing of cDNA representing mRNA of mature fruit from each line to facilitate gene expression analysis based on relative EST abundance, (4) Development of a database modeled after an existing database developed for tomato introgression lines (ILs) to facilitate online data analysis by members of this project and by researchers around the world. The main functions of the database will be to store and present metabolite and gene expression data so that correlations can be drawn between variation in target traits or metabolites across the RI population members and variation in gene expression to identify candidate genes which may impact phenotypic and chemical traits of interest, (5) Selection of RI lines for segregation and/or hybridization (crosses) analysis to ascertain whether or not genes associated with traits through gene expression/metabolite correlation analysis are indeed contributors to said traits.

The overall research strategy was to utilize an available recombinant inbred population of melon (*Cucumis melo* L.) derived from phenotypically diverse parents and for which over 800 molecular markers have been mapped for the association of metabolic trait and gene expression QTLs. Transcriptomic data were obtained by high throughput sequencing using the Illumina platform instead of the originally planned 454 platform. The change was due to the fast advancement and proven advantages of the Illumina platform, as explained in the first annual scientific report. Metabolic data were collected using both targeted (sugars, organic acids, carotenoids) and non-targeted metabolomics analysis methodologies. Genes whose expression patterns were associated with variation of particular metabolites or fruit quality traits represent candidates for the molecular mechanisms that underlie them. Candidate genes that may encode enzymes catalyzing biosynthetic steps in the production of volatile compounds of interest, downstream catabolic processes of aromatic amino acids and regulatory genes were selected and are in the process of functional analyses. Several of these are genes represent unanticipated effectors of compound accumulation that could not be identified using traditional approaches. According to the original plan, the Cucurbit Genomics Network (<http://www.icugi.org/>), developed through an earlier BARD project (IS-3333-02), was expanded to serve as a public portal for the extensive metabolomics and transcriptomic data resulting from the current project. Importantly, this database was also expanded to include genomic and metabolomic resources of all the cucurbit crops, including genomes of cucumber and watermelon, EST collections, genetic maps, metabolite data and additional information. In addition, the database provides tools enabling researchers to identify genes, the expression patterns of which correlate with traits of interest. The project has significantly expanded the existing EST resource for melon and provides new molecular tools for marker-assisted selection. This information will be opened to the public by the end of 2013, upon the first publication describing the transcriptomic and metabolomics resources developed through the project. In addition, well-characterized RI lines are available to enable targeted breeding for genes of interest. Segregation of the RI lines for specific metabolites of interest has been shown, demonstrating the utility in these lines and our new molecular and metabolic data as a basis for selection targeting specific flavor, quality, nutritional and/or defensive compounds.

To summarize, all the specific goals of the project have been achieved and in many cases exceeded. Large scale transcriptomic and metabolomic resources have been developed for melon and will soon become available to the community. The usefulness of these has been validated. A number of novel genes involved in fruit ripening have been selected and are currently being functionally analyzed. We thus fully addressed our obligations to the project. In our view, however, the potential value of the project outcomes as ultimately manifested may be far greater than originally anticipated. The resources developed and expanded under this project, and the tools created for using them will enable us, and others, to continue to employ resulting data and discoveries in future studies with benefits both in basic and applied agricultural - scientific research.

Achievements

Transcriptomic analyses

The goal of detailed transcriptomic analyses has been achieved completely according to the original plan. RNA-seq using the Illumina HiSeq platform was applied for extensive mRNA sequencing of the flesh tissue of 97 RI lines and of the parental lines of the population that were sampled at the mature stage. In addition, a sub-group of RILs, exhibiting extremely high or low levels of sucrose, as well as the parental lines, were analyzed at two earlier developmental stages. For each sample, between 5-15 million reads with the length of 100 bp were obtained and aligned to the melon reference genome (Garcia-Mas et al. 2012). This resulted in a total of 56,023 high-quality SNPs. RNA-seq data analysis and statistics are presented in the appendix (Table 1 p. 4).

Metabolomic analyses

The set of 97 RI melon lines was analyzed by targeted and non-targeted approaches for metabolite profiling: (1) Sugars were analyzed using a dedicated HPLC. (2) Carotenoid and tocopherol content and composition were analyzed utilizing a Waters 996 HPLC system equipped with two detectors: a photo diode array for the carotenoids and a fluorescence detector for the tocopherols. (3) Volatiles were quantified by split capillary GC, using fused silica open tubular columns. (4) Non-targeted metabolite profiling was performed using the high resolution UPLC-qToF-MS system (Synapt, Waters Inc.) capable of rapidly and reproducibly detecting a large number of metabolites in a given sample. Additional information concerning the various analyses was provided in the first and second annual reports. Information related to the non-targeted analyses is also provided in the Appendix (p.1 and Figure 1 p. 14).

Correlation analyses

Correlation analyses were performed for the accumulation levels of 80 metabolites directly associated with fruit quality traits and RNA-seq based expression profiles generated for ca. 27,000 melon genes (Figures 2&3, appendix p. 15&16). First, inter-metabolite association patterns were explored, leading to the observation that metabolites form two main clusters: (1) a cluster of ethylene together with several groups of volatiles (cluster I); (2) a cluster of color

determining carotenoids, sucrose (the main sweetness determinant in melon) and apo-carotenoids (carotenoids derived volatiles) (cluster II). Subsequently, association patterns of metabolites versus genes were described, demonstrating a similar clustering. Analyses using bioinformatic tools (e.g. MapMan and KEGG) indicated the possible existence of two uncorrelated cellular processes controlling the accumulation of metabolites. Intra- and inter-cluster associations were further explored, to gain a more global view of the ripening processes in melon. Candidate genes were detected based on associations of expression patterns with metabolite accumulation (for example, 14 transcription factors associated positively or negatively with sucrose and β -carotene). These genes are further studied using advanced genetic material (developed in this research for validation) and the large germplasm collection available at Newe Ya'ar. Genetic mapping was the second approach applied in this study in an attempt to link genes and metabolites. Analysis of correlations between gene expression and the untargeted metabolism data is also underway.

Mapping of mQTLs and eQTLs

More than 200 metabolite QTLs (mQTLs) and 100 expression QTLs (eQTLs) have been added to our genetic map. Two tables depict the mQTLs: (1) according to linkage groups and (2) according to metabolic pathways are presented in the appendix to this report (Tables 2&3, appendix p. 7&11). Fifty thousand SNPs are currently being analyzed, with the aim of locating at least several thousand on the genetic map. The co-localization of QTLs of several ethyl esters on chromosome 11, with both the gene *Alcohol acetyl transferase* (*CmAATI*) and the highly significant cis-eQTL of the gene is presented (Figure 4, appendix p. 17). This gene codes for the last enzyme in the biosynthetic pathway and its function in this reaction is known (El-Sharkawy et al. 2005), so that the co-localization is a proof of concept. It should be noted that mapping of aromatic volatiles and the genes involved in their biosynthesis has not been reported in melon until now.

