Review

Mutant Resources for the Miniature Tomato (*Solanum lycopersicum* L.) ‘Micro-Tom’

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Mutant-based studies have contributed to the elucidation of gene functions in plants including tomato. In this review, we introduce some outstanding research performed using spontaneous and artificially induced mutants. Tomato (*Solanum lycopersicum* L.) is a model species in the solanaceae family and research tools and information necessary to perform functional genomics study are being developed worldwide. Saturation mutagenesis is a powerful strategy that enables exploration of gene function on a genome-wide scale. We reviewed conventional mutant resources of tomato, including the ethyl methanesulfonate (EMS)-mutagenized population of the ‘M82’, which was developed in Israel (Menda et al., 2004). Recently, a miniature cultivar, ‘Micro-Tom’, has attracted the attention of tomato researchers due to its small size and rapid life cycle, which make it an ideal experimental organism for handling in the laboratory. We are developing a unique mutant resource, 10,000 EMS-mutagenized and gamma-ray-irradiated lines derived from ‘Micro-Tom’. Distinct mutant phenotypes, including plant size, leaf shape, and fruit morphology, are described. The status of newly developed tools for using this mutant resource, such as an efficient transformation method and the Targeting Induced Local Lesions In Genomes (TILLING) system, are also summarized.

Key Words: EMS, gamma ray, ‘Micro-Tom’, mutant resource, *Solanum lycopersicum* L.

Introduction

The Solanaceae family comprises more than 3000 species, including many agriculturally important vegetables such as potato, eggplant, pepper, and tomato. The genome structure of solanaceous species is generally conserved and composed of 12 haploid chromosomes (Paterson et al., 2000). Tomato has been used extensively as a target for studying fruit development and metabolite analysis related to fruit maturation (Carrari and Fernie, 2006) and is considered a model for plant species that bear fleshy berry-type fruits (Emmanuel and Levy, 2002). In studying tomato fruit quality, improvement of fruit quality and change of assimilate metabolism by salinity treatment (Saito et al., 2006; Saito et al., 2008a) and root-volume restriction (Saito et al., 2008b), screening of γ-aminobutyric acid (GABA)-rich varieties (Saito et al., 2008c), and metabolite profiling of chalcones and flavanones (Iijima et al., 2008) have been reported. Inaba (2007) reviewed physiological and molecular biological studies relating to ethylene biosynthesis and fruit softening during ripening period. The International Solanaceae Genomics Project (SOL) is a consortium of members from more than 30 countries to create a coordinated network of knowledge about the Solanaceae family (sol genomics network, http://www.sgn.cornell.edu/solanaceae-project/, November 13, 2008). In 2004, the SOL selected the tomato (*Solanum lycopersicum*) as a model for accelerating genomic studies in the Solanaceae family because of its relatively small genome size of 950 Mb. The SOL Project developed DNA markers, large-scale expressed sequence tag (EST) information, and numerous mapped traits ( Tanksley, 1993).

Mutant-based studies have contributed to the clarification of gene functions in plants, and tomato is no exception. For example, the molecular mechanisms of fruit maturation and ripening have been revealed using...
the ripening mutants *ripening inhibitor* (*rin*; Robinson and Tomes, 1968), *non-ripening* (*nor*; Tigchelaar et al., 1973), *Never-ripe* (*Nr*; Rick, 1956), and *Colorless non-ripening* (*Cnr*; Thompson et al., 1999). Biochemical and physiological features of *rin* were compared with wild-type tomatoes, and the *rin* mutation was revealed to inhibit climacteric respiration and associated ethylene evolution (Giovannoni et al., 1989), antioxidant metabolite and pigment (Torres and Andrews, 2006), glutamine and glutamate levels (Pratta et al., 2004), and softening (Smith et al., 2008).

Mutants can be classified based on the method by which they were generated, either as spontaneous or induced. Common mutagens include chemical reagents such as ethyl methanesulfonate (EMS) (Emmanuel and Levy, 2002; Hildering and Verkerk, 1965; Menda et al., 2004; Wisman et al., 1991), physical effect by X-rays, gamma-rays or fast neutrons (Emmanuel and Levy, 2002; Hildering and Verkerk, 1965; Li et al., 2001; Menda et al., 2004; Verkerk, 1971), and insertion of foreign DNA using transposons and T-DNA (Emmanuel and Levy, 2002; Knapp et al., 1994; Meissner et al., 1997; Thomas et al., 1994).

EMS is an alkylating reagent that generates a wide spectrum of mutations at high efficiency. It forms adducts with nucleotides, causing them to mispair with their complementary bases, thus resulting in base changes after replication (Greene et al., 2003). The advantage of EMS is that a broad range of alleles can be obtained within a relatively small population (several 10^3 to 10^4 lines) because many viable mutants exist per line (Emmanuel and Levy, 2002). EMS has frequently been used to induce point mutations in tomatoes, but no mutant stock has thus far been created for forward or reverse screens (Emmanuel and Levy, 2002).

