Comprehensive Resources for Tomato Functional Genomics Based on the Miniature Model Tomato Micro-Tom

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Abstract: Tomato (Solanum lycopersicum L., Solanaceae) is an excellent model plant for genomic research of solanaceous plants, as well as for studying the development, ripening, and metabolism of fruit. In 2003, the International Solanaceae Project (SOL, www.sgn.cornell.edu) was initiated by members from more than 30 countries, and the tomato genome-sequencing project is currently underway. Genome sequence of tomato obtained by this project will provide a firm foundation for forthcoming genomic studies such as the comparative analysis of genes conserved among the Solanaceae species and the elucidation of the functions of unknown tomato genes. To exploit the wealth of the genome sequence information, there is an urgent need for novel resources and analytical tools for tomato functional genomics. Here, we present an overview of the development of genetic and genomic resources of tomato in the last decade, with a special focus on the activities of Japan SOL and the National Bio-Resource Project in the development of functional genomic resources of a model cultivar, Micro-Tom.

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INTRODUCTION

The Solanaceae family comprises many agriculturally valuable crops, including eggplant, potato, pepper, tobacco, and tomato. Among them, tomato is one of the most important crops in the fresh vegetable market and the food-processing industry [1]. For genetic and genomic studies, tomato has many advantages over other Solanaceae, such as the moderate size of its diploid genome (950 Mb, n = 12), having numerous mapped traits, developed DNA markers, abundant collections of germplasm and mutants, and an increasing number of expressed sequence tags (ESTs) [2–6]. These advantages have made tomato an excellent model organism for investigating fruit development [7], ripening processes [8–11], sugar metabolism [12–14], carotenoid biosynthesis [15, 16] in a fleshy berry-type fruit, quantitative trait locus (QTL) analyses [17, 18], and plant–pathogen interactions [19, 20]. For these reasons, tomato was chosen for the genome sequencing as a model species of the Solanaceae family.

The genome structures of most of the solanaceous plants are relatively well conserved [21]. Thus, the tomato genome sequence will serve as a reference to study the evolution of sequence and function of orthologous genes of solanaceous plants, which then allows researchers to investigate molecular mechanisms underlying diversification and adaptation. Additionally, a large-scale analysis of tomato ESTs revealed that approximately 30% of tomato genes do not have significant similarity with Arabidopsis genes [4]. Functional analysis of these genes will provide novel insights into the mechanisms controlling biological functions that are unique to tomato. To achieve these goals, it is necessary to develop resources and analytical tools for tomato functional genomics.

Small organisms with short generation time have been the choice for model systems in functional genomics, exemplified by Drosophila and Arabidopsis. From this point of view, a miniature tomato cultivar ‘Micro-Tom’ attracts an attention as a model cultivar for tomato genomics. Micro-Tom can grow at high density in a Arabidopsis-like manner, which allows large-scale production of mutagenized lines. Since EMS mutagenized lines and transposable element-based enhancer- and gene-trap lines of Micro-Tom were reported [22], potential of Micro-Tom as a genomic tool has been recognized widely, and various resources for tomato functional genomics has been developed in Micro-Tom-background.

In this review, we first describe the current status of tomato genomics, with the summary of the development of genetic and genomic resources. We then focus on the genetic resources developed by using Micro-Tom, and describe the activities of Japan SOL (JSOL) and the National Bio-Resource Project in organizing the Micro-Tom genomic resources.

CURRENT STATE OF TOMATO GENOMICS

Tomato genomic resources have been developed in the last two decades in the form of linkage maps with various...
markers, ESTs, full length cDNA sequences, gene expression profiles, and genome sequences with annotations. Currently, most information from these resources has been released to databases in the public domain such as the National Center for Biotechnology Information (NCBI), the DNA Data Bank of Japan (DDBJ), the Solanaceae Genome Project Network (SGN), and the J. Craig Venter Institute (JCVI), formed through the merger of several organizations including The Institute for Genomic Research, TIGR). The current status of these tomato genomics databases has been reviewed by [23].