To summarize, a large scale metabolic and transcriptomic resource was developed with both scientific and agricultural implications. Exploring this resource, a first step towards the identification of the respective regulatory mechanisms that regulate the intricate association between color, sweetness and aroma has been described.

Description of the resulting publications

The list of publications is presented below. Of the three joint manuscripts that have been published so far, the first two describe work that began as part of our earlier BARD project (IS-3877-06) but was completed at the earlier stage of the current project. One of the two (Diaz et al 2010) describes the development of a merged reference map for melon, developed as part of a wide international efforts. The genetic RI map that was developed by us and used under the current project had been one of the merged maps. The reference maps as well as all the merged maps are presented in the melon data base described above. The third manuscript (Gonda et al. 2013) describes a study of a major metabolic pathway determining melon aroma, the catabolism of methionine into aroma volatiles. The manuscript describes a novel gene, *CmGML*, coding for an enzyme with L-methionine-c-lyase activity. Functional analysis of the gene was performed using an *E. coli* system. A crucial support for the findings came from the metabolomics and transcriptomic data of the current project. Figure 7 in Gonda *et al.* (2013) depicts the co-segregation, in the RIL population, of the expression of *CmGML* and the levels of S-compounds enriched with +1 m/z unit accumulation (Gonda *et al.* 2013a, Appendix). The figure demonstrates the applicability of the data obtained and could be considered as the first validation of the project concept.

A review of fruit ripening was published by the US PI (J. Giovannoni). The US CO-PI (Z. Fei) is a co-author of two additional manuscripts of the genome and transcriptome of watermelon. Dr. Fei contributed to the bioinformatics of these two works and the genomic and transcriptomic data are presented through the Cucurbit Genomics Network (<http://www.icugi.org/>) and are most useful in comparative mapping of melon, watermelon and the additional cucurbit crops.

Three manuscripts that are currently in preparation deal with the major topics resulting from the project as described above: (1) Expression data analysis (Table 1, appendix p. 4); (2) Network approaches for exploring the intricate associations between color, sweetness and aroma in melon fruits (Figures 2&3, appendix p. 15&16); (3) High resolution QTL map of melon (Tables 2&3 appendix p. 7&11).

Details of cooperation

Cooperation among the participants was excellent: The participating researchers met several times to discuss and coordinate the project: N. Katzir visited Cornell in May 2010, J. Giovannoni visited Israel in December 2012 and Z. Fei visited Israel in June 2013. The two PIs and several of the Co-PIs met at the Plant and Animal Genome meeting, San Diego, in January 2011, 2012 and 2013 and in the SOL-ICuGI meeting in Kobe, Japan in November 2011. Material (RNA) was transferred from Neve Ya'ar to Cornell where libraries for the RNA-seq were constructed and the sequencing was performed. Plant material was also transferred to the Weizmann Institute for untargeted metabolomics analyses. Transcriptomic and metabolomics data was transferred back and forth and data analysis was discussed throughout the project. The frequent meetings and discussions also affected the further development of the Cucurbit Genomics Network (<http://www.icugi.org/>) with new tools implemented to enhance the usefulness of the data deposited. For example, at the ICuGI meeting in Japan, J.G. Z.F. and N.K. met with the Spanish group that developed the melon genome (Garcia-Mas *et al*, 2013) a meeting that enabled early access, ahead of publication, to the melon genome, which opened the possibility of mapping of the RNA-seq data to the genome instead of de-novo assembly.

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Gonda, I., Lev, S., Bar, E., Sikron, N., Portnoy, V., Davidovich-Rikanati, R., Burger, J., Schaffer, A.A., Tadmor, Y., Giovannoni, J.J., Huang, M., Fei, Z., Katzir, N., Fait, A. and Lewinsohn, E. (2013 a). Catabolism of L-methionine in the formation of sulfur and other volatiles in melon (*Cucumis melo* L.) fruit. *The Plant Journal* 74: 458-472.

Publications IS-4223-09C (Reviewed)

Joint Publications

Clepet, C., Joobeur, T., Zheng, Y., Jublot, D., Huang, M., Truniger, V., Boualem, A., Hernandez-Gonzalez, M.E., Dolcet –Sanjuan, R., Portnoy, V., Creus, A.M., Caño-Delgado, A., **Katzir, N., Giovannoni, J.J.**, Bendahmane, A., Aranda, M.A., Garcia-Mas, J., and **Fei, Z.** (2011). Analysis of expressed sequence tags generated from full-length enriched cDNA libraries of melon. *BMC Genomics* 12:252

Diaz, A., Fergany, M., Formisano, G., Ziarsolo, P., Blanca, J., **Fei, Z.**, Staub, J.E., Zalapa, J.E., Cuevas, H.E., Dace, G., Oliver, M., Boissot, N., Dogimont, C., Pitrat, M., Hofstede, R., van Koert, P., Harel-Beja, R., Tzuri, G., Portnoy, V., Cohen, S., **Schaffer, A., Katzir, N.**, Xu, Y., Zhang, H., Fukino, N., Matsumoto, S., Garcia-Mas, J., Monforte, A.J. (2011). A consensus linkage map for molecular markers and Quantitative Trait Loci associated with economically important traits in melon (*Cucumis melo* L.). *BMC Plant Biol.* 11:111

Gonda, I., Lev., S., Bar, E., Sikron, N., Portnoy, V., Davidovich-Rikanati, R., Burger, J., **Schaffer, A.A., Tadmor, Y., Giovannoni, J.J.**, Huang, M., **Fei, Z., Katzir, N.**, Fait, A. and **Lewinsohn, E.** (2013 a). Catabolism of L-methionine in the formation of sulfur and other volatiles in melon (*Cucumis melo* L.) fruit. *The Plant Journal* 74: 458–472.

