TOMATOMA Update: Phenotypic and Metabolite Information in the Micro-Tom Mutant Resource

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Introduction

Tomato is an economically important crop with widespread production worldwide. Tomato is a member of the Solanaceae family, which consists of potato, eggplant, pepper, tobacco, petunia and others. The Solanaceae family is distantly related to model plants used in molecular biology, such as Arabidopsis thaliana (Brassicaceae), Oryza sativa (Poaceae) and Lotus japonicus (Fabaceae). Compared with these model plants which bear dry fruits, development of fleshy fruit is an exclusive feature of tomato. Therefore, tomato is also important scientifically and is used as a model plant for Solanaceae and fruit development. To accelerate tomato research, the tomato genome was sequenced in 2012 (Tomato Genome Consortium 2012). Many databases for tomato have been developed, including Sol Genomics Network (SGN) (Bombarely et al. 2011), MiBase (Yano et al. 2006), KaFTom (Aoki et al. 2010), the Tomato Functional Genomics Database (TFGD) (Fei et al. 2011), miSolRNA (Bazzini et al. 2010) and the Tomato Genomic Resource Database (TGRD) (Suresh et al. 2014). These databases provide sequence and expression information for tomato, such as genome sequences, full-length cDNAs, expressed sequence tags (ESTs), small RNAs, microRNAs, quantitative trait loci (QTLs) and single nucleotide polymorphisms (SNPs), which are useful for reverse genetics research. However, forward genetics approaches can be used to identify useful traits for breeding. Collections of mutants are a powerful tool for forward genetics research. Several groups have developed tomato mutant collections in the following cultivars: M82, Red Setter and TPAADASU (Menda et al. 2004, Gady et al. 2009, Minoia et al. 2010). However, these cultivars have large space requirements for cultivation.

Among tomato cultivars, Micro-Tom has advantages as a model plant. These include small plant size, a short life cycle and the existence of functional genomics tools. Micro-Tom can grow under fluorescent light at high density (Scott and Harbaugh 1989, Ariizumi et al. 2011), while commercial cultivars require vast fields. These growth conditions are almost the same as those of Arabidopsis, a representative model plant. It may be difficult to assess yield or fruit quality in Micro-Tom research for commercial use. Nevertheless, the results obtained from Micro-Tom research are valuable, and useful traits can be easily transferred to commercial cultivars by crossing (Mubarok et al. 2015). The number of articles where Micro-Tom is used as a plant material is increasing rapidly (Supplementary Fig. S1), indicating that Micro-Tom has been recognized as a model cultivar of tomato. To facilitate tomato breeding and functional genomics research, we have been developing tomato biorepositories that are focused on Micro-Tom since 2007 within the

TOMATOMA (http://tomatoma.nbrp.jp/) is a tomato mutant database providing visible phenotypic data of tomato mutant lines generated by ethylmethane sulfonate (EMS) treatment or γ-ray irradiation in the genetic background of Micro-Tom, a small and rapidly growing variety. To increase mutation efficiency further, mutagenized M3 seeds were subjected to a second round of EMS treatment; M3M1 populations were generated. These plants were self-pollinated, and 4,952 lines of M3M2 mutagenized seeds were generated. We checked for visible phenotypes in the M3M2 plants, and 618 mutant lines with 1,194 phenotypic categories were identified. In addition to the phenotypic information, we investigated Brix values and carotenoid contents in the fruits of individual mutants. Of 466 samples from 171 mutant lines, Brix values and carotenoid contents were between 3.2% and 11.6% and 6.9 and 37.3 μg g⁻¹ FW, respectively. This metabolite information concerning the mutant fruits would be useful in breeding programs as well as for the elucidation of metabolic regulation. Researchers are able to browse and search this phenotypic and metabolite information and order seeds of individual mutants via TOMATOMA. Our new Micro-Tom double-mutagenized populations and the metabolic information could provide a valuable genetic toolkit to accelerate tomato research and potential breeding programs.

Keywords: Database • Fruit • Micro-Tom • Mutant • Resource • Tomato.

