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Invited Review

Research tools for functional genomics in melon (*Cucumis melo* L.): Current status and prospects

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Abstract Melon (*Cucumis melo* L.) is an excellent model species for the study of functional genomics in the Cucurbitaceae, a plant family that includes melon, cucumber, watermelon, and squash, because of its unique traits such as a lianous structure of the plant body and unusual mode of sex determination. Following the first workshop on Cucurbit genomics in Barcelona in 2005, the International Cucurbit Genomics Initiative (ICuGI: http://www.icugi.org/) was established at an academic level, and melon became the model species for cucurbit study as a result of existing genomic resources for this species. The ICuGI is currently overseeing three projects concerned with the development of research tools for functional genomics in melon: sequencing of ESTs, merging of existing melon genetic maps, and development of an ICuGI webpage containing specific genomic tools available to the cucurbit research community. Additional efforts are also underway to develop tools for the study of melon functional genomics. Availability of these resources will promote research activities in the Cucurbitaceae, especially melon. This review summarizes the current status of these tools and discusses future aspects of the study of melon functional genomics.

Key words: DNA marker, EST, functional genomics, linkage-map, melon.

The Cucurbitaceae family comprises important crop species, including melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrulus lanatus* (Thunb.) Matsum. & Nakai), and squash (*Cucurbita pepo* L., *Cucurbita maxima* Duch.). Members of the Cucurbitaceae have several unique traits; these include a lianous structure of the plant body, the development of fleshy fruits, and a mode of sex determination that is not found in other model plants for which comprehensive genomics tools have been developed. Therefore, the Cucurbitaceae are an important target for both plant science and crop improvement. Following the first workshop on Cucurbita genomics in Barcelona in 2005, the International Cucurbit Genomics Initiative (ICuGI: http://www.icugi.org/) was established at an academic level, and melon became the Cucurbit model species because of previously established genomic resources for this species. The ICuGI is currently overseeing three projects concerned with the development of research tools for functional genomics in melon: sequencing of ESTs, merging of existing melon genetic maps, and development of an ICuGI webpage containing specific genomic tools available to the cucurbit research community.

Melon is a diploid species with 12 chromosomes (2x=2n=24) and an estimated genome size of 450 to 500 Mb (Arumuganathan and Earle 1991), which is half the size of the tomato genome (950 Mb) (Arumuganathan and Earle 1991) and about three times the size of the Arabidopsis genome (125 Mb) (Arabidopsis Genome Initiative 2001). The current tomato model is excellent for studying fruit development given the availability of comprehensive resources (e.g., Matsukura et al. 2008). Melon has significant potential to also become a model plant for the elucidation of key traits in fruit development, considering its morphological, physiological, and biochemical diversity in flavor development and textural changes during fruit ripening (Kirkbride 1993; Liu et al. 2004; Nunez-Palenius et al. 2008). Modern melon cultivars are categorized into two types based on their fruit ripening pattern, climacteric and non-climacteric. Elucidation of fruit ripening is a major target of the study of fruit development, and its diverse fruit ripening patterns make melon a valuable model for these studies (Ezura and Owino 2008). Comparative studies between climacteric and non-climacteric melons have provided an understanding of the molecular mechanisms of aroma formation (Flores et al. 2002; Lucchetta et al. 2007; Shalit et al. 2001) and cell wall disassembly (Bennett 2002; Nishiyama et al. 2007). Melon is also a useful plant in which to study other aspects of plant systems

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such as the transportation of macromolecules through vasculature (Gomez et al. 2005; Haritatos et al. 1996) and sex determination (Boualem et al. 2008).

This review summarizes the current status of functional genomics tools for the study of melon; these include expressed sequence tag (EST) collections, an "omics" database, genetic linkage maps, DNA markers, bacterial artificial chromosome (BAC) libraries, a mutation library, a targeted induced local lesions in genomes (TILLING) platform for mutation screening, and transformation techniques are being developed by the ICuGI and the research community. Future directions of genomic research on melon are also discussed.