US publications IS-4223-09C:

Gapper, N.E., Ryan P. McQuinn, R.P. and **Giovannoni J.J.** (2013). Molecular and genetic regulation of fruit ripening. *Plant Mol. Biol.* DOI 10.1007/s11103-013-0050-3

Guo, S., Liu, J., Zheng, Y., Huang, M., Zhang, H., Gong, G., He, H., Ren, Y., Zhong, S., **Fei, Z.**, Xu, Y. (2011) Characterization of transcriptome dynamics during watermelon fruit development: sequencing, assembly, annotation and gene expression profiles. *BMC Genomics* 12:454

Guo, S., Zhang, J., Sun, H., Salse, J., Lucas, W., Zhang, H., Zheng, Y., Mao, L., Ren, Y., Wang, Z., Min, J., Guo, X., Murat, F., Ham, B., Zhang, Z., Gao, S., Huang, M., Xu, Y., Zhong, S., Bombarely, A., Mueller, L., Zhao, H., He, H., Zhang, Y., Zhang, Z., Huang, S., Tan, T., Pang, E., Lin, K., Hu, Q., Kuang, H., Ni, P., Wang, B., Liu, J., Kou, Q., Hou, W., Zou, X., Jiang, J., Gong, G., Klee, K., Schoof, H., Huang, Y., Hu, X., Dong, S., Liang, D., Wang, J., Wu, K., Xia, Y., Zhao X, Zheng Z, Xing M, Liang X, Huang B, Lv T, Wang J, Yin Y, Yi H, Li R, Wu M, Levi A., Zhang, X., Giovannoni, J., Wang, J., Li, Y., **Fei, Z.**, Xu, Y. (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. *Nature Genetics* 45:51-58

Book chapter IS-4223-09C (Israel):

Gonda, I., Burger, Y., **Schaffer, A.A.**, Ibdah, M., **Tadmor, Y., Katzir., N.**, Fait, A. and **Lewinsohn, E.** (2013b). Biosynthesis and perception of melon aroma. In: “Biotechnology in Flavor Production” 2nd Edition. (eds. D. Havkin Frenkel and N. Dudai). Blackwell Publishing, Oxford. (In Press).

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Appendix

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US publications	
Guo <i>et al.</i> 2011	
Gapper <i>et al.</i> 2013	
Guo <i>et al.</i> 2013	

Unpublished material

RNA-seq analysis and statistics

Library preparation and sequencing

A total of 110 strand-specific RNA-seq libraries were constructed using a protocol developed in the Giovannoni lab (Zhong et al., 2011). The libraries were barcoded and around 8 and 20 libraries were pooled and sequenced on one lane of the Illumina GAII and HiSeq 2000 systems, respectively. Around 5-10 million reads were obtained for each library. Please check Table 1 for the complete list of RNA-seq libraries

RNA-seq data analysis

RNA-Seq reads were first aligned to the ribosomal RNA database (Quast et al., 2013) using Bowtie allowing two mismatches (Langmead et al., 2009), to remove any possible rRNA sequence contaminations. The resulting cleaned reads were aligned to the melon genome (Garcia-Mas et al., 2012) using TopHat (Trapnell et al., 2009) allowing 1 segment mismatch. Following alignments, raw counts for each melon gene were normalized to reads per kilobase of exon model per million mapped reads (RPKM).

To identify SNPs between each RIL and their donor and recurrent parents, respectively, RNA-seq reads were aligned to the melon reference genome using BWA (Li and Durbin, 2009) and only uniquely mapped reads were kept. Following mapping, SNPs were identified based on the mpileup files generated by SAMtools (Li et al., 2009). The identified SNPs were supported by at least three reads and have allele frequency of at least 0.8.

Untargeted metabolomics analyses

The set of 97 RI melon lines (5 fruits for each) was profiled by the non-targeted metabolite profiling platform, established at the Aharoni lab. This profiling system is based on the high resolution UPLC-qTof-MS system (Synapt, Waters Inc.) capable of rapidly and reproducibly detecting a large number of metabolites in a given sample. A

major advantage of using this technology is the possibility to obtain a reasonable level of structural elucidation for unknown peaks. Most of the metabolites detected by this method will belong to different classes of secondary metabolites such as phenolics (phenolic acids, flavonoids and other phenylpropanoid pathway derivatives), alkaloids, non-volatile terpenoids, polyamines and more. Flesh of mature fruit tissue from single RI lines was isolated, grinded to a fine powder in liquid nitrogen, and extracted as described by Mintz-Oron et al. (2008). Data analysis is currently underway by the metabolomics data analysis pipeline for LC-MS analysis currently used at the Aharoni lab. The final goal of the data analysis will be to detect metabolites that are significantly reduced or increased in a particular RI line (or more) as compared to the rest of the RI population (Figure 1).

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Table 1 Statistics of melon RIL RNA-seq libraries

line	tissue	total reads	rRNA	clean	mapped	% mapped
Dulce	10_20DAA	15107585	144210	14963375	13648377	91.21
PI414723	10_20DAA	4130602	23739	4106863	3533767	86.05
RIL13	10_20DAA	7082449	77498	7004951	6132169	87.54
RIL30	10_20DAA	8675841	113433	8562408	7557840	88.27
RIL35	10_20DAA	3574237	21702	3552535	3171755	89.28
RIL54	10_20DAA	9611272	115990	9495282	8764490	92.3
RIL77	10_20DAA	9652374	97895	9554479	8405268	87.97
RIL82	10_20DAA	7171843	94062	7077781	6141103	86.77
RIL93	10_20DAA	9582523	113970	9468553	8687419	91.75
RIL97	10_20DAA	9577409	61771	9515638	8021555	84.3
RIL103	10_20DAA	12962811	115755	12847056	11102505	86.42
RIL111	10_20DAA	10401723	104380	10297343	9248718	89.82
Dulce	Mature	17089846	508911	16580935	12773906	77.04
PI414723	Mature	7381577	53524	7328053	6548063	89.36
RIL1	Mature	12791126	51898	12739228	5991374	47.03
RIL2	Mature	11959652	39598	11920054	5645996	47.37
RIL3	Mature	11346061	37454	11308607	6120232	54.12
RIL4	Mature	10845920	36541	10809379	5742201	53.12
RIL5	Mature	12034673	44515	11990158	6412400	53.48
RIL6	Mature	10865611	43055	10822556	6228440	57.55
RIL7	Mature	12810212	38663	12771549	7211164	56.46
RIL9	Mature	10042403	36382	10006021	5272583	52.69
RIL10	Mature	14010812	48572	13962240	7801781	55.88
RIL11	Mature	11301107	41592	11259515	5646761	50.15
RIL12	Mature	10710907	56177	10654730	5550880	52.10
RIL13	Mature	9561569	121422	9440147	8459857	89.62
RIL14	Mature	6606138	90916	6515222	6039951	92.71
RIL15	Mature	4919227	69440	4849787	4232545	87.27
RIL16	Mature	3972869	12739	3960130	3628901	91.64
RIL17	Mature	12540743	62258	12478485	6901213	55.30
RIL18	Mature	10708277	27564	10680713	4671666	43.74
RIL19	Mature	10915993	36637	10879356	5937838	54.58
RIL20	Mature	11537567	29762	11507805	5677735	49.34
RIL21	Mature	9791858	33848	9758010	5323576	54.56
RIL22	Mature	10483049	46756	10436293	5771476	55.30
RIL23	Mature	10267725	49344	10218381	5817307	56.93
RIL24	Mature	11018326	42406	10975920	5946193	54.17
RIL25	Mature	9266147	16657	9249490	4133380	44.69
RIL26	Mature	8835101	56500	8778601	6334740	72.16
RIL27	Mature	9346656	30351	9316305	7547224	81.01
RIL28	Mature	10241309	26749	10214560	7606114	74.46
RIL30	Mature	10552067	132660	10419407	9566912	91.82