Physical mutagens, such as gamma-rays and fast neutrons, are widely used. Since they usually cause large deletions in the genome, a knockout phenotype is more frequently observed compared to EMS-induced mutation (Sato et al., 2006). Additionally, the mutant population generated by physical mutagenesis exhibits high lethality due to the large-scale deletions induced; up to 6 Mb (Naito et al., 2005). The lower mutation frequency may be due to the lethality of such large deletions. Control of dose level is important when generating a useful mutant population with a normal yield property (Dumanovic et al., 1968).

Insertional mutagenesis has been carried out using maize *Activator (Ac)/Dissociation (Ds)* transposable elements (Carroll et al., 1995; Osborne et al., 1991; Rommens et al., 1992; Yoder et al., 1988). *Agrobacterium*-mediated T-DNA insertional mutagenesis for gene identification has been effectively demonstrated in tomato, with targeted tagging yielding mutants at a high rate of about 1/1000 (Jones et al., 1994).

In this review, we describe the conventional tomato mutant resources developed worldwide, and then report new resources to facilitate functional genomics in tomato, with special emphasis on a miniature model cultivar, ‘Micro-Tom’.

**Conventional spontaneous mutant resources**

Spontaneous mutations are caused by copying errors in genetic material during cell division, or by exposure to ultraviolet or ionizing radiation. Several spontaneous mutants have been used to elucidate the molecular function of tomato genes. Among them, *high-pigment 1* (*hp1*; Reynard, 1956) and *high-pigment 2* (*hp2*; Soressi, 1975), *Beta-Carotene* (*B*; Harris and Spurr, 1969), and *tangerine* (*t*; Zechmeister et al., 1941) are well-known flower and fruit color mutants. These mutants have been used for studying developmental changes in carotenoid pigments and antioxidant metabolites in fruit (Baker and Tomes, 1964; Jarret et al., 1984; Ronen et al., 2000; Thompson, 1955, 1961; Torres and Andrews, 2006). To study the regulation of homeobox gene expression in plants, Avivi et al. (2000) analyzed a spontaneous recessive mutant, *clausa*, which phenocopies several features of transgenic plants overexpressing class I *knox* genes, exhibiting abnormal leaf morphology, epiphyllous inflorescences, and ectopic meristems; however, spontaneous mutation is not suitable for systematic genome-wide mutagenesis.

**Conventionally induced mutant resources**

Induced mutagenesis has contributed to identifying several important mutants related to plant growth regulators, such as *flacca* (*flc*) and *dwarf* (*d*). A wilted mutant, *flc*, was induced by X-ray irradiation (Stubbe, 1957) and the genetic lesion was shown to be impaired in the last step of abscisic acid (ABA) biosynthesis (Sindhu and Walton, 1988; Taylor et al., 1988). The mutant has played an invaluable role in elucidating many important features of ABA biosynthesis (Taylor et al., 2005). A transposon-tagged loss-of-function mutant, *d*, displayed severe dwarfism due to a block in the brassinosteroid-6-oxidation step, which is the penultimate step in the brassinosteroid biosynthetic pathway (Bishop et al., 1996).

The C. M. Rick Tomato Genetics Resource Center at the University of California, Davis (http://tgrc.ucdavis.edu/, November 13, 2008), has an important role as a seed stock center of tomato mutants, providing phenotypic information on 1022 monogenic mutants at 622 loci collected from various cultivar backgrounds.

A mutant tomato population that enables comprehensive exploration of gene function was established in Israel. ‘M82’ was used to generate 13,000 M2 families derived from EMS and fast neutron mutagenesis (Menda et al., 2004). ‘M82’ is a small plant that forms medium-sized blocky fruit with relatively low Brix; it is a common inbred variety in Israel (Eshed et al., 1996). Thousands of mutant phenotypes are being cataloged in a database,
The Genes That Make Tomatoes (http://Zamir.sgn.cornell.edu/mutants/, November 13, 2008), aimed at performing in silico screening of phenotypic categories (Menda et al., 2004). Important findings have been made using this mutant population, for example, Galpaz et al. (2008) isolated 64 monogenic mutants associated with flower and fruit pigments.

The ‘M82’ mutant population is a powerful tool for accelerating tomato functional genomics, but drawbacks in using this cultivar in limited spaces include its large size (about 1 m) and a relatively long life cycle (90–110 days from seed germination to fruit maturation) (Emmanuel and Levy, 2002; Meissner et al., 1997).