ESTs and "omics" studies

ESTs have been used to select candidate genes implicated in traits of interest. Microarrays for identifying sets of plant genes expressed during different developmental stages or in response to environmental stimuli can be constructed using EST collections (Alba et al. 2004; Rudd 2003). In addition, EST collections are good sources of simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) that can be applied in the creation of saturated genetic maps (Morgante et al. 2002; Rafalski 2002). EST collections have been generated for many plant species; the most comprehensive are those for Arabidopsis (Arabidopsis Genomics Initiative 2001) and rice (Ouyang 2007). Fruit crops have been less extensively surveyed, but important collections are publicly available for several species, including tomato (Fei et al. 2006), apple (Newcomb et al. 2006), grape (da Silva et al. 2005), and citrus (Forment et al. 2005).

Before the establishment of the ICuGI in 2005, fewer than 4,000 cucurbit ESTs, primarily contributed by Katzir and Giovannoni, were deposited in the publicly accessible GenBank database. This is in sharp contrast to the data available for families of other important food crops such as the Solanaceae (1,064,706 sequences), Fabaceae (2,794,198 sequences), Brassicaceae (2,710,569 sequences), Vitaceae (381,631 sequences), and Rosaceae (499,759 sequences). In more recent years, Gonzalez-Ibeas et al. (2007) have reported 30,675 high-quality ESTs sequenced from eight normalized melon cDNA libraries and corresponding to different tissues under different physiological conditions. The ESTs were clustered into 16,637 non-redundant sequences or unigenes, which comprised 6,023 tentative consensus sequences (contigs), and 10,614 unclustered sequences (singletons). Many potential molecular markers were identified in the melon dataset; these included 1,052 potential SSRs and 356 SNPs. Among the melon unigenes, 69% showed significant sequence similarity to proteins in databases. The unigenes were functionally classified according to a gene ontology scheme. In total, 9,402 unigenes were mapped to one or more ontologies. The distributions of the melon and Arabidopsis unigenes followed a similar tendency, suggesting that the melon dataset represents the whole melon transcriptome. Bioinformatic analyses focused primarily on potential precursors of melon microRNAs in the melon dataset, but many other genes potentially controlling disease resistance and fruit quality traits were also identified. The patterns of transcript accumulation for 20 of these genes were characterized by real-time quantitative PCR. Currently, the ICuGI is sequencing 100,000 ESTs, which will be released as a publicly accessible database.

EST collections have contributed to the discovery of genes responsible for the volatile sesquiterpene content in melon rinds (Portnoy et al. 2008). Sesquiterpenes are present mainly in the rinds of climacteric varieties and show a great diversity of composition among varieties. Melon EST database mining yielded two novel cDNAs, termed CmTpsNY and CmTpsDul, that code for members of the Tps gene family and are 43.2% similar to each other. Heterologous expression of CmTpsNY in *E. coli* produced primarily δ-copaene, α-copaene, β-caryophyllene, germacrene D, α-muurolene, γ-cadinene, δ-cadinene, and α-cadinene, whereas CmTpsDul produced α-farnesene only. CmTpsNY was mostly expressed in 'Noy Yizre'el' rind, whereas CmTpsDul expression was specific to 'Dulce' rind. None of these genes was expressed in the rind of the non-climacteric 'Tam Dew' cultivar. These results indicate that different sesquiterpene synthases encoded by different members of the Tps gene family are active in different melon varieties and that this specificity modulates the accumulation of sesquiterpenes. The genes are differentially and transcriptionally regulated during fruit development and according to variety, and are likely to be associated with chemical differences responsible for the unique aromas of melon varieties.

A melon cDNA microarray (ver. 1.0) was designed based on the melon unigene build (ver. 1.0) EST collection. The array contains 9,216 spots; 12 spots are negative controls consisting of only printing buffer, while the remaining 9,204 spots represent 3068 unique genes, each printed in triplicate on the array (ICuGI: http://www.icugi.org/). This preliminary version of the microarray is not widely used. After the completion of EST sequencing by the ICuGI, a practical cDNA microarray will be designed.

Recently, a metabolomics approach combining 1H NMR and gas chromatography–electrospray ionization time-of-flight mass spectrometry (GC-EI-TOFMS) profiling was employed to characterize melon fruit (Biais et al. 2009). The data analyses revealed several metabolite gradients related to differences in metabolism in fruit flesh and demonstrated the suitability of

multiblock hierarchical principal component analyses for correlation of data from two metabolomics platforms. The metabolomic approach combined with transcriptome analysis will contribute to a comprehensive understanding of melon fruit development.