RIL31	Mature	9309545	39195	9270350	8149679	87.91
RIL32	Mature	7515377	52172	7463205	6365695	85.29
RIL34	Mature	7759040	39579	7719461	6728335	87.16
RIL35	Mature	10488809	177396	10311413	9185302	89.08
RIL36	Mature	7019405	34197	6985208	6076878	87
RIL37	Mature	9120774	30607	9090167	7900404	86.91
RIL38	Mature	6823251	28371	6794880	5840092	85.95
RIL39	Mature	6455866	18806	6437060	5634871	87.54
RIL40	Mature	5633740	45213	5588527	4828064	86.39
RIL41	Mature	7915150	56390	7858760	6577464	83.7
RIL42	Mature	6031672	37260	5994412	5086979	84.86
RIL43	Mature	3314443	22789	3291654	2751038	83.58
RIL44	Mature	7582623	29682	7552941	6467445	85.63
RIL45	Mature	6394964	24895	6370069	5458583	85.69
RIL46	Mature	4974001	30368	4943633	4198329	84.92
RIL48	Mature	6489649	38106	6451543	5521932	85.59
RIL50	Mature	5518009	43070	5474939	4644078	84.82
RIL51	Mature	5419010	29022	5389988	4561225	84.62
RIL52	Mature	6570968	44085	6526883	5500982	84.28
RIL53	Mature	7478484	30181	7448303	6455302	86.67
RIL54	Mature	13900510	174272	13726238	12409628	90.41
RIL55	Mature	3088229	33912	3054317	2634500	86.25
RIL61	Mature	4458800	22681	4436119	3718080	83.81
RIL62	Mature	6891057	27149	6863908	4391525	63.98
RIL63	Mature	6203583	30311	6173272	5166135	83.69
RIL64	Mature	5831330	20837	5810493	4751877	81.78
RIL65	Mature	6711221	24363	6686858	5382728	80.5
RIL66	Mature	7145523	25009	7120514	5645580	79.29
RIL68	Mature	8648760	33163	8615597	7274282	84.43
RIL69	Mature	8409200	35392	8373808	6991969	83.5
RIL71	Mature	7741543	29548	7711995	6021052	78.07
RIL72	Mature	7660934	20331	7640603	6501944	85.1
RIL73	Mature	6870675	15802	6854873	5695288	83.08
RIL74	Mature	6511779	18393	6493386	4883319	75.2
RIL76	Mature	8731062	33356	8697706	7212689	82.93
RIL77	Mature	7868657	29218	7839439	6567847	83.78
RIL79	Mature	6403787	16085	6387702	5376813	84.17
RIL80	Mature	5263972	18447	5245525	4298916	81.95
RIL81	Mature	6535637	17388	6518249	5523128	84.73
RIL82	Mature	11171625	132281	11039344	9551182	86.52
RIL83	Mature	8277600	19475	8258125	6721436	81.39
RIL84	Mature	5502106	15649	5486457	4163912	75.89
RIL85	Mature	4803385	11562	4791823	4055751	84.64
RIL87	Mature	4963812	22339	4941473	4159190	84.17
RIL88	Mature	4398018	10930	4387088	3327647	75.85

RIL89	Mature	7496587	28208	7468379	6326205	84.71
RIL91	Mature	11265574	24951	11240623	9138501	81.3
RIL92	Mature	6054740	72763	5981977	5539474	92.6
RIL93	Mature	3082766	13670	3069096	2670737	87.02
RIL94	Mature	10000500	42175	9958325	8073580	81.07
RIL96	Mature	9286184	32323	9253861	7448694	80.49
RIL97	Mature	4900044	51342	4848702	4425620	91.27
RIL98	Mature	10201112	29518	10171594	8271822	81.32
RIL99	Mature	10158123	29844	10128279	8090184	79.88
RIL100	Mature	9356385	33874	9322511	7541948	80.9
RIL101	Mature	8694630	24156	8670474	7000217	80.74
RIL102	Mature	9406425	27262	9379163	7645619	81.52
RIL103	Mature	13348280	56976	13291304	11998102	90.27
RIL107	Mature	9543778	22025	9521753	7539428	79.18
RIL108	Mature	3470812	41543	3429269	2948564	85.98
RIL110	Mature	9071849	32588	9039261	5845324	64.67
RIL111	Mature	13894376	69681	13824695	12150370	87.89
RIL112	Mature	9124904	21427	9103477	6686287	73.45
RIL113	Mature	12076541	50159	12026382	9283376	77.19
RIL114	Mature	10075283	30624	10044659	7806553	77.72
RIL115	Mature	6147677	79932	6067745	5065926	83.49
RIL116	Mature	12204872	36730	12168142	9040389	74.3
RIL117	Mature	11959186	52255	11906931	9576858	80.43

Table 2: QTL analysis (MQM) of 80 metabolites and phenotypes, (sorted by metabolites and phenotypes)