Abbreviations: EMS, ethylmethane sulfonate; NBRP, National BioResource Project; SNP, single nucleotide polymorphism; TILLING, target induced local lesions in genomes.

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framework of the National BioResource Project (NBRP) in Japan (Ariizumi et al. 2011). Within the NBRP tomato project, we have developed Micro-Tom mutant resources; this includes the generation of ethylmethane sulfonate (EMS) mutants and \( \gamma \)-ray irradiation mutants (TOMJPE and TOMJPG series, respectively) and the propagation \( M_2 \) seeds from 8,598 TOMJPE lines and 6,422 TOMJPG lines (Matsukura et al. 2007, Watanabe et al. 2007, Saito et al. 2011). We have examined phenotypic alterations in \( M_2 \) plants. Visible phenotypes were classified into 15 major categories (plant size, leaf...
Thus far, we have generated 8,598 and 6,422 M2 families by EMS double EMS-mutagenized lines: TOMJPW series; experimental strains (TOMJPF series); these include tomato cultivars (Solanum lycopersicum) such as Micro-Tom and Moneymaker, wild tomato species (S. pennelli, S. peruvianum, S. pimpinellifolium, etc.), and introgression lines (ILs) provided by Dr. Zamir (Eshed and Zamir 1994).

As fleshy fruit development is an important feature of tomato that does not exist in other model plants, many studies concerning tomato fruits have been conducted; these studies have focused on aspects such as ripening, parthenocarpy and metabolites (Akihiro et al. 2008, Terao et al. 2013, Takizawa et al. 2014, Shinozaki et al. 2015, Reuscher et al. 2014). Tomato fruits contain many functional metabolites involved in human health, such as lycopene, 
\[\text{\textgamma-aminobutyric acid (GABA)}\] and vitamins A and C (Inoue et al. 2003, Abdou et al. 2006, Raiola et al. 2015, Takayama et al. 2015). Sweetness is a favorable consumer trait for tomatoes; Brix values are used to indicate sweetness. Therefore, information concerning metabolite content in tomato fruits is useful for tomato researchers and breeders.

Here we introduce an updated version of TOMATOMA. Additional EMS mutant lines were generated via a second round of EMS treatment to increase the mutation frequency, and a bulk set of mutant seeds was prepared for screening. Furthermore, we collected additional information concerning Brix values and carotenoid contents in mutant fruits for TOMATOMA. This metabolite information is valuable for both basic and applied tomato research. These Micro-Tom bioresources will support the further acceleration of tomato research.

### Results

#### Double EMS-mutagenized lines: TOMJPW series

Thus far, we have generated 8,598 and 6,422 M2 families by EMS and \(\gamma\)-ray irradiation (TOMJPE and TOMJPJG series), respectively. To date, M3 seeds have been harvested from 8,059 EMS and 4,896 \(\gamma\)-ray mutant families. Visible phenotypes of these mutants are available from the database TOMATOMA. Accumulation of information on visible phenotypes would be useful to breeding programs, and we expected that increased mutation frequency would allow us to obtain further phenotypic alteration such as quantitative traits which were regulated by multiple genes. To increase the mutation frequency further, bulked M3 seeds from TOMJPE M2 plants were submitted to a second round of mutagenesis with 1.0% or 1.5% EMS for 12 h (TOMJPW series) (Fig. 1; Table 1). The resulting M3 M1 seeds were sown and grown, and M3 M2 seeds from 4,593 lines were harvested. We sowed 10 seeds per M3 M2 line, checked for visible phenotypes and classified the lines into 15 major phenotypic categories and 55 subcategories, as in our investigations of the TOMJPE and TOMJPJG series (Saito et al. 2011). Note that we have added seven subcategories since our previous report, such as trichome, leaf margin and other flower/fruit color. In total, we identified 1,194 phenotype subcategories in 618 M3 M2 mutants. There were no differences in phenotype distribution between lines from the TOMJPE and TOMJPJG series (Supplementary Table S1).

