Genetic and physiological characterization of tomato cv. Micro-Tom

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Abstract

Based on its compact habit, Micro-Tom, a dwarf cultivar of tomato (Solanum lycopersicum L.), has been proposed as a preferred variety to carry out molecular research in tomato. This cultivar, however, is poorly characterized. It is shown here that Micro-Tom has mutations in the SELF-PRUNING (SP) and DWARF (D) genes. In addition to this, it is also shown that Micro-Tom harbours at least two independently segregating resistance loci to the plant pathogen Cladosporium fulvum. The presence of the self-pruning mutation in Micro-Tom, that generates a determinate phenotype, was confirmed by crossing and sequence analysis. It was also found that Micro-Tom has a mutation in the DWARF gene (d) that leads to mis-splicing and production of at least two shorter mRNAs. The d mutation is predicted to generate truncated DWARF protein. The d sequence defect co-segregates with dark-green and rugose leaves, characteristics of brassinosteroid biosynthesis mutants. Micro-Tom also carries at least another mutation producing internode length reduction that affects plant height but not active gibberellin (GA) levels, which were similar in dwarf and tall Micro-Tom × Severianin segregants. GAs and brassinosteroids act synergistically in Micro-Tom, and the response to GA depends on brassinosteroids because the elongation of internodes was at least six times higher when GA3 was applied simultaneously with brassinolide. A novel variety, Micro-0 that is fully susceptible to C. fulvum, has been generated from the cross of Cf0 × Micro-Tom. This line represents a valuable resource for future analysis of Cf resistance genes through breeding or transformation.

Key words: Brassinosteroids, Cladosporium fulvum, Dwarf, gibberellins, Micro-Tom, Self-Pruning, tomato.

Introduction

The tomato cultivar Micro-Tom was produced for ornamental purposes by crossing Florida Basket and Ohio 4013-3 cultivars, and displays a very dwarf phenotype with small and red ripened fruits (Scott and Harbaugh, 1989). Its small size, rapid growth, and easy transformation has led to its proposal as a convenient model system for research on the regulation of berry fruit development (Meissner et al., 1997; Eyal and Levy, 2002). It has been suggested, based on its pedigree, that the phenotype of Micro-Tom is the result of two major recessive mutations: dwarf (d) and miniature (mnt) (Meissner et al., 1997). It has also been reported that allelism tests support that Micro-Tom carries a mutation in gene D (Lima et al., 2004). The determinate phenotype of Micro-Tom suggests that it has also a mutation in the SELF-PRUNING (SP) gene (Pnueli et al., 1998). However, there is no genetic or molecular evidence in the literature supporting those conclusions.

SP belongs to the CETS family of regulatory genes encoding 23 kDa modulator proteins that determine the potential for continuous growth of the shoot apical meristem (Pnueli et al., 2001). SP controls the processes in which vegetative and reproductive shoots alternate in the sympodial
together, were applied in 10 μl per plant of 10% ethanolic solution containing 0.1% Tween-20 to the youngest expanding leaves of Micro-Tom. Paclobutrazol (Duchefa, Haarlem, The Netherlands), an inhibitor of GA biosynthesis, was applied as 10−7 M aqueous solution to the pot. BL was also added in 5 ml of 10−8 M aqueous solution to germinating seeds grown in Petri dishes over three layers of filter paper.

Plant inoculation

Inoculation of plants with C. fulvum was performed as described by Dickinson et al. (1993) and involved controls of susceptible Cf0 tomatoes (lacking any Cf resistance genes) and the resistant Cf5 line (containing the Cf-5 resistance gene). Cladosporium fulvum race 4GUS, a race that expresses the uidA reporter gene (Oliver et al., 1993), was used for the experiments. β-Glucuronidase (β-GUS) reporter enzyme activity was determined according to Jefferson et al. (1986).

Nucleic acid isolation and cDNA synthesis

Total RNA was isolated from homogenized, frozen tissues using the TRIzol (Invitrogen, Paisley, UK) method. cDNA was synthesized from total RNA using the First-Strand cDNA Synthesis Kit (Amersham Biosciences).

Genomic DNA was isolated from young leaves according to Dellaporta et al. (1983) or Thomas et al. (1994).