Category	Sub1	Mean(weight)	Group	LOD
Weight	wgt2	Mean(weight)	LG2	7.38
Weight	wgt7-1	Mean(weight)	LG7	3.97
Weight	wgt6	Mean(weight)	LG6	3.02
Weight	wgt7-2	Mean(weight)	LG7	2.72
Category	Sub2	pH	Group	LOD
pH	pH8	pH	LG8	28.1
Category	Sub3	Lutein µg/g F.W.	Group	LOD
cartenoids	Lut2-1	Lutein µg/g F.W.	LG2	7.53
cartenoids	Lu8	Lutein µg/g F.W.	LG8	4.6
cartenoids	Lut11	Lutein µg/g F.W.	LG11	4.18
cartenoids	Lut2-2	Lutein µg/g F.W.	LG2	4.07
Category	Sub4	β-Carotene mg/g F.W.	Group	LOD
carotenoids	β-car8	β-Carotene µg/g F.W.	LG8	5.61
carotenoids	β-car5	β-Carotene µg/g F.W.	LG5	3.74
carotenoids	β-car6	β-Carotene µg/g F.W.	LG6	3.13
Category	Sub5	z-Carotene mg/g F.W.	Group	LOD
carotenoids	ζ-car8	z-Carotene mg/g F.W.	LG8	5.48
carotenoids	ζ-car5	z-Carotene mg/g F.W.	LG5	2.8
Category	Sub6	a-Carotene mg/g F.W.	Group	LOD
carotenoids	α-car8	a-Carotene mg/g F.W.	LG8	8.03
carotenoids	α-car5	a-Carotene mg/g F.W.	LG5	2.66
Category	Sub7	Phytoene mg/g F.W.	Group	LOD
carotenoids	Phy8	Phytoene mg/g F.W.	LG8	4.11
carotenoids	Phy6	Phytoene mg/g F.W.	LG6	3.45
Category	Sub8	Mean(Total Carotenes mg/g F.W.)	Group	LOD
carotenoids	T-car8	Mean(Total Carotenes mg/g F.W.)	LG8	6.86
carotenoids	T-car5	Mean(Total Carotenes mg/g F.W.)	LG5	3.77
carotenoids	T-car6	Mean(Total Carotenes mg/g F.W.)	LG6	3.23
Category	Sub9	d-tocopherole	Group	LOD
tocopherols		δ-tocopherole		
Category	Sub10	g-tocopherole mg/g F.W.	Group	LOD
tocopherols	γ-toc8	γ-tocopherole mg/g F.W.	LG8	3.8
Category	Sub11	a-tocopherole mg/g F.W.	Group	LOD
tocopherols		a-tocopherole mg/g F.W.		
Category	Sub12	Mean(Total tocopheroles mg/g F.W.)	Group	LOD
tocopherols	T-toc8	Mean(Total tocopheroles mg/g F.W.)	LG8	4.31
tocopherols	T-toc5	Mean(Total tocopheroles mg/g F.W.)	LG5	2.13
Category	Sub13	ethylene	Group	LOD
ethylene	Ethy6	ethylene	LG6	4.28
ethylene	Ethy2	ethylene	LG2	3.33
ethylene	Ethy11	ethylene	LG11	3.18
ethylene	Ethy12-2	ethylene	LG12-2	2.5
Category	Sub14	sucrose	Group	LOD
sugars	Suc5	sucrose	LG5	5.12
sugars	Suc2	sucrose	LG2	3.58
sugars	Suc6	sucrose	LG6	2.88
sugars	Suc8	sucrose	LG8	2.79
Category	Sub15	glucose	Group	LOD
sugars	Glu8	glucose	LG8	5.93
sugars	Glu4-3	glucose	LG4-3	2.25
Category	Sub16	fructose	Group	LOD
sugars	Fru8	fructose	LG8	2.9
sugars	Fru9	fructose	LG9	2.12
Category	Sub17	benzaldehyde	Group	LOD
aldehyde	BenAld8	benzaldehyde	LG8	6.97

aldehyde	BenAld5	benzaldehyde	LG5	1.91
Category	Sub18	benzyl alcohol	Group	LOD
alcohol	BenAlc8	benzyl alcohol	LG8	2.14
Category	Sub19	phenylacetaldehyde	Group	LOD
aldehyde	PheAceAld8	phenylacetaldehyde	LG8	9.25
aldehyde	PheAceAld1	phenylacetaldehyde	LG1	2.49
Category	Sub 20	methyl benzoate	Group	LOD
methyl ester	MetBen8	methyl benzoate	LG8	2.13
Category	Sub 21	phenetyl alcohol	Group	LOD
phenolic derivative	PheAlc5	phenetyl alcohol	LG5	2.63
Category	Sub 22	benzenacetoneitrill	Group	LOD
phenolic derivative	BenAceNit2	benzenacetoneitrill	LG2	4.19
phenolic derivative	BenAceNit4-2	benzenacetoneitrill	LG4-2	2.62
phenolic derivative	BenAceNit12-1	benzenacetoneitrill	LG12-1	2.42
Category	Sub 23	benzyl acetate	Group	LOD
acetate ester	Ben_Ace8	benzyl acetate	LG8	7.8
acetate ester	Ben_Ace11	benzyl acetate	LG11	3.26
acetate ester	Ben_Ace4-2	benzyl acetate	LG4-2	3.07
Category	Sub 24	benzoic acid	Group	LOD
volatile acid	BenAci5	benzoic acid	LG5	2.41
Category	Sub 25	benzenpropanol	Group	LOD
alcohol	BenPro8	benzenpropanol	LG8	3.03
Category	Sub 26	phenethyl acetate	Group	LOD
acetate ester	Phe_Ace8-1	phenethyl acetate	LG8	9.81
acetate ester	Phe_Ace8-2	phenethyl acetate	LG8	2.66
Category	Sub 27	benzyl propanoate	Group	LOD
ester	Ben_Pro4-2	benzyl propanoate	LG4-2	4.21
ester	Ben_Pro6	benzyl propanoate	LG6	3.16
ester	Ben_Pro3	benzyl propanoate	LG3	2.76
Category	Sub 28	(E)-cinnamaldehyde	Group	LOD
aldehyde	(E)-Cin_De8	(E)-cinnamaldehyde	LG8	1.42
Category	Sub 29	cinnamyl alcohol	Group	LOD
phenolic derivative	Cin_Alc11	cinnamyl alcohol	LG11	2.06
Category	Sub 30	eugenol	Group	LOD
phenolic derivative	Eug7	eugenol	LG7	2.86
Category	Sub 31	3-phenyl-1-propanol acetate	Group	LOD
acetate ester	3-Phe-1-Pro_Ace8	3-phenyl-1-propanol acetate	LG8	3.56
acetate ester	3-Phe-1-Pro_Ace11	3-phenyl-1-propanol acetate	LG11	2.46
Category	Sub 32	methyl cinnamate	Group	LOD
methyl ester	Met_Cin9	methyl cinnamate	LG9	1.86
Category	Sub 33	cinnamic acid	Group	LOD
volatile acid	Cin_Aci12-2	cinnamic acid	LG12-2	3.57
volatile acid	Cin_Aci6	cinnamic acid	LG6	2.77
Category	Sub 34	cinnamyl acetate	Group	LOD
acetate ester	Cin_Ace8	cinnamyl acetate	LG8	2.94
Category	Sub 35	ethyl benzoate	Group	LOD
ethyl ester	Eth_Ben11	ethyl benzoate	LG11	1.89
Category	Sub 36	ethyl phenyl acetate	Group	LOD
ethyl ester	Eth_Phe_Ace8	ethyl phenyl acetate	LG8	1.55
Category	Sub 37	ethyl cinnamate	Group	LOD
Ethyl ester	Eth_Cin4-2-1	ethyl cinnamate	LG4-2	4.28
Ethyl ester	Eth_Cin4-2-2	ethyl cinnamate	LG4-2	4.27
Category	Sub 38	ethanol	Group	LOD
Alcohol	Eth4-1-1	ethanol	LG4-1-1	2.33
Alcohol	Eth12-1	ethanol	LG12-1	1.62
Category	Sub 39	ethyl acetate	Group	LOD
ethyl ester	Eth_Ace12-1	Ethyl acetate	LG12-1	3.25
Category	Sub 40	ethyl propanoate	Group	LOD
ethyl ester	Eth_pro9	Ethyl propanoate	LG9	2.23
Category	Sub 41	ethyl 2-methyl propanoate	Group	LOD