For the distribution of mutant seeds, one or two M3 M2 plants per line with a visible phenotype were independently harvested to obtain M3 M3 seeds. Other siblings that may include plants with the same visible phenotype as the independently harvested plant were harvested as an M3 M3 seed mix. To date, 3,947 mutant families of M3 M3 seeds have been harvested.

Pictures of visible phenotypes of M3 M3 plants from the TOMJPW series as well as M3 plants from the TOMJPE and TOMJPJG series were uploaded to TOMATOMA. Phenotypic information is available from the ‘Strain Detail’ page, which linked from ‘Resource Information’ on the top page or from ‘Strains’ on the ‘Browse & Search’ page. The ‘Strain Detail’ page includes some information concerning the germination rate, seed number, etc. (Fig. 2; Saito et al. 2011). Users are able to order mutant seeds from this page. We distribute 3–15 seeds from M3 M3 mutants. If the seeds of the displayed plant are out of stock because of sterility, lethality or related reasons, we will send mixed seeds from sibling plants.

#### Image gallery for visual search

The TOMJPW, TOMJPE and TOMJPJG mutant data contained in TOMATOMA are searchable by strain ID (TOMJPExxx) and keywords from the ‘Quick Search’ at the upper right of the TOMATOMA interface or from ‘Strain Search’ on the ‘Browse & Search’ page (Fig. 2A, B). Phenotypic categories are listed under ‘Phenotype’ to show mutant strains with altered phenotypes in the corresponding category (Saito et al. 2011). To allow

### Table 1 Second round of EMS treatment on Micro-Tom EMS mutants

<table>
<thead>
<tr>
<th>EMS treatment</th>
<th>Treated seed</th>
<th>Strains</th>
<th>Seeds</th>
<th>EMS concentration</th>
<th>TOMJPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMS-W1</td>
<td>TOMJPE6301-7200</td>
<td>793</td>
<td>3,172</td>
<td>1.5%</td>
<td>0001–2052</td>
</tr>
<tr>
<td>EMS-W2</td>
<td>TOMJPE7201-8190</td>
<td>850</td>
<td>3,400</td>
<td>1.5%</td>
<td>2053–2678</td>
</tr>
<tr>
<td>EMS-W3</td>
<td>EMS bulk set D</td>
<td>406</td>
<td>2,592</td>
<td>1.0%</td>
<td>2679–3321</td>
</tr>
<tr>
<td>EMS-W4</td>
<td>EMS bulk set C</td>
<td>117</td>
<td>ND</td>
<td>1.0%</td>
<td>3322–3947</td>
</tr>
</tbody>
</table>

*ND, not determined.*
Fig. 2 The TOMATOMA interface. (A) Top page of the TOMATOMA interface. A contents menu is shown on the left side. ‘Quick Search’ is on the upper right. ‘Resource Information’ is at the top of the page. These are indicated by arrowheads. (B) ‘Browse & Search’ page. ‘Strain list’ and ‘Metabolite Info’ pages are linked from the ‘show all’ seen below ‘Phenotype’ and ‘Metabolite Info’, respectively. The list of bulk sets is shown by clicking on ‘EMS and gamma irradiation-induced mutant lines’ (indicated by an arrowhead).
for visual searches for mutant phenotypes, we added an ‘Image Gallery’ to the contents. The ‘Image Gallery’ consists of the ‘Phenotype List’ and the ‘Growth Stage List’ (Fig. 3A, B). Representative pictures of each category are shown, and all pictures can be seen on the ‘Image List’ page linked from the categories (Fig. 3C).

**Bulk set of Micro-Tom mutants**

Mutant strains without visible phenotype are not registered to TOMATOMA. Such mutants may represent abnormal phenotypes under specific conditions. For acquisition of such potential mutants, screening mutant seeds is a valuable approach. For example, salt-tolerant mutants can be found when screening for surviving plants under high salinity conditions. We collected 117–471 M2 and M3M3 families of EMS mutants and 1,354 M3 families of γ-ray mutants to make bulk sets (Table 2). The bulk sets include seeds derived from plants without any visible phenotypic alterations. In TOMATOMA, the list of bulk sets available for seed requests is found under ‘EMS and gamma irradiation-induced mutant lines’ in the ‘Resource information’ on the top page or under ‘Strains’ in the ‘Browse & Search’ page (Fig. 2). For orders, we estimate 100 seeds in a 1 ml volume and pack a corresponding volume for the number of seeds calculated by the number of lines × 4 (Table 2).