Genetic linkage maps and DNA markers

Studies on the development of DNA markers and construction of linkage maps of the Cucurbitaceae have focused on melon, and a number of DNA markers and linkage maps in melon have been published. Melon is an economically important vegetable and is widely distributed in temperate, subtropical, and tropical climates. Given the high price of fruit and F_1 hybrid seed, there is a great demand for top-quality seed. Breeders have high expectations of DNA markers applicable to F_1 seed purity testing and marker-assisted selection (MAS). Recently, an attempt to sequence the whole melon genome was begun (Gonzalez et al. Plant & Animal Genomes 2009). A complete genome sequence of the closely related cucumber (*Cucumis sativus* L.) has been built and will be published soon (Huang et al. Plant & Animal Genomes 2009). Substantial progress in genomic studies of the Cucurbitaceae is imminent.

Until the early 2000s, DNA markers used in melon genetic research were mainly restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) markers. RFLP analysis has been a valuable tool in mapping the genomes of various species, but it is laborious, cumbersome, and not available to all laboratories. In addition, the level of polymorphism by RFLP analysis is low in melon (Shattuck-Eidens et al. 1990). RAPD, AFLP, and inter-simple sequence repeat (ISSR) markers are easy to use and reveal large sets of genetic loci, but they are dominant markers and cannot be readily transferred to other populations. Recently, SSR markers and SNP-based genetic markers have attracted significant attention. They are hypervariable, multiallelic, codominant, locus-specific, and evenly distributed throughout the genome. Therefore, they are widely used for applications such as cultivar identification, hybrid seed purity testing, and linkage map construction. At present, more than 2,000 melon SSR markers are available (Chiba et al. 2003; Danin-Poleg et al. 2000; Fukino et al. 2007; Gonzalo et al. 2005; International Cucurbit Genomics Initiative database, http://www.icugi.org/; Kong et al. 2007; Ritschel et al. 2004).

A genetic linkage map is a prerequisite to the study of inheritance of both qualitative and quantitative traits and to integration of the molecular information necessary for MAS and map-based gene cloning techniques (Morgante and Salamini 2003). In melon, a number of genetic linkage maps have been produced based on a range of marker types, including RFLPs, RAPDs, AFLPs, ISSRs, and SSRs (Baudracco-Arnas and Pitrat, 1996; Cuevas et al. 2008; Fukino et al. 2008b; Gonzalo et al. 2005; Oliver et al. 2001; Périn et al. 2002; Pitrat 1991; Wang et al. 1997). Several genes and quantitative trait loci (QTLs), most of which confer resistance to diseases and pests, have been mapped to those linkage maps. Construction of a higher density map is desirable in order to expand the application of the genetic map to a larger group of breeding populations, but it is costly and laborious. A strategy for improving the efficiency of genetic mapping saturation (bin mapping) by reducing the size of the mapping population was proposed. This strategy has recently been applied to melon by Fernandez-Silva et al. (2008), and its implementation for efficient and accurate map saturation has been demonstrated. A survey of published linkage maps, including the genes and QTLs mapped to them, are summarized in Table 1.

Although various linkage maps of melon are rapidly being developed, molecular information from the various genotypic maps must be integrated to allow their efficient use. In accordance with one of its objectives, the ICuGI has begun merging the existing melon genetic maps. Owing to the demonstrated usefulness of SSR-based linkage maps for the alignment of different genetic maps in melon (Gonzalo et al. 2005) and other species (Wu and Huang 2006), SSR markers were selected as anchor markers localized in common to different maps. The maps being used for construction of a consensus map are those developed by research groups in Israel, Japan, Spain, USA, France, and China. The consensus map and relevant information will be available on the ICuGI webpage.

BAC libraries

Bacterial artificial chromosome (BAC) libraries are useful tools in plant genomic studies for constructing linkage maps, sequencing whole genomes, identifying molecular markers, performing map-based cloning, and analyzing microsynteny (Budiman et al. 2000; Cregan et al. 1999; Morales et al. 2005; Morishige et al. 2002; Nam et al. 2005; Sasaki et al. 2005).

Two BAC libraries for the multi-disease resistant melon line MR-1 were the first BAC libraries constructed from members of the Cucurbitaceae family (Luo et al. 2001). The *Hin*dIII library consists of 177 microtiter plates in a 384-well format; approximately 95.6% of the *Hin*dIII library clones contain nuclear DNA inserts (average size, 118 kb), providing coverage of 15.4 genome equivalents. Similarly, 96% of the clones in the *Eco*RI library, which consists of 222 microtiter plates, contain nuclear DNA inserts (average size, 114 kb), accounting for 18.7 genome equivalents. The MR-1 BAC

Table 1. Survey of published linkage maps in melon.