ethyl ester	Eth-2-Met_Pro11	Ethyl 2-methyl propanoate	LG11	2.11
Category	Sub 42	ethyl butanoate	Group	LOD
ethyl ester	Eth_But3	Ethyl butanoate	LG3	1.72
Category	Sub 43	ethyl 2-methylbutanoate	Group	LOD
ethyl ester	Eth-2-Met_But11	Ethyl 2-methylbutanoate	LG11	3.7
Category	Sub 44	ethyl pentanoate	Group	LOD
ethyl ester	Eth_Pen3	Ethyl pentanoate	LG3	1.34
ethyl ester	Eth_Pen4-2	Ethyl pentanoate	LG4-2	1.33
Category	Sub 45	ethyl tiglate	Group	LOD
Ethyl ester	Eth_Tig11	Ethyl tiglate	LG11	3.42
Category	Sub 46	6-Methyl-5-hepten-2-one (MHO)	Group	LOD
direct apo-carotenoids	MHO8	MHO	LG8	3.87
direct apo-carotenoids	MHO5	MHO	LG5	2.75
Category	Sub 47	ethyl hexanoate	Group	LOD
ethyl ester			LG4-2	2.15
ethyl ester	Eth_Hex4-2	Ethyl hexanoate	LG4-2	1.78
Category	Sub 48	ethyl heptanoate	Group	LOD
ethyl ester	Eth_Hep4-2	Ethyl heptanoate	LG4-2	2.66
Category	Sub 49	ethyl octanoate	Group	LOD
ethyl ester			LG11	2.37
ethyl ester	Eth_Oct4-2	ethyl octanoate	LG4-2	2.29
Category	Sub 50	beta-cyctocitral	Group	LOD
			LG4-3	1.66
direct apo-carotenoids	Bet_Cyc4-3	beta-cyctocitral	LG4-3	1.63
Category	Sub 51	beta-(E)-damascenone	Group	LOD
			LG8	11.54
direct apo-carotenoids	Bet-E-Dam8	beta-(E)-damascenone	LG8	10.92
Category	Sub 52	ethyl decanoate	Group	LOD
			LG4-2	2.98
ethyl ester	Eth_Dec4-2	ethyl decanoate	LG4-2	2.06
Category	Sub 53	alpha ionone	Group	LOD
			LG8	6.94
direct apo-carotenoids	Alp_Ion8	alpha ionone	LG8	5.75
Category	Sub 54	geranyl acetone	Group	LOD
			LG5	3.62
direct apo-carotenoids	Ger_Ace5	geranyl acetone	LG5	3.23
Category	Sub 55	beta-ionone	Group	LOD
direct apo-carotenoids	Bet_Ion4-3	beta-ionone	LG4-3	1.52
Category	Sub 56	beta-ionone epoxide	Group	LOD
			LG8	3.68
direct apo-carotenoids	Bet_Ion_Epo8	beta-ionone epoxide	LG8	3.38
Category	Sub 57	2-methylpropyl acetate	Group	LOD
acetate ester	2-Met_Ace12-1	2-methylpropyl acetate	LG12-1	3.99
Category	Sub 58	methyl-2-methylbutanoate	Group	LOD
methyl ester	Met-2-Met6	methyl-2-methylbutanoate	LG6	2.52
Category	Sub 59	hexanal	Group	LOD
			LG3	2.1
aldehyde	Hex3	hexanal	LG3	1.87
Category	Sub 60	butyl acetate	Group	LOD
acetate ester	But_Ace11	butyl acetate	LG11	2.85
Category	Sub 61	2-methylbutyl acetate	Group	LOD
acetate ester	2-Met_But_Ace11	2-methylbutyl acetate	LG11	2.96
Category	Sub 62	hexyl acetate	Group	LOD
			LG5	9.19
acetate ester	Hex_Ace5-1	hexyl acetate	LG5	7.44
acetate ester	Hex_Ace5-2	hexyl acetate	LG5	5.66
acetate ester	Hex_Ace12-1	hexyl acetate	LG12-1	2.36
Category	Sub 63	2,6-(E,Z)-nonadienal	Group	LOD
aldehyde	2,6-(E,Z)_Non8	2,6-(E,Z)-nonadienal	LG8	7.61
Category	Sub 64	methanethiol	Group	LOD