**Genome Sequencing by SOL**

SOL started genome sequencing of 12 tomato chromosomes in 2004, focusing on 220 Mbp gene-rich euchromatic regions [24, 25]. By this approach, SOL predicts that approximately 87% of a total of 35,000 genes [4] could be sequenced, although the euchromatic region only covers 23% of all chromosomes. The progress of the project and the sequence information are provided on the SGN website (http://soldb.cit.cornell.edu/about/tomato_sequencing.pl)

Prior to this project, a bacterial artificial chromosome (BAC) library was constructed with the tomato cultivar Heinz 1706 [26]. This library is composed of approximately 129,000 clones containing HindIII, MboI and EcoRI-digested mega-size DNAs. The average insert size of the BAC clones is 117.5 Kb and the BAC library covers the haploid genome by 15-fold. The 88,642 BACs were fingerprinted and anchored to the high-density genetic linkage map “F2-2000” to generate a physical map [24]. Seed BACs on each chromosome were anchored to mapped markers by hybridizing oligo DNA probes developed from marker sequences to BAC-arrayed filters. Thirty to sixty seed points per chromosome were selected in this way, and BAC-by-BAC sequencing is in progress. Currently the project goal is to sequence 2,500 BACs in total to cover the estimated 220 Mbp euchromatin.

The sequencing proceeds on a clone by clone basis in the ten participating countries (China, France, India, Italy, Korea, Japan, Netherlands, Spain, USA, and UK). Using the fingerprint contig physical map and a BAC end sequence database, which were constructed as a part of the project, the BAC sequences are assembled together in the euchromatin tiling path. By May 3 2008, 28.1% of the sequencing was completed. The number of genome survey sequences (GSS) accumulating in the International Nucleotide Sequence Databases (INSD) is rapidly increasing with the progress of the project. By Apr. 25, 2008, it constitutes 319,461 sequences, which is the sixth largest found among plants.

**Genetic Linkage Maps and DNA Markers**

SGN provides information for the high-density genetic linkage map of the tomato genome with DNA markers, including cleaved amplified polymorphic sequences (CAPS), restriction fragment length polymorphisms (RFLPs), single nucleotide polymorphisms (SNPs), and simple sequence repeats (SSRs; http://soldb.cit.cornell.edu/cview/) (Table 1). Currently, five maps constructed using segregation populations and inbred lines derived from crossing between cultivars and wild or wild derivatives are available. “Tomato-EXPEN 1992” is based on S. lycopersicum (cv. VF36) x S. pennellii (LA716) F₂ population, including 1,005 of the DNA markers (RFLP and CAPS) and also some isozyme and morphological markers [2]. “Tomato-EXHIR 1997” is derived from the interspecific backcross of S. lycopersicum (TA209) x S. habrochaites (also known as S. hirsutum; LA1777), including 135 RFLP markers [27]. “Tomato-EXPEN 2000” is based on 80 F₂ individuals from the crossing of S. lycopersicum (LA925) x S. pennellii (LA716), including 2,586 CAPS, RFLP, SNPs, and SSR markers. This map is also assisted by conserved ortholog set (COS) markers generated by a comparison of the tomato EST database to the Arabidopsis genome [28]. “Tomato-EXPIMP 2001” is based on three populations derived from the crossing of S. lycopersicum (cv. E6203) x S. pimpinellifolium (LA1589) BC₁, BC₂ and backcross recombinant inbred lines (BCRILs), including 144 CAPS and RFLP markers [21, 29, 30]. “Tomato-EXPIMP 2008” is based on S. lycopersicum (TA492) x S. pimpinellifolium (LA1589), which includes 181 CAPS, RFLP, and SSR markers. The physical maps showing the anchored positions of the BACs used for genome sequencing and the introgression line (IL) maps based on EXPEN1992 and EXPEN2000 are also available on the SGN website. These ILs were developed by successive backcrosses, and each line carries a genetically defined chromosome segment derived from S. pennellii (LA716) in the background of S. lycopersicum cv. M82 (LA3475; [31]).