Parents	Type of the population	Number of the individuals	Markers[a]	Total length (cM)	Genes	QTL	Reference
Védrantais × PI161375	RIL	163	346 AFLP 113 IMA	1,411	disease resistance, andromonoecious, green flesh color, and so on	—	Périn et al. 2002
Védrantais × PI414723	RIL	63	233 AFLP 65 ISSR 5 SSR 2 RFLP	1,180			
PI124112 × Védrantais	RIL	120	465 AFLP 17 SSR 26 ISSR	1,150	andromonoecious, *Papaya ringspot* virus resistance	downy mildew resistance, powdery mildew resistance	Perchepied et al. 2005a
Isabelle × Védrantais	RIL	120	AFLP, ISSR, SSR	—	—	*Fusarium oxysporum melonis* resistance	Perchepied et al. 2005b
PI414723 × TopMark	F_2	113	41 RFLP 74 RAPD 3 ISSR 16 SSR 42 AFLP	1,421	andromonoecious, seed-coat color, *Aphis gossypii* resistance	—	Silberstein et al. 2003
Piel de Sapo × PI 161375	F_2	93	52 SSR 235 RFLP	1,240	Melon *necrotic spot virus* resistance	fruit quality traits	Gonzalo et al. 2005, Monforte et al. 2004
	DHL	77	90 SSR 79 RFLP 3 SNP	1,223			
	DHL (bin mapping)	14	80 RFLP 212 SSR 3 SNP	1,261	Melon *necrotic spot virus* resistance	—	Fernandez-Silva et al. 2008
USDA 846-1 × Top Mark	RIL	81	114 RAPD 43 SSR 32 AFLP	1,116	andromonoecious	yield-related traits	Zalapa et al. 2007
						fruit quality traits	Paris et al. 2008
			104 SSR 7 CAPS 4 SNP 140 other	1,180	andromonoecious	quantity of beta-carotene in fruit	Cuevas et al. 2008
Melon Chukanbohon Nou 4 Gou × Harukei 3	F_2	94	94 SSR 2 CAPS 18 AFLP	700	—	short lateral branching	Fukino et al. 2008a
AR 5 × Harukei 3	RIL	93	157 SSR 7 SCAR/CAPS	877	*Aphis gossypii* resistance, flesh color, Melon *necrotic spot virus* resistance	Powdery mildew resistance	Fukino et al. 2008b

[a] *SCAR*, sequence characterized amplified region; CAPS, cleaved amplified polymorphic sequences.

libraries are currently available from the BAC/EST Resource Center, Genomics Institute, Clemson University (http://www.genome.clemson.edu/, 2009.04.20).

Fom-2 is the gene responsible for resistance to *Fusarium* wilt, which is one of the most destructive diseases in melon production worldwide. Two PCR-based codominant DNA markers (AM, AFLP marker; FM, *Fusarium* marker) that cosegregate with *Fom-2* were previously identified by Wang et al. (2000), who used the two markers to screen the *Hin*dIII BAC library mentioned above (Wang et al. 2002). Fingerprinting analysis showed that clones identified by each marker assembled into two separate contigs at high stringency. GenBank searches using the end-sequencing results for the identified clones produced matches to leucine-rich repeats (LRRs) of resistance genes (R genes); to retroelements and cellulose synthase in clones identified

by FM; and to nucleotide-binding sites (NBSs) of R genes, retroelements, and cytochrome P-450 in clones identified by AM. A 6.5-kb fragment containing both NBS and LRR sequences was found to share high sequence similarity to the Toll-interleukin-1 receptor (TIR)/NBS/LRR class of R genes such as the N gene, exhibiting 42% identity and 58% similarity with the TIR–NBS and LRR regions, respectively.

A BAC library from the dihaploid melon line PIT92 was constructed, with six-fold coverage of the haploid melon genome and an average insert size of 139 kb (van Leeuwen et al. 2003). Using this BAC library, a contig of four BACs around the MRGH63 (Garcia-Mas et al. 2001) resistance gene homolog fragment was created (van Leeuwen et al. 2005). A detailed analysis of four regions of the melon genome, including two sequenced BACs, identified 14 TIR/NBS/LRR genes, which appear to be clustered in the melon genome. They contain all the conserved motifs previously described for their counterparts in other species, although differences were also detected. These and similar results may contribute to a better understanding of the variability, genomic distribution, and evolution of this group of resistance gene homologs.