PCR amplification and cloning of PCR products

Oligonucleotides based on the published sequences of SP (accession no. U841440) and D (accession no. U54770) genes were used for PCR amplification. The pairs 5′-ATGGCTTCTCAAAAATGTGTGAAC-3′ (sense) and 5′-TCAACGCTTCTAGGGGACGT-3′ (antisense) were used as primers to amplify by RT-PCR the entire coding region of SP, and 5′-ATGGCCTTCTTCTAATTCTTCTCATCC-3′ (sense) and 5′-TTAGTGAGCTGAAACTCTAATCCGTA-3′ (antisense) to amplify the coding region of D in Micro-Tom. The same primers were used to isolate genomic clones of D. The thermocycling conditions used were: 94°C 2 min+40×(94°C 1 min+55°C 1.5 min+72°C 3 min)+72°C 10 min. PCR of genomic sequences of different dwarf (d) alleles was carried out using primers DW30 (5′-AGTCTATATAACGAGGACAC-3′, sense) and DW31 (5′-TGATCTTCAAAATCTCTCTC-3′, antisense).

Oligonucleotide primers were used to amplify cDNA and characterize the presence of D and d transcripts after separating the amplified bands by 5% PAGE [A=5′-GGGTATATCATTCCT-GTACCCATGGGAGATGGAG-3′ (sense), and B=5′-TTAGTGAGCTGAAACTCTAATCCGTA-3′ (antisense)] or by 4% agarose gel electrophoresis [C=5′-GGAACCTTGTTAGCAAGAAATTTCTCACTTT-AC-3′ (antisense)] or by 4% agarose gel electrophoresis [C=5′-GGAACCTTGTTAGCAAGAAATTTCTCACTTT-AC-3′ (antisense)] or by 4% agarose gel electrophoresis [C=5′-GGAACCTTGTTAGCAAGAAATTTCTCACTTT-AC-3′ (antisense)] or by 4% agarose gel electrophoresis [C=5′-GGAACCTTGTTAGCAAGAAATTTCTCACTTT-AC-3′ (antisense)] or by 4% agarose gel electrophoresis [C=5′-GGAACCTTGTTAGCAAGAAATTTCTCACTTT-AC-3′ (antisense)]. The thermocycling conditions were: 94°C 5 min+15×(94°C 45 s/62°C 45 s/72°C 30 s)+20×(94°C 45 s/62°C 45 s/72°C 7 min. cDNA and genomic clones of GAI of Micro-Tom were isolated following amplification with the primers 5′-ATGAAGAGAGATCGAGATCGAG-3′ (sense) and 5′-CATCTTCTCTGTTCTCTC-3′ (antisense) (accession no. AY269087).

PCR amplification products were cloned in p-GEM-T Easy Vector Systems (Promega, Madison, WI, USA) (when separated by agarose gel electrophoresis) or in pTZ57R (Fermentas, Burlington, Ontario, Canada) (when separated by PAGE).

Polyacrylamide gel electrophoresis

Non-denaturing (in TAE buffer, at 250 V) 5% PAGE was used to separate amplified PCR products to characterize D and d transcripts. The DNA from the gel was extracted as described in Sambrook et al. (1989) before cloning.
**DNA sequence analysis**

DNA sequencing was carried out using internal primers on PCR products that had been purified using a Qiagen PCR column (Qiagen, Chatsworth, CA, USA). Sequencing reactions were performed using cycle sequencing dye terminator kits from Applied Biosystems (ABI, La Jolla, CA, USA) and carried out according to the manufacturer’s instructions. Sequencing reactions were analysed using an ABI 373A or an ABI 1337 sequencing system. Contiguous sequences were generated using Macintosh-based ABI software Autoassembler™.

**Quantification of gibberellins**

A mixture of developing leaves from five plants (three leaves per plant) was used in each replicate for GA quantification. Frozen material (5 g fresh weight aliquots) was used for extraction, purification and quantification of GAs by GC–SIM essentially as described before (Fos et al., 2000). [2H]GA1, [2H]GA4, [2H]GA19, [2H]GA20, [2H]GA29, and [2H]GA44 (purchased from Professor L. Mander, Australian National University, Canberra) were used as internal standards. The extracts were passed, after QAE-Sephadex A-25 column chromatography, through SAX columns (BondElut SS-SAX, 500 mg; Varian-Scharlau) before C18-BondElut cartridge purification and reverse-phase HPLC fractionation.