**Table 1. Tomato Genetic Linkage Maps Provided by the Solanaceae Genome Project Network (SGN)**

| Map Name       | Cross Combination                     | Population | References |
|----------------|--------------------------------------|------------|------------|
| Tomato-EXPEN 1992 | S. lycopersicum (cv. VF36) x S. pennellii (LA716) | F₂         | [2]        |
| Tomato-EXHIR 1997 | S. lycopersicum (TA209) x S. habrochaites (LA1777) | BC₁        | [27]       |
| Tomato-EXPEN 2000 | S. lycopersicum (LA925) x S. pennellii (LA716) | F₂         | [2, 28]    |
| Tomato-EXPIMP 2001 | S. lycopersicum (cv. E6203) x S. pimpinellifolium (LA1589) | BC₁, BC₂ and BCRILs | [21, 29, 30] |
| Tomato-EXPIMP 2008 | S. lycopersicum (TA492) x S. pimpinellifolium (LA1589) | BC₁, BC₂ and BCRILs | [21, 29, 30] |
| Introgression line (IL) map based on the EXPEN 1992 | S. lycopersicum (cv. M82) x S. pennellii (LA716) | NILs       | [31]       |
| Introgression line (IL) map based on the EXPEN 2000 | S. lycopersicum (cv. M82) x S. pennellii (LA716) | NILs       | [31]       |

x, y, z means backcross, backcross recombinant inbred lines and nearly isogenic lines, respectively.
Expressed Sequence Tags, Full-Length cDNA, and Gene Expression Data

ESTs provide comprehensive information reflecting gene expression patterns in certain tissues/organs at various developmental stages, as well as sequence information. Thus far (Apr 25, 2008), 257,940 tomato EST sequences have been deposited in the INSD, the largest such database among vegetable crops (tenth in plants). These ESTs were assembled into non-redundant consensus sequence sets called ‘unigenes’ or “tentative consensus” (TC). SGN [25], the DFCI Tomato Gene Index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=tomato; [32]), and the MiBASE (http://www.kazusa.or.jp/jsol/microtom/indexe.html; [33]) provide ESTs and unigene sequences on the basis of their own in silico construction methods. To date, SGN delivers 34,829 unigenes assembled from 223,441 ESTs (Tomato 200607), the Tomato Gene Index delivers 41,425 unigenes assembled from 215,990 ESTs (Release 11.0, June 21, 2006), and the MiBASE delivers 26,363 unigenes assembled from 186,405 ESTs, including 36,427 in-house generated ESTs (ver. Mar. 2007). These databases also support corresponding gene ontology (GO) terms, predicted peptide sequences, and DNA marker information. As described above, SGN has mapped these ESTs as COS markers on the “Tomato XPEN2000” based on comparisons to the Arabidopsis genome to estimate the level of genome synteny between tomato and Arabidopsis. By using these resources, the following DNA microarrays have been developed: the Tom1 12K cDNA array and the Tom2 11K oligo array offered by the Center for Gene Expression Profiling (CGEP), and the GeneChip® Tomato Genome Array from Affymetrix, Inc. (Santa Clara, CA, USA). These include more than 10,000 probes designed on the basis of information from UniGene Build #20 (3 October 2004) and GenBank mRNA (5 November 2004). The Tomato Expression Database collects and releases gene expression data obtained from DNA microarray experiments with Tom1, Tom2 and the Affymetrix GeneChip on the website (http://ted.bti.cornell.edu/). This database is a part of the tomato genome project, and contains basic microarray information including SGN-supported probe sequences and annotation information as well as microarray data for fruit development [11, 34-36]. Several other databases, including ArrayExpress [37] and MiBASE [33, 38, 39], also have open gene expression data obtained from microarray experiments. These databases will continue to be updated with additional expression results. Aside from ESTs and unigenes, full-length cDNA libraries derived from fruit and leaves, including pathogen-treated tissue, have been developed [40] and their sequence information is released to INSD and KaFTom (http://www.pgb.kazusa.or.jp/kaftom/). Together with the comprehensive gene expression analysis data, full-length cDNA resource accelerates the application of reverse-genetics approach to elucidate the function of tomato genes.