In melon, andromonoecy is controlled by the identity of the alleles at the andromonoecious (*a*) locus (Kenigsbuch and Cohen 1990). The *a* locus was cloned by constructing high-resolution genetic and physical maps, using chromosome walking to construct a BAC contig anchored to the genetic map (Boualem et al. 2008). Cloning of the *a* gene revealed that andromonoecy results from a mutation in the active site of 1-aminocyclopropane-1-carboxylic acid synthase. Expression of the active enzyme inhibits the development of the male organs and is not required for carpel development. A causal SNP associated with andromonoecy was identified, which suggests that the a allele has been under recent positive selection and may be linked to the evolution of this sexual system.

BAC libraries are powerful tools for functional genomics in melon.

Mutation libraries and TILLING

Large collections of induced mutations in a common genetic background are designated as mutation libraries, and mutants isolated from plant mutation libraries have made major contributions to both basic and agricultural research (Ahloowalia et al. 2004). Recently, technologies such as TILLING that allow high-throughput mutant isolation have been developed (Henikoff et al. 2004), increasing the value of mutation libraries for functional genomics in plants.

Ethyl methanesulfonate (EMS) is a widely employed chemical mutagen with high mutagenicity, low mortality, and simple application. EMS treatment causes primarily G/C to A/T transitions in DNA, which ultimately may result in an amino acid change or transcription termination. Mutation libraries established by EMS treatment have been reported for several important plant species, including tomato (cv M82, Menda et al. 2004; cv. Micro-Tom, Saito et al. 2009), wheat (Slade et al. 2005), sorghum (Xin et al. 2008), and soybean (Cooper et al. 2008). These mutation libraries have subsequently used for TILLING.

The production of a melon mutation library was first reported by Israeli researchers (Tadmor et al. 2007). Although melon is a diploid species (2n=24) with a small genome size of 450 Mb, it exhibits relatively high levels of sequence and fruit shape polymorphism. Seeds of 'Noy Yizre'el', a Galia-type melon parental line, were treated with the EMS, and the resulting M-1 plants were self-pollinated to produce about 3,000 M-2 families. Phenotypic analyses revealed newly induced variation, mostly governed by single recessive mutations; different plant organs, including cotyledon, leaves, flowers, and fruit, were affected at different growth stages, from emergence to mature fruit. Several mutations showed phenotypic similarities to mutations found in other plant species. This melon mutation library is a valuable source of new traits. Moreover, it also serves as an essential infrastructure for the discovery of important genes (map-based cloning), the annotation of unknown sequences (TILLING), and the comparison of phenotypic and genetic characteristics among plant mutation libraries.

Spanish research groups have also initiated the establishment of a mutant collection and a TILLING platform (Puigdomènech et al. 2007). In the 'Piel de Sapo' M62-113 line, 20,000 melon seeds were mutagenized by 0.5 to 1.5% EMS, resulting in a collection of 5,000 M2 mutant families. DNA bulks prepared from these families will be examined for allelic variation.

Japanese researchers have begun preliminary studies to establish a melon mutation library and a TILLING platform (Ezura et al. unpublished results). We used 'Earl's Favourite (Harukei 3)', a Japanese cultivar that is an important breeding material for netted melon cultivars in Japan. The seeds were tested for EMS response, and the appropriate mutagenizing concentration was estimated at 0.5 to 1% based on mortality and deformity. Currently, a collection of approximately 600 M2 families has been obtained and will be evaluated by TILLING for mutation frequency. As a preliminary study for establishing a TILLING platform in melon, EcoTILLING, which allows the identification of allelic variants within natural populations, has been performed. In the first attempt at EcoTILLING in melon (Nieto et al. 2007), a collection of *Cucumis* spp. was characterized for susceptibility to Melon necrotic spot virus (MNSV)