**Results**

**Micro-Tom has a mutation in the gene SP**

Micro-Tom has a very short stature at the time of flowering as a result of very short internodes and a clear determinate phenotype (see Fig. 1A for comparison with Ailsa Craig, an indeterminate cultivar, and UC-82 and Rutgers, determinate cultivars). To confirm that the determinate phenotype was due to an alteration in the SP gene, oligonucleotide primers spanning the entire coding sequence were used to isolate several (seven) independent cDNA clones of SP by RT–PCR. Sequencing of these clones showed that, compared with the sequence of indeterminate cultivars (see accession no. U841410), they had a T instead of a C at position 227 of the coding sequence. This mutation, found previously in the determinate line M82 of tomato, produces the change of the proline in position 76 to a leucine (Pnueli et al., 1998). The analysis of four independent cDNA clones of Severianin, another determinate cultivar used later in this work for crossing with Micro-Tom, showed that it also had the same mutation in the SP gene.

**Role of brassinosteroids on the vegetative phenotype of Micro-Tom**

Plants of Micro-Tom are dwarf and of bushy appearance (Fig. 1A) and their leaves are small, with deformed leaflets, rugose, and of deep green colour compared with diverse wild-type cultivars (Fig. 1B). Those phenotypic characteristics are similar to those described for BR-deficient mutants (Altmann, 1998).

Seedlings of Micro-Tom germinated and grown in Petri dishes in the dark for 5 d showed a weak photomorphogenic phenotype (disappearance of hook and cotyledon opening) (data not presented) when compared with UC-82 (a tall determinate cultivar probably bearing a wild-type D allele) seedlings. Interestingly, cotyledons of 5-d-old Micro-Tom seedlings grown in the light for the last 3 d showed more expansion than UC-82 seedlings (Fig. 2A), suggesting that light-induced photomorphogenesis takes place earlier in Micro-Tom. The application of BL at 10⁻⁸ M reduced cotyledon expansion in Micro-Tom and stimulated hypocotyl length both in the light and in darkness, but had no visible effect on UC-82 growth (Fig. 2A, B).

BL applied to developing leaves was sufficient to increase the internode length of light-grown plants, an effect that was dose dependent (Fig. 2C, D). These results suggested that Micro-Tom carries a mutation in a gene of BR biosynthesis.

**Micro-Tom has a mutation in the gene D leading to mis-splicing**

The analysis of D transcript levels by RT–PCR, using oligonucleotides spanning the entire coding sequence as primers, showed that Micro-Tom also contained transcripts of the expected size (~1.4 kb) (data not presented). Therefore, in order to discern whether the D gene of Micro-Tom had a mutation that could alter its transcript...
sequence, genomic clones from Micro-Tom and wild-type (Moneymaker and Madrigal) cultivars (two from each cultivar) were isolated by PCR and sequenced. The three genes (DQ374444, DQ374445, and DQ374446) contained eight introns and nine exons (Fig. 3A), and had identical sequences except for the presence of a T in Micro-Tom at position 2468 (starting from the first coding ATG codon) instead of an A (mutation d). This base corresponded to the 3′-consensus splicing AG of intron 8 and should therefore affect splicing. To confirm this possibility, seven cDNA clones from two independent PCR-amplified bands from Micro-Tom were isolated and sequenced. All of these clones were eight bases shorter (d type I transcripts; DQ374442) than those corresponding to wild type (U54770 and DQ374447) (Fig. 3B). This means that splicing occurred using the AG pair of bases located eight bases downstream to the AG corresponding to intron 8 in the wild-type gene (Fig. 3A). This produced a premature stop leading to a truncated protein 24 amino acids shorter (d type I protein; Fig. 3C). Sequencing of genomic DNA of the d mutant tomato showed that the sequence was identical to that from Micro-Tom, indicating that the d allele had been bred into Micro-Tom.

The d mutation of Micro-Tom co-segregates with a brassinosteroid-deficient phenotype

To identify whether the d mutation in Micro-Tom is the cause of the phenotype described previously for diverse mutations in the D gene (Bishop et al., 1996, 1999), a cross between Micro-Tom and Severianin (a determinate wild-type cultivar) was carried out. The F1 plants were tall and with smooth leaves, similar to Severianin. Plants of the F2 generation were classified according to their leaf phenotype, and 217 plants out of a total of 287 were considered as smooth (Sm)-leaf plants, while 70 were dark-green, wrinkle-leaf plants. The 3:1 ratio (χ², P > 0.5) was expected for the recessive BR-related mutation responsible of the dark-green, rugose (Ru)-leaf phenotype.