Collection of Tomato Germplasm Stock

One direct way to identify a gene and understand its function involves a forward genetic approach based on mutation analysis. Currently, 1,017 monogenic mutants at 622 loci have been collected in the Tomato Genetics Resource Center (TGRC) at the University of California, Davis (http://tgrc.ucdavis.edu). These monogenic mutants contain spontaneous and induced mutations affecting many aspects of plant development. In addition to the mutants, TGRC has stored wild relatives, including representatives of all nine Lycopersicon species, four related Solanum species, and approximately 1,500 miscellaneous genetic stocks including landraces, cultivars, prebred lines, introgression lines, backcross recombinant inbred lines, stress-tolerant stock, and cyto genetic stock containing trisomics, tetraploids, and translocations. Except for rare stock with low fertility, TGRC distributes the seed stock gratis to researchers for research purposes. Additionally, an isogenic mutation library containing more than 3,400 mutations has been developed in the genetic background of the inbred variety ‘M82’ and cataloged in the SGN on a site named “Genes That Make Tomatoes” (http://Zamir.sgn.cornell.edu/mutants/; [5]). However, since a current rough estimation suggests that the number of genes in the tomato genome ranges from 30,000 to 35,000, the current mutant population is insufficient for saturated mutagenesis. Considerable efforts have been made to generate a large M2 population through chemical (e.g., ethyl methane sulfonate; EMS), physical (e.g., X-ray or fast-neutron irradiation), and insertional (e.g., transposable elements or T-DNA) mutagenesis [5, 22, 41-45].

MODEL CULTIVAR MICRO-TOM FOR FUNCTIONAL GENOMICS

cv. Micro-Tom

The miniature and dwarf S. lycopersicum cultivar Micro-Tom (TGRC accession # LA3911) was bred for home gardening purposes by crossing cv. Florida Basket and Ohio 4013-3 [46]. From the late 1990s, the Micro-Tom has received attention as a model cultivar for molecular research on tomato. Compared to other cultivars, it has several unique features, such as small plant size (15–20 cm high), rapid life cycle (70–90 days), and easy transformation [22, 45]. It also exhibits relatively high fertility and fruit set even under normal fluorescent lighting. Meissner et al. [22] showed that it can grow at high density (up to 1,357 individuals/m²) and can produce three or four generations in a year. These utilities, which most ordinary tomato cultivars lack, allow us to handle this cultivar in an Arabidopsis-like manner, making it successful for large-scale and high-throughput work in functional genomics.

The miniature growth phenotype of Micro-Tom is attributed to at least two major recessive mutations, dwarf (d) and miniature (mnt) derived from its ancestors [22]. The D gene encodes cytochrome P450 protein, which is a brassinosteroid biosynthetic enzyme [47]. Reduced brassinosteroid content in Micro-Tom results in its rugose deep-green leaves and shortened internode phenotype [48]. Although the mnt mutation has not been well characterized, it is suggested to be associated with gibberellin (GA) signaling without affecting GA metabolism [48]. A mutation in the SELF PRUNING (SP) gene is responsible for the determinate phenotype of Micro-Tom [48]. SP is an ortholog of the Arabidopsis TFL1 (TERMINAL FLOWER 1) gene, which is involved in continuous growth of the shoot apical meristem [49].

Micro-Tom Mutagenized Lines

During the last decade, a large number of mutagenized population resources have been developed in Micro-Tom:
Ac/Ds transposon insertional tagging lines and T-DNA inser-
tional activation tagging lines, EMS-mutagenized lines, and
gamma irradiation mutagenized lines. The transposon tag-
ging lines proved that an Ac/Ds system derived from maize
is active in the Micro-Tom genome. A total of 2,932 lines
harboring two to three Ds elements (approximately 7,500
insertions in total) were generated, and the Ds insertion was
preferentially inserted into genes [50]. A novel transcription
factor, ANT1, which was isolated from a population of
10,427 independent T-DNA tagging lines, encodes a MYB
transcription factor regulating gene expression of a group
of anthocyanin biosynthetic enzymes in tomato [51]. These
results show the feasibility of insertional mutagenesis as a
reverse genetic approach via a knockout strategy, as found in
other tomato cultivars [47, 52, 53]. However, based on the
number of genes in the tomato genome, it is estimated that
200,000–300,000 lines harboring two or three insertions in
each line will be necessary for construction of a population
containing sufficient mutations to knock out a target gene
[50]. This scale requirement would be a disadvantage for
insertional mutagenesis strategies. International collabora-
tions will be necessary for the saturated mutagenesis of to-
mando by such approaches.