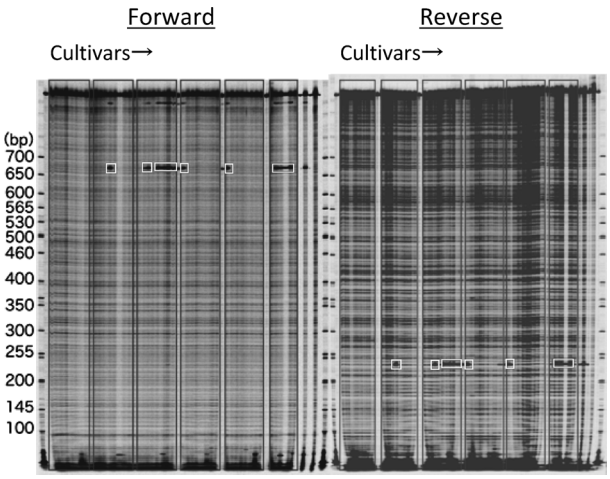

Figure 1 Detection of polymorphisms in melon ethylene receptor gene *Cm-ETR1*. Gel images from LI-COR analyzer. Each lane displays the 900 bp amplified PCR product of transmembrane regions digested with endonuclease *Cel1*. Heteroduplexes were produced after melting and anniling PCR products with the DNA of the reference genotype (culivar Harukei 3). Cleaved products, indicated by boxes, correspond to sequence polymorphisms. True polymorphisms should give rise to two complementary bands.

in order to identify new allelic variants of eukaryotic translation initiation factor 4E (eIF4E). A single point mutation in melon eIF4E controls resistance to MNSV. A high conservation of eIF4E exonic regions was found, with six polymorphic sites identified by EcoTILLING 113 accessions.

We also performed Eco-TILLING experiments using 50 melon varieties with different phenotypes for fruit shelf life (Ezura et al. unpublished results). We designed several PCR primers for EcoTILLING the melon ethylene receptor gene Cm-ETR1, which is expressed during fruit maturation and may be responsible for melon fruit ripening (Sato-Nara et al. 1999). Significant allelic variations in Cm-ETR1 were identified among the melon varieties tested (Figure 1).

These results demonstrate that EcoTILLING and TILLING are applicable to melon and will be useful in screening melon mutation libraries for genes of interest.

Genetic transformation

Genetic transformation is an alternative technique for characterizing the functions of genes of interest. Various transformation procedures have been reported for melon (reviewed by Guis et al. 1998), and an *Agrobacterium*-mediated procedure has been found to be practical for the production of transgenic melon plants. The first successful *Agrobacterium*-mediated transformation conferred kanamycin resistance to melon by the introduction of the *NPT-II* (Fang and Grumet 1990) and GUS genes (Dong et al. 1991; Valles and Lasa 1994).

These early studies revealed two difficulties in melon transformation. First, many transgenic melon plants were found to be tetraploid. In response, Guis et al. (2000) developed a simple and efficient regeneration system that facilitated the production of diploid transformants at a high rate. Second, the frequency of transformation events was low due to the occurrence of "escapes" (Guis et al. 1998). In initial studies, transgenic plants were generated via adventitious shoot organogenesis. To reduce the frequency of escapes, an alternative regeneration system was needed. Several groups reported the production of somatic embryos from melon cell suspension cultures (e.g., Oridate and Oosawa, 1986). Although somatic embryogenesis can lead to problems such as abnormal embryos and hyperhydricity, the liquid-culture system is useful for the efficient selection of transformed tissues. Akasaka-Kennedy et al. (2004) reported an efficient transformation and plant regeneration system using somatic embryogenesis. With their protocol, transgenic plants were successfully produced at a rate greater than 2.3%, which was sufficient for practical use.

Other approaches for improving the frequency of transformation events have been proposed. Galperin et al. (2003) screened melon genotypes for ease of transformation and regeneration, and noted a variation of between 0.4 to 1.5 transgenic shoots per explant. Ezura et al. (2000) observed that during *Agrobacterium* inoculation, explants produced ethylene. By adding an ethylene biosynthesis inhibitor, AVG, to the co-cultivation medium, they reduced ethylene production by the explants, resulting in increased transformation efficiency. Nonaka et al. (2008a) demonstrated that the ethylene evolved from a plant inoculated with *A. tumefaciens* inhibited *vir* gene expression in *A. tumefaciens* via ethylene signal transduction in the plant, consequently inhibiting genetic transformation. To suppress ethylene evolution, they introduced 1-aminocyclopropane-1-carboxylate (ACC) deaminase into *A. tumefaciens* (Nonaka et al. 2008b). The enzyme cleaves ACC (the immediate precursor to ethylene) to a-ketobutyrate and ammonia, and as a result, ethylene levels are decreased. *Agrobacterium tumefaciens* with ACC deaminase activity, named Super-*Agrobacterium*, has shown a reduction in ethylene evolution and enhanced gene transfer into melon explants.