thiol	Met_Eth	methanethiol	LG8	3.9
thiol	Met_Eth8-1	methanethiol	LG8	3.82
thiol	Met_Eth4-2	methanethiol	LG4-2	2.91
thiol	Met_Eth8-2	methanethiol	LG8	2.51
Category	Sub 65	S-methylthio acetate	Group	LOD
thio-ester	S-Met_Ace1	S-methylthio acetate	LG1	9.47
thio-ester	S-Met_Ace8	S-methylthio acetate	LG8	3.2
Category	Sub 66	dimethyl disulfide	Group	LOD
sulfide	Dim_Dis8	dimethyl disulfide	LG8	1.99
sulfide	Dim_Dis4-1-2	dimethyl disulfide	LG4-1-2	1.72
Category	Sub 67	S-methyl propanethioate	Group	LOD
thio-ester	S-Met-Pro8	S-methyl propanethioate	LG8	9.66
thio-ester	S-Met-Pro6	S-methyl propanethioate	LG6	6.59
thio-ester	S-Met-Pro2	S-methyl propanethioate	LG2	4.99
thio-ester	S-Met-Pro11	S-methyl propanethioate	LG11	3.81
thio-ester	S-Met-Pro7	S-methyl propanethioate	LG7	3.08
Category	Sub 68	2-(methylthio)ethanol	Group	LOD
			LG11	4.25
alcohol	2-Met_Eth11	2-(methylthio)ethanol	LG11	4.25
alcohol	2-Met_Eth7	2-(methylthio)ethanol	LG7	3.68
Category	Sub 69	S-methyl 2-methylpropanethioate	Group	LOD
thio-ester	S-Met-2-Met_Pro_Eth8	S-methyl 2-methylpropanethioate	LG8	4.07
thio-ester	S-Met-2-Met_Pro_Eth2	S-methyl 2-methylpropanethioate	LG2	3.29
thio-ester	S-Met-2-Met_Pro_Eth11/2	S-methyl 2-methylpropanethioate	LG11/2	2.84
Category	Sub 70	S-methyl thiobutanoate	Group	LOD
			LG6	4.03
thio-ester	S-Met-2-Thi_But_6	S-methyl thiobutanoate	LG6	3.97
Category	Sub 71	3-(methylthio)propanal	Group	LOD
			LG7	2.75
aldehyde	3-Pro7	3-(methylthio)propanal	LG7	2.57
aldehyde	3-Pro2	3-(methylthio)propanal	LG2	2.26
Category	Sub 72	methyl 2-methylthio acetate	Group	LOD
thio-ether ester	Met-2-Met_Ace2	methyl 2-methylthio acetate	LG2	4.37
thio-ether ester	Met-2-Met_Ace5-2	methyl 2-methylthio acetate	LG5	4.03
thio-ether ester	Met-2-Met_Ace6	methyl 2-methylthio acetate	LG6	3.03
Category	Sub 73	S-methyl 2-methylbutanethioate	Group	LOD
thio-ester	S-Met-2-Met_But_Thi6	S-methyl 2-methylbutanethioate	LG6	2.53
Category	Sub 74	dimethyl trisulfide	Group	LOD
			LG8	2.8
sulfide	Dim_Tri_Sul8	dimethyl trisulfide	LG8	2.72
sulfide	Dim_Tri_Sul4-1-2	dimethyl trisulfide	LG4-1-2	1.95
Category	Sub 75	3-(methylthio)propanol	Group	LOD
			LG8	4.03
alcohol	3-Pro8	3-(methylthio)propanol	LG8	3.99
alcohol	3-Pro11	3-(methylthio)propanol	LG11	3.55
Category	Sub 76	ethyl (methylthio) acetate	Group	LOD
ethyl ester and thio-ether ester	Eth_(Met)_Ace4-2-1	ethyl (methylthio) acetate	LG4-2	5.12
ethyl ester and thio-ether ester	Eth_(Met)_Ace4-2-2	ethyl (methylthio) acetate	LG4-2	2.76
Category	Sub 77	2-(methylthio) ethyl acetate	Group	LOD
thio-ether ester	2-(Met)_Eth_Ace8	2-(methylthio) ethyl acetate	LG8	4.73
Category	Sub 78	methyl 3-(methylthio)propanoate	Group	LOD
thio-ether ester	Met 3-(Met_Thi)_Pro11	methyl 3-(methylthio)propanoate	LG11	4.52
thio-ether ester	Met 3-(Met_Thi)_Pro6	methyl 3-(methylthio)propanoate	LG6	2.69
Category	Sub 79	ethyl 3-(methylthio)propanoate	Group	LOD
ethyl ester and thio-ether ester	Eth 3-(Met_Thi)_Pro6	ethyl 3-(methylthio)propanoate	LG11	3.17
Category	Sub 80	3-(methylthio)propyl acetate	Group	LOD
acetate and thio-ether ester	3-(Met_Thi)_Pro_Ace11	3-(methylthio)propyl acetate	LG11	2.91

Table 3: QTL analysis (MQM) of 80 metabolites and phenotypes, (sorted by linkage groups)

Category	Metabolite	Group	LOD
thio-ester	S-methylthio acetate	LG1	9.47
aldehyde	phenylacetaldehyde	LG1	2.49
Category	Metabolite	Group	LOD
thio-ether ester	methyl 2-methylthio acetate	LG2	4.37
thio-ester	S-methyl 2-methylpropanethioate	LG2	3.29
sugars	sucrose	LG2	3.58
thio-ester	S-methyl propanethioate	LG2	4.99
ethylene	ethylene	LG2	3.33
Weight	Mean(weight)	LG2	7.38
phenolic derivative	benzenacetoneitrill	LG2	4.19
carotenoids	Lutein µg/g F.W.	LG2	7.53
carotenoids	Lutein µg/g F.W.	LG2	4.07
aldehyde	3-(methylthio)propanal	LG2	2.26
Category	Metabolite	Group	LOD
ester	benzyl propanoate	LG3	2.76
Category	Metabolite	Group	LOD
Ethyl ester	ethyl cinnamate	LG4-2	4.27
Ethyl ester	ethyl cinnamate	LG4-2	4.28
ethyl ester and thio-ether ester	ethyl (methylthio) acetate	LG4-2	2.76
ethyl ester	Ethyl heptanoate	LG4-2	2.66
phenolic derivative	benzenacetoneitrill	LG4-2	2.62
ethyl ester and thio-ether ester	ethyl (methylthio) acetate	LG4-2	5.12
ester	benzyl propanoate	LG4-2	4.21
acetate ester	benzyl acetate	LG4-2	3.07
Category	Metabolite	Group	LOD
carotenoids	a-Carotene mg/g F.W.	LG5	2.66
carotenoids	Mean(Total Carotenes mg/g F.W.)	LG5	3.77
direct apo-carotenoids	geranyl acetone	LG5	3.23
thio-ether ester	methyl 2-methylthio acetate	LG5	4.03
direct apo-carotenoids	MHO	LG5	2.75
carotenoids	β-Carotene µg/g F.W.	LG5	3.74
carotenoids	z-Carotene mg/g F.W.	LG5	2.8
sugars	sucrose	LG5	5.12
phenolic derivative	phenetyl alcohol	LG5	2.63
acetate ester	hexyl acetate	LG5	5.66
acetate ester	hexyl acetate	LG5	7.44
volatile acid	benzoic acid	LG5	2.41
Category	Metabolite	Group	LOD
ester	benzyl propanoate	LG6	3.16
ethylene	ethylene	LG6	4.28
thio-ether ester	methyl 3-(methylthio)propionate	LG6	2.69