The specific primers A and B (see Materials and methods), spanning a 329 bp region of D, were used to amplify cDNA from Severianin and Micro-Tom, and the amplified products were analysed by non-denaturing
As expected, a single band of 329 bp (confirmed by sequencing) was found in Severianin, whereas in the case of Micro-Tom two bands were detected. The major band from Micro-Tom (band a) contained only products of 321 bp (corresponding to the \(d\) type I transcripts of Micro-Tom described before; Fig. 3B) while the minor band (band b) contained products of 315 bp. These corresponded to transcripts 14 bp shorter (\(d\) type II transcripts; DQ374443) than those of \(D\) (Fig. 3B), extending from the AG located 14 bp further than the first splicing AG of intron 8 in \(D\) (Fig. 3A), and should produce a peptide 26 amino acids shorter (\(d\) type II protein) than that corresponding to \(D\) (Fig. 3C).

Twenty-three Sm-leaf and 23 Ru-leaf \(F_2\) plants from Severianin×Micro-Tom were analysed by RT–PCR for the presence of \(d\) alleles. Primers C and D, that amplified cDNA regions of smaller size, i.e. 145 bp from \(D\), and 137 and 131 bp from \(d\), were used. All the Sm-leaf plants had either one band of 145 bp (lane 3) (similar to Severianin, lane S) or an additional band of 137 bp (lanes 1 and 2; two bands larger than 145 bp of unknown origin were also amplified in this case). By contrast, all the Ru-leaf plants had only the 137 bp band (lanes 4–6) (similar to Micro-Tom; lane MT) (Fig. 4B). An additional band of >145 bp of unknown origin was also amplified in Micro-Tom and in all the Ru-leaf segregants (lanes 4–6). No band of 131 bp, corresponding to \(d\) type II transcripts, could be seen in any Ru-leaf plant nor in Micro-Tom, probably because of its low abundance. The co-segregation of the rugose phenotype with the \(d\) mutation indicates that the BR-deficient phenotype of Micro-Tom is the result of the \(d\) mutation.

**Dwarf segregants of the Micro-Tom×Severianin cross contain a mutation that does not affect active gibberellin content**

The population of \(F_2\) plants from the Micro-Tom×Severianin cross was also analysed for segregation of plant height by measuring the length of the first five internodes (starting from cotyledons). The mean height of the plant population with the Sm-leaf phenotype (Fig. 5A) was
9.3±0.2 cm (n=217), and that of those with the Ru-leaf phenotype (Fig. 5B) was 6.1±0.2 cm (n=70). This indicates that the d mutation reduces plant height, as expected. The height pattern distribution of plants with Sm-leaf phenotype (Fig. 5B) was 6.1±0.2 cm (n=70). This indicates that the d mutation reduces plant height, as expected. The height pattern distribution of plants with Sm-leaf phenotype (Fig. 5A) suggests that in addition to d, Micro-Tom carries at least another mutation also producing internode length reduction. This mutation might correspond to the mnt mutation previously suggested to be present in Micro-Tom (Meissner et al., 1997). The absence of bimodality in Fig. 5B may be due to the strong dwarfing effect of d masking the additional dwarfing effect of putative mnt in plants with Ru-leaf phenotype.

To determine whether the putative mnt mutation reduces internode length by altering GA metabolism, representative short (Sh) and tall (Ta) plants within the Sm- and the Ru-leaf populations of the Severianin×Micro-Tom cross (Fig. 5) were selected for quantification of GA levels. The short and tall phenotypes of the selected plants were maintained in the next generation (Table 2). Determination of GAs of the early C-13-hydroxylation pathway, the main GA biosynthesis pathway in tomato (Fos et al., 2000), showed that there was no significant content difference of GA1 (the active GA) and their immediate precursors (GA44, GA19, and GA20) between Sh and Ta plants in Ru- and Sm-leaf phenotypic background (Table 1). Interestingly, the content of GA29 and GA8, catabolic products of GA20 and GA1, respectively, were 2–4-fold higher in Ru than in Sm plants (Table 1), raising the possibility that BR deficiency may enhance the GA deactivation pathways.

All the segregant plants from the Micro-Tom×Severianin cross elongated in response to GA3, and their stem length was reduced by paclobutrazol, an effect that could be reversed by GA3 application (Table 2). This indicates that internode elongation of plants from both Ru-leaf and Sm-leaf phenotype populations depends on GAs. Interestingly, although there was no significant difference in GA content between segregants, GA3-treated Sh

![](https://academic.oup.com/jxb/article-abstract/57/9/2037/622929/fig5)

**Fig. 5.** F2 height segregation of Micro-Tom×Severianin. (A) Distribution of the F2 population of smooth-leaf plants. (B) Distribution of the F2 population of rugose-leaf plants. Height was determined as length of the first five internodes. * Selected dwarf plants; +, selected tall plants.