Development of mutant resources based on ‘Micro-Tom’

A miniature tomato cultivar ‘Micro-Tom’ (Fig. 1), which was originally bred by crossing ‘Florida Basket’ and ‘Ohio 4013-3’ for home gardening (Scott and Harbaugh, 1989), is well suited as an experimental strain because of its small size (about 10–20 cm height) and rapid life cycle (Emmanuel and Levy, 2002; Meissner et al., 2000). The small size of ‘Micro-Tom’ enables cultivation at high density, up to 1357 plants/m², which is ideal for cultivation in most plant biology laboratories. Its rapid life cycle, with fruit maturity 70–90 days after sowing, allows three or four growing cycles per year. Additionally, a highly efficient Agrobacterium-mediated transformation method has been established for ‘Micro-Tom’ (Sun et al., 2006), which is indispensable for investigating gene function. These conditions make ‘Micro-Tom’ an attractive model cultivar for the next generation of tomato studies.

Recently, several mutant population resources, such as Ac/Ds transposon insertional tagging lines, T-DNA tagging lines, activation-tagging lines, and EMS mutagenized lines (Mathews et al., 2003; Meissner et al., 1997, 2000), were developed using ‘Micro-Tom’. In maize Ac/Ds transposable element insertional lines, 2932 F₂ families with transposed and stabilized Ds elements were phenotypically screened (Meissner et al., 2000). In activation-tagging lines, 1338 transgenic lines from a population of 10,427 independent lines (12.38%) exhibited phenotypically observable characteristics (Mathews et al., 2003). In EMS mutagenized lines, 70 M₂ families derived from 9000 individual M₁ plants were phenotypically screened (Meissner et al., 1997).

Our group (University of Tsukuba) developed 3845 EMS mutagenized M₂ families (Watanabe et al., 2007) and 6422 gamma-ray-irradiated M₂ families (Matsukura et al., 2007) using ‘Micro-Tom’. Many mutant phenotypes were obtained in the course of screening. Mutant phenotypes were categorized into 15 major and 48 subclasses. The number of mutants classified into the 15 major categories is summarized in Table 1. We have obtained 813 phenotypically categorized mutants at the time of writing (November 2008). Some distinct phenotypes obtained from EMS-mutagenized and gamma-irradiated populations are shown in Figures 2 and 3, respectively. In the plant size category, extremely small mutants were selected (Figs. 2A, 3A, and 3G). In the plant habit category, a very short internode mutant was obtained (Fig. 2B). In the leaf morphology categories, mutants with a broad leaf (Fig. 2C), wilty curl leaf (Fig. 2D), simple leaf (Fig. 3B), narrow leaf without serration (Fig. 3C), deformed leaf (Fig. 3D) were obtained. In the leaf color category, a yellow-green leaf mutant was selected (Figs. 2E, and Fig. 3E). In the inflorescence and flower morphology categories, mutants with a long peduncle (Fig. 3H) and a narrow petal and leaf (Fig. 3F) were obtained. In the flower color category, a mutant with pale-yellow flowers (Fig. 2G) was obtained. In the fruit size and morphology and color categories, mutants with small fruit (Fig. 3J), long fruit (Figs. 2H, and 3K), imbricate fruit (Fig. 2I), variegated (Fig. 3I), and dark green fruit (Fig. 2J), as compared to

Table 1. Number of mutant lines in each phenotypic category.

<table>
<thead>
<tr>
<th>Phenotypic category</th>
<th>Number of mutant lines</th>
</tr>
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<tbody>
<tr>
<td>Seed germination</td>
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<tr>
<td>Plant size</td>
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<tr>
<td>Plant morphology</td>
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</tr>
<tr>
<td>Leaf morphology</td>
<td>83</td>
</tr>
<tr>
<td>Leaf color</td>
<td>79</td>
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<tr>
<td>Flowering</td>
<td>3</td>
</tr>
<tr>
<td>Flower morphology</td>
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</tr>
<tr>
<td>Flower color</td>
<td>15</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>15</td>
</tr>
<tr>
<td>Fruit size</td>
<td>11</td>
</tr>
<tr>
<td>Fruit morphology</td>
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<tr>
<td>Fruit color</td>
<td>5</td>
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<tr>
<td>Fruit ripening</td>
<td>1</td>
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<tr>
<td>Sterility</td>
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<tr>
<td>Disease and stress response</td>
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</tr>
<tr>
<td>Total</td>
<td>813</td>
</tr>
</tbody>
</table>

Fig. 1. *Solanum lycopersicum* L. ‘Micro-Tom’.  

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Fig. 2. Mutant phenotypes obtained from EMS mutant lines. A, extremely small plant with pear-shaped fruit; B, very short internodes; C, broad leaf; D, curled leaf; E, yellow-green leaf; F, short and curled trichomes; G, pale-yellow flower; H, long fruit; I, ovules in fruit; J, dark green fruit; WT, wild-type ‘Micro-Tom’; mt, mutant.