![Image gallery](https://academic.oup.com/pcp/article-abstract/57/1/e11/2470174)

**Table 2** Bulk set of Micro-Tom mutants

<table>
<thead>
<tr>
<th>Bulk set name</th>
<th>No. of lines</th>
<th>Seed volume for distribution (ml)</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMS bulk set A</td>
<td>240</td>
<td>12</td>
<td>TOMJPE 6301–7050, 7051–7150</td>
</tr>
<tr>
<td>EMS bulk set B</td>
<td>241</td>
<td>12</td>
<td>TOMJPE 7151–8190</td>
</tr>
<tr>
<td>EMS bulk set C</td>
<td>117</td>
<td>6</td>
<td>TOMJPE 1001–1002</td>
</tr>
<tr>
<td>EMS bulk set D</td>
<td>406</td>
<td>20</td>
<td>TOMJPE 0002–4936</td>
</tr>
<tr>
<td>EMS bulk set E</td>
<td>131</td>
<td>6.5</td>
<td>TOMJPE 8191–8993</td>
</tr>
<tr>
<td>EMS bulk set F</td>
<td>456</td>
<td>23</td>
<td>TOMJPE 2–1838</td>
</tr>
<tr>
<td>EMS bulk set G</td>
<td>471</td>
<td>24</td>
<td>TOMJPW 0002–1862</td>
</tr>
<tr>
<td>EMS bulk set H</td>
<td>398</td>
<td>20</td>
<td>TOMJPW 1224–1972</td>
</tr>
<tr>
<td>γ-Irradiation bulk set</td>
<td>1,354</td>
<td>60</td>
<td>TOMJPW</td>
</tr>
</tbody>
</table>
Metabolite content of mutant fruits

One of the most important traits in tomato is fruit development. Therefore, information concerning fruit metabolite content is useful for both basic and applied tomato research in addition to visible phenotypes. To improve our mutant resource, we collected information concerning metabolites in mutant fruits, namely Brix values and carotenoid contents. We investigated $M_3$ and $M_2M_3$ plants from 171 lines that showed visible phenotypes in the $M_2$ or $M_2M_3$ generation. Wild-type Micro-Tom (MT-J) had a Brix value of 4.5 ± 0.5% and a carotenoid content of $16.4 \pm 1.9 \mu g \cdot g^{-1} FW$. Of 466 samples from 171 mutant lines, the Brix value ranged between 3.2% and 11.6%, and the carotenoid contents ranged between 6.9 and 37.3 $\mu g \cdot g^{-1} FW$ (Fig. 4). The fresh fruit weight was between 0.3 and 7.1 g. The fresh fruit weight was not correlated with the Brix value or the carotenoid content (Supplementary Fig. S2).

This metabolite information concerning the fruits of Micro-Tom mutants was entered into TOMATOMA. Users can see the whole list of metabolite information for the mutant fruits, which contains the number of fruits per measurement, the fruit fresh weight, the Brix value, carotenoid content and harvest season (Fig. 5A). Detailed information concerning each mutant line can be shown by clicking the strain ID (Fig. 5B). Clicking a title in green font on the headings allows for the sorting of data by column. All the metabolic data are available for downloading as a text file in tab-delimited format from the ‘Download’ page. Histograms created from the entire mutant data set are displayed by clicking the graph icon shown in the fruit fresh weight, Brix value or carotenoid content headings (Fig. 5C). The number of histogram bars can be changed by entering a number in the text box at the upper left of the modal window; the strain list corresponding to each bar is shown by clicking any bar. Histograms can also be displayed from the ‘Strain Detail’ page for each strain. In this case, bars including the mutant line samples are indicated by a white arrowhead. For comparison, the average Micro-Tom values and the median of all samples are represented by a black arrowhead and black circle, respectively (Fig. 5D).