Agrobacterium-mediated genetic transformation has been used to validate the functions of isolated melon genes (Pitrat, personal communication). Three genes, *Vat*, *Nsv*, and *Fom-2*, which are responsible for major disease resistance in melon, have been isolated by map-based cloning. *Vat* confers a double resistance: resistance to plant colonization by an important pest, the melon/cotton aphid *Aphis gossypii*, and resistance to virus transmission by *A. gossypii* (Pauquet et al. 2004). The second gene, Nsv, confers resistance to MNSV (Garcia-Mas et al. 2004), a single-stranded RNA virus

that infects cucurbits grown under glass. *Fom-2* confers resistance to races 0 and 1 of the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. melonis (Joobeur et al. 2004), which causes significant losses in cultivated melon. Functional validation was obtained by the stable transformation of susceptible melons with these genes.

Another major fungal pathogen, *Pseudoperonospora cubensis*, is the causal agent of downy mildew in cucurbits (i.e., cucumber, melon, watermelon, and squash) and can be controlled through transgenic manipulation. The wild melon line PI 124111F is highly resistant to *P. cubensis* owing to its enhanced expression of the resistance genes *At1* and *At2*, which encode glyoxylate aminotransferases. These enzymes are important in photorespiration. Transgenic melon plants overexpressing either *At1* or *At2* displayed enhanced glyoxylate aminotransferase activity and remarkable resistance against *P. cubensis* (Taler et al. 2004), demonstrating the function of these genes.

Genetic transformation is a significant tool for melon functional genomics studies, as evidenced by the foregoing examples. The accumulation of sequence information in melon will allow the identification of unique melon genes with unknown functions. The elucidation of their functions should provide a comprehensive understanding of melon development. However, in a recent study (Nieto et al. 2006), to confirm the function of the *Cm-eIF4E* gene, which was expected to confer resistance to MNSV in melon, the gene was transiently expressed in melon plants or stably expressed in a heterologous plant species, *Nicotiana benthamiana*. This suggests that the production of stable transgenic melon plants expressing target genes is a bottleneck for melon functional genomics studies. We are trying to improve the Super-*Agrobacterium* with ACC deaminase activity and will establish a reproducible transformation protocol for melon functional genomics study.

Perspectives

Fleshy fruit development is a fundamental process that evolved with higher plants. Currently, tomato is the primary model species for studying fleshy fruit development owing to the availability of comprehensive resources for functional genomics approaches (Ezura, 2009; Matsukura et al. 2008). Although resources and tools for functional genomics studies in melon are not yet adequate, melon will provide an alternative model species for studying fleshy fruit development through the accumulation of comprehensive genomics resources and development of functional genomics tools.

The ICuGI will soon release more than 100,000 ESTs of melon, and transcriptomic analysis of melon using a next-generation sequencer is in progress (Katir N., personal communication). These activities should provide a collection of ESTs suitable for further studies. A cucumber genomics study is in progress, and the information will be adaptable to melon because of the high similarity between the cDNA sequences of both species. Comparisons of ESTs between melon and other model plant species will enable the selection of genes unique to melon. Elucidation of the functions of these melon-specific genes will contribute to our knowledge of unique Cucurbitaceae traits and will allow the application of the information for Cucurbitaceae improvement.

There are two obstacles to using melon as a model plant for fruit development. One is the inefficiency of genetic transformation techniques, although several methods have previously been reported. The establishment of efficient and reproducible methods for genetic transformation has a profound effect on research activities, as demonstrated for *Arabidopsis* (Clough and Bent 1998), rice (Toki et al. 2006), and tomato (Sun et al. 2006). Similarly, genetic transformation methods should be developed in melon; the super-Agrobacterium described above may have great potential in this regard. A second obstacle is the relative lack of mutation libraries and mutant collections for melon. Mutants are essential tools for elucidating the molecular mechanisms of target traits and the functions of target genes. Although preliminary studies on the production of mutation libraries have been conducted in melon, only one study has been reported (Tadmor et al. 2007). The cucurbit research community must establish publicly available mutation libraries and mutant databases, similar to those that exist for other model plants.

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