Fig. 3. Mutant phenotypes obtained from gamma-ray irradiated mutant lines. A, extremely small; B, simple leaf; C, narrow leaf without serration; D, deformed leaf; E, yellow-green leaf; F, narrow petal and leaf; G, extremely small plant with spotted leaf; H, long peduncle; I, variegated fruit (at mature green and red stage); J, small fruit; K, long fruit; L, pink fruit; WT, wild-type ‘Micro-Tom’; mt, mutant.
fruit of wild-type plants in the mature green stage and pink fruit were obtained (Fig. 3L).

The phenotypic data are scored and saved in an in-house database “TOMATOMA,” which is maintained with the support of the National Institute of Genetics. This database will be available to the public in 2009. We have initiated the distribution of M$_3$ generation seeds for bulk screening, and the latest information is available at http://tomato.nbrp.jp/ (November 13, 2008).

Beginning in April 2007, we started sharing our EMS-mutagenized resource with the French National Institute for Agricultural Research (INRA), which has already generated approximately 8000 EMS-mutagenized M$_2$ populations of ‘Micro-Tom’ (Matsukura et al., 2008). This international collaboration is expected to expand the scale of the mutagenized population to a level required for reverse genetics approach-based mutant analyses.

**Current status of development of tools to utilize the mutant resources**

*TILLING*

Recently, a high-throughput and highly sensitive point mutation detection technique, named Targeting Induced Local Lesions IN Genomes (TILLING), was developed and is available to identify point mutations in plants, including tomato (Colbert et al., 2001; McCallum et al., 2000a, 2000b; Rothan, personal communication). This technique visualizes single-base nucleotide exchange occurring in a target gene by heteroduplex formation and consequent specific digestion by Cell enzyme (Colbert et al., 2001). By constructing a system combining EMS-mutagenized resources and TILLING, we can expect to obtain a desirable mutant through large-scale screening. The mutation frequency of our EMS-mutagenized resource, which was estimated with several known genes by TILLING, is one mutant allele per 1 Mb. The increasing genome sequence information supplied from SOL makes this technique a powerful tool for clarifying the function of a specific gene.

**Genome sequencing**

The goal of the International Tomato Genome Sequencing Project launched in 2004 is to decipher the euchromatic region of the genome, the estimated size of which is 220 Mb, using a clone-by-clone strategy. The project is approximately 30% completed at the time of writing. A Japanese team (Kazusa DNA Research Institute and National Institute of Vegetable and Tea Sciences) is making good progress in the sequencing of chromosome 8 (sol genomics network, http://www.sgn.cornell.edu/about/tomato_sequencing.pl, November 13, 2008). The Kazusa group is also performing whole-genome shotgun sequencing based on selected bacterial artificial chromosome (BAC) mixtures as a complementary effort for filling gaps in a BAC tiling path (Asamizu, 2007). Together with this effort, the majority of the tomato euchromatic genome should be sequenced in the next few years. There is an urgent need to develop reverse genetic tools such as TILLING to fully utilize genome sequence data.

**cDNA**

In tomato, as many as 330,000 EST sequences have been deposited in public databases. Clustering performed by the Dana Farber Cancer Institute (DFCI) Tomato Gene Index has generated 46,849 unigenes (http://compbio.dfci.harvard.edu/cgi-bin/tgi/tgi/gimain.pl?gudb=tomato, November 13, 2008), and in Japan, the Kazusa group has developed a unique ‘Micro-Tom’ full-length cDNA resource (Tsugane et al., 2005). Sequence data and annotation information are available from the original database, Kazusa Full-length Tomato cDNA Database (KafTom, http://www.pgb.kazusa.or.jp/kaftom/, November 13, 2008). Fifty-seven thousand clones are included in the current release.

**Future activity**

The National Bio-Resource Project (NBRP) funded by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan is collecting, preserving, and providing bioresources (such as animals and plants) that are basic materials for life sciences research. In 2007, tomato was selected as a target of NBRP. The University of Tsukuba and the Kazusa DNA Research Institute are the core organizations of this project. Our group is in charge of developing individual-level tomato biological resources, and we collect, propagate, and distribute tomato resources based on ‘Micro-Tom’, including EMS and gamma-ray mutant populations as the core resource of our project. In addition, we will include major experimental strains, related species, and
transgenic lines, including T-DNA insertional lines. Kazusa DNA Research Institute is in charge of developing DNA-level tomato biological resources, and their major activities include the collection, maintenance, and distribution of full-length cDNA clones derived from ‘Micro-Tom’. The latest release information is provided in the database KaFTom (http://www.pgb.kazusa.or.jp/kaftom/, November 13, 2008). We are developing new tools aside from NBRP activity, such as a TILLING platform and mapping tools, and encourage the research community to use the developed resources, tools, and information.

**Literature Cited**


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