Users can search for mutants with specific metabolite data (Fig. 5E). The search page is linked at the top of the strain list on the metabolite info page (Fig. 5A) and the ‘Browse & Search’ page (Fig. 2B). Users can refine the data by shifting the slide bars corresponding to each value. For example, mutants with a fresh weight < 3.0 g and a Brix value > 7.0 will be listed in Fig. 5E.

Discussion

Micro-Tom as a model cultivar of tomato

We have developed a Micro-Tom-based mutant resource and provided information via the database TOMATOMA. Some other Micro-Tom databases are available; these include KaFTom and MiBASE, which contain full-length cDNA and EST sequence information, respectively (Yano et al. 2006, Aoki et al. 2010). Structural annotations for Micro-Tom and Heinz 1706 genomes are accessible via the TOMATOMICS genome browser (Kobayashi et al. 2014). A comparison of genome sequences between these cultivars revealed 1.23 million SNPs. Recently, Shirasawa et al. (2015) analyzed the genome sequences of several Micro-Tom lines from different institutes and revealed that there were SNPs between them. To avoid confusion, we named our Micro-Tom line MT-J; this is an abbreviation of Micro-Tom Japan. Micro-Tom (MT-J) should be used as a control plant when analyzing our mutants, as the mutant resources in TOMATOMA have been generated from MT-J.

Micro-Tom mutant resources

We have generated approximately 17,000 $M_3$ and $M_2M_3$ mutant families from Micro-Tom by EMS treatment and $\gamma$-ray irradiation. Other groups have also generated Micro-Tom mutants. In France, 8,000 Micro-Tom mutant families were generated by EMS treatment (Rothan and Causse 2007, Just et al. 2013). In Brazil, Perez and his colleagues created 2,800 mutants by EMS treatment (Rothan and Causse 2007, Just et al. 2013). In Brazil, Perez and his colleagues created 2,800 and 280 mutants by EMS treatment and $\gamma$-ray irradiation, respectively (Pino-Nunes et al. 2009). This group also created near-isogenic lines (NILs) in which natural genetic variation was introgressed into the Micro-Tom background (Carvalho et al. 2011). In Israel, approximately 9,000 strains were produced by EMS treatment and harvested in bulk (Meissner et al. 1997). Of these Micro-Tom mutants, our mutant collection in the NBRP tomato is the largest Micro-Tom resource in the world. Moreover, our resource is both a collection and an easily accessible database containing phenotypic and metabolite information named ‘TOMATOMA’. In addition, users can request mutant
Fig. 5 Metabolite information. (A) The entire list of metabolite information. (B) Metabolite information for each mutant was added to the bottom of the 'Strain Detail' page. (C) A histogram is displayed by clicking the graph icon in (A) or (E). Bars corresponding to median of all samples and average values for Micro-Tom are indicated by a black circle and a black arrowhead, respectively. (D) A histogram is displayed by clicking the graph icon on the 'Strain Detail' page (B). Bars representing the corresponding strain are indicated by white arrowheads. (E) Metabolite information search linked from the 'Browse & Search' page (Fig. 2B). Users can freely search for mutants with specific data by shifting the slide bars.
seeds via TOMATOMA. By August 2014, NBRP tomato had distributed approximately 1,000 lines of Micro-Tom mutant seeds and > 50 sets of bulk seeds to researchers in various countries. Our mutant resources are useful in both forward and reverse genetics approaches. Using forward genetics, novel mutants with specific traits such as pale colored petals, parthenocarpy and elongated fruits have been screened from the mutant collection and characterized (Ariizumi et al. 2014, Chuasaeeom et al. 2014, Shinozaki et al. 2015). The genes responsible have also been identified. We have also developed a platform for Target Induced Local Lesions IN Genomes (TILLING) using our Micro-Tom mutant collection generated by EMS treatment (Okabe et al. 2011, Okabe et al. 2013). Users can screen mutants for their gene of interest from 8,448 lines and expect to obtain approximately 10 mutant alleles per 1 kb of genome sequence.