In tomato, EMS has frequently been used to induce point
mutations. One of the big advantages of EMS mutagenesis is
that it causes high-frequency and wide-spectrum mutations
in the entire genome. This means that near-saturated muta-
genesis can be expected with a relatively small population
(several 10^10 lines). To date, several groups, including
our own, have generated EMS mutagenized populations and
recovered a broad range of mutant alleles [22, 54 and C.
Rothan, personal communication]. Recently, a highly sensi-
tive point mutation detection technique, named Targeting
Induced Local Lesions IN Genomes (TILLING), was developed
and is available for use in plants to identify a point mu-
tation [55, 56]. By constructing a system combining EMS
mutagenized resources and TILLING, we can expect to ob-
tain a desirable mutant through large-scale and high-through-
put screening. A gamma-ray (γ-ray) irradiated M_2 population
was also generated in Micro-Tom, and several dozen of se-
vere phenotypic mutants have been obtained to date through
consequent screening [57]. Such a mutation resource caused
by large-size deletions would be applicable for reverse ge-
netic approaches supported by PCR-based screening meth-
ods as well as for forward genetics approaches, as estab-
lished in Arabidopsis and rice [44]. Considering the re-
quirements for regulatory approval of genetically modified
organisms (GMOs), non-transgenic mutant resources will be
preferred as parental germplasm for a breeding program.
Overall, all mutagenesis strategies reported in other cultivars
and species are also applicable to Micro-Tom. The unique
features of this cultivar are very profitable for taking advan-
tage of scale to reach saturated mutagenesis in tomato.

Development of a High-Efficiency Transformation Sys-
tem with Micro-Tom

Efficient genetic transformation is an essential technol-
ogy for functional genomics. This experimental tool is in-
valuable to elucidate and verify the function of a target gene
through transgenic plants, T-DNA insertion tagging lines,
and complementation analyses. Although Agrobacterium-
mediated transformation is applicable to S. lycopersicum [58],
it’s efficiency had been relatively lower than that of other
model species such as Arabidopsis and rice. High transform-
ation frequencies of up to 60–80% have been reported in
tomato [22, 51], but there are no detailed descriptions of the
transformation methods. According to other early studies,
the efficiencies range from 6 to a maximum of 37% [59–65].
Furthermore, large plant size and long life cycle (120–150
days) of ordinary tomato cultivars has discouraged research-
ers from scale expansion of transformation experiments,
which require a large-scale facility which meets the regula-
tions for GMOs. Recently, we and another group independ-
ently developed a highly efficient transformation protocol
for Micro-Tom to break this stalemate [66, 67]. The proto-
cols use cotyledons as a starting explant, and the transforma-
tion efficiency based on independent transgenic events per
inoculated explant stably exceeded 40% in the study by [67]
and ranged from 24 to 80% (on average 56%) in the study by
[66]. Our group modified and refined a protocol that had
been reported for other tomato cultivars [13]. In this proto-
col, the efficient elimination of chimeric status by repeated
shoot elongation on the selection medium is critical for effi-
cient generation of stable transgenic plants. [66] also made
modifications to existing protocols; they indicated that opti-
mal initial infection of Agrobacterium is critical for high
frequency and stable transformation, and suggested to use
the figwort mosaic virus (FMV) promoter [68] to drive target
genome. It is noteworthy that both methods enabled us to obtain
a transgenic plant in about 3 months after in vitro seed
germination for explant preparation. These protocols for Micro-
Tom will provide a powerful tool for functional genomics of
tomato.