methyl ester	methyl-2-methylbutanoate	LG6	2.52
Weight	Mean(weight)	LG6	3.02
thio-ester	S-methyl thiobutanoate	LG6	3.97
thio-ether ester	methyl 2-methylthio acetate	LG6	3.03
carotenoids	Phytoene mg/g F.W.	LG6	3.45
carotenoids	β -Carotene μ g/g F.W.	LG6	3.13
sugars	sucrose	LG6	2.88
thio-ester	S-methyl propanethioate	LG6	6.59
thio-ester	S-methyl 2-methylbutanethioate	LG6	2.53
carotenoids	Mean(Total Carotenes mg/g F.W.)	LG6	3.23
volatile acid	cinnamic acid	LG6	2.77
Category	Metabolite	Group	LOD
alcohol	2-(methylthio)ethanol	LG7	3.68
aldehyde	3-(methylthio)propanal	LG7	2.57
Weight	Mean(weight)	LG7	2.72
Weight	Mean(weight)	LG7	3.97
phenolic derivative	eugenol	LG7	2.86
thio-ester	S-methyl propanethioate	LG7	3.08
Category	Metabolite	Group	LOD
thiol	methanethiol	LG8	2.51
tocopherols	γ -tocopherole mg/g F.W.	LG8	3.8
tocopherols	Mean(Total tocopheroles mg/g F.W.)	LG8	4.31
alcohol	3-(methylthio)propanol	LG8	3.99
thio-ester	S-methyl 2-methylpropanethioate	LG8	4.07
thio-ester	S-methyl propanethioate	LG8	9.66
sugars	fructose	LG8	2.9
acetate ester	phenethyl acetate	LG8	9.81
aldehyde	(E)-cinnamaldehyde	LG8	1.42
sugars	sucrose	LG8	2.79
alcohol	benzenpropanol	LG8	3.03
thio-ether ester	2-(methylthio) ethyl acetate	LG8	4.73
aldehyde	benzaldehyde	LG8	6.97
aldehyde	2,6-(E,Z)-nonadienal	LG8	7.61
aldehyde	phenylacetaldehyde	LG8	9.25
direct apo-carotenoids	beta-(E)-damascenone	LG8	10.92
acetate ester	cinnamyl acetate	LG8	2.94
acetate ester	3-phenyl-1-propanol acetate	LG8	3.56
thio-ester	S-methylthio acetate	LG8	3.2
sugars	glucose	LG8	5.93
acetate ester	benzyl acetate	LG8	7.8
thiol	methanethiol	LG8	3.82
direct apo-carotenoids	MHO	LG8	3.87
carotenoids	Phytoene mg/g F.W.	LG8	4.11
cartenoids	Lutein μ g/g F.W.	LG8	4.6
carotenoids	z-Carotene mg/g F.W.	LG8	5.48
carotenoids	β -Carotene μ g/g F.W.	LG8	5.61
carotenoids	Mean(Total Carotenes mg/g F.W.)	LG8	6.86

carotenoids	a-Carotene mg/g F.W.	LG8	8.03
acetate ester	phenethyl acetate	LG8	2.66
direct apo-carotenoids	beta-ionone epoxide	LG8	3.38
direct apo-carotenoids	alpha ionone	LG8	5.75
sulfide	dimethyl trisulfide	LG8	2.72
Category	Metabolite	Group	LOD
methyl ester	methyl cinnamate	LG9	1.86
Category	Metabolite	Group	LOD
ethylene	ethylene	LG11	3.18
thio-ester	S-methyl 2-methylpropanethioate	LG11/2	2.84
acetate ester	3-phenyl-1-propanol acetate	LG11	2.46
ethyl ester and thio-ether ester	ethyl 3-(methylthio)propanoate	LG11	3.17
ethyl ester	Ethyl 2-methylbutanoate	LG11	3.7
thio-ether ester	methyl 3-(methylthio)propionate	LG11	4.52
acetate ester	butyl acetate	LG11	2.85
Ethyl ester	Ethyl tiglate	LG11	3.42
carotenoids	Lutein µg/g F.W.	LG11	4.18
acetate and thio-ether ester	3-(methylthio)propyl acetate	LG11	2.91
acetate ester	2-methylbutyl acetate	LG11	2.96
acetate ester	benzyl acetate	LG11	3.26
alcohol	2-(methylthio)ethanol	LG11	4.25
alcohol	3-(methylthio)propanol	LG11	3.55
thio-ester	S-methyl propanethioate	LG11	3.81
Category	Metabolite	Group	LOD
volatile acid	cinnamic acid	LG12-2	3.57
acetate ester	hexyl acetate	LG12-1	2.36
ethyl ester	Ethyl acetate	LG12-1	3.25
acetate ester	2-methylpropyl acetate	LG12-1	3.99
phenolic derivative	benzenecetonitrile	LG12-1	2.42
ethylene	ethylene	LG12-2	2.5

Figures

Figure 1: PCA of melon flesh analyzed with UPLC-QTOF-MS in negative mode

Melon tissue was extracted with methanol containing formic acid. The samples were concentrated 6.6 times and filtered before injection to the QTOF-MS. Data analysis was done using XCMS peak-picking program followed by MetaboStat in-house software. Peak areas after log transformation and quantile normalization were used for PCA presentation.

Figure 2: Association between metabolites accumulation and cellular processes. (A) Metabolites versus metabolites correlation matrix (Spearman's rho). Accumulation values were recorded for 76 metabolites directly associated with fruit quality (sweetness, aroma, color) as well as Ph values (acidity phenotype). (B) Pathway enrichment of genes whose expression values are correlated with the accumulation level of metabolites.

Figure 3: Correlation matrix of metabolite accumulation versus gene expression (A), showing an overall agreement between the clustering patterns of the main metabolite groups as derived from the metabolite-versus-metabolite analysis (Figure 2). Pathway enrichment (B). Enzymes from all groups have been highlighted on top of the generic KEGG metabolic pathway, in order to gain a network perspective of the functional significance of the gene clusters (C).

Figure 4: Co-localization of acetate esters and *CmAAT1*, the last enzyme in the pathway of their biosynthesis. Highly significant cis-eQTL of *CmAAT1* was located to the same region.