Because tomato is used as a model for Solanaceae, Micro-Tom can be utilized to research the universality of function of orthologous genes in Solanaceae species (Sawai et al. 2014). We are also providing wild-type Micro-Tom and additional cultivars, including Moneymaker, Ailsa Craig and M82, as experimental strains. ILS between M82 and Solanum pennellii, which were provided by Dr. Zamar, are also available (Eshed and Zamir 1994). We have distributed > 1,100 sets of seeds from the experimental strains. Papers related to the NBRP tomato resource are registered to the resource research circulation (RRC) database (Yamazaki et al. 2010), which can be accessed from the ‘Reference feedback’ link on the TOMATOMA menu (Fig. 2A).

Metabolite information

Because tomato is used as a model plant for fruit development research, we provided Brix values and carotenoid content values for mutant fruits via TOMATOMA. The Brix value is an indicator of sweetness, and sweet tomato is one of the preferable traits for consumers. Conversely, carotenoids are essential components for human health. Carotenoids serve as fruit pigments and as antioxidants and precursors of vitamin A and many volatile flavor compounds (Liu et al. 2015). The metabolism content of mutant fruits is useful for breeding tomatoes with high Brix values and nutrient contents as well as understanding the mechanisms of fruit development. We now provide data for 466 samples from 171 mutant lines that were harvested in the summer of 2012; we will add more data from other seasons in the future. Furthermore, we are now collecting information concerning amino acid composition. Glutamate and aspartate are the major amino acid components in tomatoes, and the amino acid composition in tomato fruits is an important factor in taste. In combination with the Brix value data, our tomato mutant resource will assist in the development of delicious and original tasting tomatoes.

Materials and Methods

Plant material and growth conditions

The tomato (S. lycopersicum L.) dwarf cultivar Micro-Tom (MT-J, TOMIP100001) was used in this study. Wild-type and mutant seeds were sown in 5 × 5 cm cell trays filled with Jiffy-Mix (Sakata seed Co., Ltd.) in a greenhouse located at the University of Tsukuba. A nutrient solution (Hyponex; Hyponex Japan Co., Ltd.) was provided weekly at a concentration of 0.2% (before anthesis) or 0.5% (after anthesis). After flowering, self-fertilization was assisted by vibration with electric pollinators (Bunbun-Tasuke, TS-550; Takii & Co., Ltd.).

Production of double EMS-treated mutant strains

We performed four sets of second-round EMS treatments (EMS-W1–EMS-W4) (Table 1). For EMS-W1 and EMS-W2, bulked seeds were prepared by collecting four M3 seeds from 793 and 850 strains each. For EMS-W3 and EMS-W4, EMS bulk sets D and C were used (Table 2). It was roughly estimated that around 6–7 seeds of each strain were present in these bulk sets. EMS treatments were performed as described previously (Saito et al. 2011).

Preparation of mutant fruits for metabolic profiles

To collect metabolite information concerning mutant fruits, M3 or M3M3 plants whose parents showed visible phenotypes were grown and phenotypic reproducibility was confirmed. Each mutant family was categorized by phenotypes, and 15 mature red fruits (5 fruits in bulk × 3 sets) were collected after fruit setting. The first set of fruits from each plant was excluded from these bulk collections owing to a tendency towards abnormal fruit production. Seeds with gelatinous tissue were removed; fruits were then frozen in liquid nitrogen and stored at −80 °C.

Weight, Brix value and total carotenoid content measurements

The bulked fruits were weighed and ground to powder using a pestle and mortar. Brix values were measured with a Brix refractometer (Atago Co., Ltd.). To extract carotenoids, approximately 100 mg of frozen fruit tissue was suspended in 600 μl of 100% acetone and 200 μl of H2O were added, and the sample was mixed by vortexing and centrifuged. The supernatant was purified with a 0.45 μm filter (Millipore Co., Ltd.). The absorbance was measured at 470, 645, 663 and 750 nm. The total carotenoid content was calculated using equations described by Lichtenthaler and Wellburn (1983).

Supplementary data

Supplementary data are available at PCP online.

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Disclosures

The authors have no conflicts of interest to declare.
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