Application for Pathology

Tomato is a well-established model plant to investigate
interactions between plants and pathogens. Tomato is sus-
ceptible to a wide range of plant diseases induced by over 60
fungi, bacteria, viruses, viroids, and nematodes [69]. On the
other hand, over 20 disease-resistance loci have been identi-
fied, and these traits have been introduced into S. lycoper-
sicum cultivars from various wild species [70]. Micro-Tom
is susceptible to at least four fungal and two bacterial species
and two viruses [71]. It also exhibits resistance to Fusaria-
num wilt race 1 and grey leafspot, which were derived from an
original variety [46]. Additionally, [48] reported that Micro-
Tom is resistant to tomato leaf mold caused by Cladospo-
rium fulvum. However, the resistance does not correspond to
the previously reported Cf-2, Cf-4, Cf-5, and Cf-9 genes,
which control resistance to various races of C. fulvum in a
dose-dependent manner [52, 72–74]. Because many unchar-
acterized Cf-like genes in the tomato genome have been re-
ported [75], Micro-Tom represents a new target cultivar to
study resistance to tomato leaf mold. The unique features of
Micro-Tom, increasing genomic resources, and sequence
information will facilitate its use as a model plant in the field
of plant pathology.

Micro-Tom Resource Development by Japanese Solana-
ceae Research Community

Japan has contributed to the SOL genome sequencing pro-
ject through JSOL activities. JSOL was voluntarily formed

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Comprehensive Resources for Tomato Functional Genomics

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in 2004 by Japanese tomato-related researchers from universities, public research organizations and private companies. Alongside the efforts in genome sequencing, we have developed the tomato genomic resources based on Micro-Tom: i) collection of ESTs and full-length cDNAs; ii) construction of BAC libraries (in progress); iii) generation of mutagenized M2 populations by EMS and γ-ray irradiation and consequent mutant screening [54, 57]; iv) establishment of advanced tools for functional genomics research, including the high-throughput genetic transformation protocol [67], DNA arrays [38], the TILLING screening system and T-DNA-insertional tagging lines; and v) database construction of these resources in the public domain (Fig. 1). To date (April 25 2008), we have obtained 36,427 ESTs and 2,268 putative full-length cDNA sequences derived from fruit and leaves, including from pathogen-treated tissues [6, 40]. Information on these resources has been released on the Kazusa DNA Research Institute websites, MiBASE and KaFTom, with DNA array profiling data [33, 39]. We also have a total stock of 10,267 M2 families (EMS mutagenesis 3,845; gamma irradiation mutagenesis 6,422, as of 3 May 2008), and 489 phenotypically categorized definitive mutants screened from the populations [54, 57]. We have already shared these genomic resources among JSOL members. The Kazusa DNA Research Institute has released a large part of the resources and information into the public domain since 2005. Additionally, from 2008, our group (University of Tsukuba) will begin sharing the EMS-mutagenized M2 resources with the French National Institute for Agricultural Research (INRA), which has already generated approximately 8,000 M2 populations in Micro-Tom and concomitantly established a TILLING screening system (C. Rothan, personal communication). This international research collaboration will greatly facilitate comprehensive genomic analyses for exploring gene function by taking advantage of the scale of the program.

In 2007, tomato was designated as a national biological resource by the National Bio-Resource Project (NBRP) funded by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The University of Tsukuba and the Kazusa DNA Research Institute are the core organizations in this tomato project, and the above-mentioned genomic resources are core project resources. During the next 10 years, we will continue to collect and distribute tomato genomic resources based on Micro-Tom. We will also develop and improve new bioresources and concomitant analytical tools, including large-scale T-DNA tagging lines and mapping populations, a reverse-genetic screening system, and metabolome databases (http://webs2.kazusa.or.jp/komics/index.php) [76], through the activities of JSOL and NBRP (Fig. 1).

Fig. (1). Current state of tomato functional genomics in Japan through the JSOL and NBRP activities. The activities based on Micro-Tom are marked with asterisks.
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ABBREVIATIONS

BAC = Bacterial artificial chromosome  
CAPS = Cleaved amplified polymorphic sequences  
EMS = Ethyl methane sulfonate  
EST = Expressed sequence tag  
JSOL = Japan SOL  
QTL = Quantitative trait locus  
RFLP = Restriction fragment length polymorphism  
SGN = Solanaceae genome project network  
SNPs = Single nucleotide polymorphisms  
SOL = International Solanaceae Project  
SSR = Simple sequence repeat  
TGRC = Institute for genomic research  
TILLING = Targeting induced local lesions in genomes

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