<table>
<thead>
<tr>
<th>GA44</th>
<th>GA19</th>
<th>GA20</th>
<th>GA29</th>
<th>GA1</th>
<th>GA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rugose-short</td>
<td>0.22±0.09</td>
<td>0.07±0.04</td>
<td>0.33±0.08</td>
<td>4.2±0.9</td>
<td>0.38±0.08</td>
</tr>
<tr>
<td>Rugose-tall</td>
<td>0.24±0.05</td>
<td>0.08±0.02</td>
<td>0.82±0.08</td>
<td>5.3</td>
<td>0.26±0.05</td>
</tr>
<tr>
<td>Smooth-short</td>
<td>0.32±0.10</td>
<td>0.16±0.04</td>
<td>0.45±0.16</td>
<td>1.7±0.8</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td>Smooth-tall</td>
<td>0.28±0.04</td>
<td>0.10±0.05</td>
<td>0.55±0.16</td>
<td>1.5±0.2</td>
<td>0.24±0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control</th>
<th>GA3</th>
<th>PCB</th>
<th>GA3 + PCB</th>
<th>ΔGA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rugose-leaves, short</td>
<td>19±1.5</td>
<td>38±3</td>
<td>9±0.5</td>
<td>32±3</td>
</tr>
<tr>
<td>Rugose-leaves, tall</td>
<td>34±2</td>
<td>72±2</td>
<td>11±1</td>
<td>59±3</td>
</tr>
<tr>
<td>Smooth-leaves, short</td>
<td>20±1</td>
<td>64±3</td>
<td>9±0.5</td>
<td>66±4</td>
</tr>
<tr>
<td>Smooth-leaves, tall</td>
<td>46±3</td>
<td>101</td>
<td>16±1</td>
<td>79±5</td>
</tr>
</tbody>
</table>

Table 1. Gibberellin content (ng g⁻¹ fresh weight) of selected short and tall plants within the rugose- and smooth-leaf plant populations from the F2 Micro-Tom×Severianin cross

Values are means of 3–6 biological replicates ±SE (except GA29 in Rugose-Tall which is the mean of only two replicates). See Fig. 5 for the origin of selected rugose- and smooth-leaf lines.
plants did not attain the height of GA3-treated Ta plants, further supporting the idea that the short phenotype produced by \( d \) and putative \( mnt \) was not due to GA deficiency. While the response of RuSh and RuTa plants to GA3 was similar (100% and 112%, respectively), that of SmSh plants was about twice that of SmTa plants (220% and 120%, respectively) (Table 2). This suggested the existence of an interaction between BR and the GA response.

**Effect of interaction of gibberellins and brassinosteroids on vegetative growth**

The application of GA3 stimulated internode elongation of Micro-Tom plants (Fig. 6A, upper row, and B). Paclobutrazol, an inhibitor of GA biosynthesis, reduced the height of the plants, and the application of GA3 fully counteracted the effect of 10\(^{-8}\) M, only partially that of 10\(^{-7}\) M, and had no effect on 10\(^{-6}\) M paclobutrazol (Fig. 6A). Paclobutrazol reduced the size of the leaf (estimated by the product of its length by maximum width), an effect that could be reversed (even that of 10\(^{-7}\) M) by GA3 (Fig. 6B). Also, the application of GA3 made the margin of the leaves less serrate (Fig. 6C), as described before for wild-type tomato (Hay et al., 2002). Interestingly, leaf size was apparently more sensitive than internode length to GA content modification. These results show that Micro-Tom architecture depends partially on GA and that this cultivar responds to GA application.

The comparison of GA content in shoots of Micro-Tom and the tall cultivars UC-82, Ailsa Craig, and Rutgers did not show significant differences for GA1, GA19, GA20, and GA29 (Table 3). The level of GA8 (a GA1 inactivation product) was about four times higher in Micro-Tom (Table 3), in agreement with the data obtained in selected segregating lines (Table 1). However, Micro-Tom presented a response to GA3 about three times lower than UC-82 (Table 4), suggesting that the response to GA may be reduced in that cultivar. Cloning and sequencing of the gene \( GAI \) of tomato, that encodes a negative regulator of GA signal transduction (Bassel et al., 2004), however, showed that Micro-Tom had no mutation in this gene (data not presented).

The stem of Micro-Tom elongated in response to separate GA3 and BL applications, although less to the former (\( ~\)10 mm with 1 \( \mu \)g of GA3) than to the latter (\( ~\)18 mm

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>GA3 (ng plant(^{-1}) per application)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-Tom</td>
<td>11.4±0.3  11.0±0.7  13.0±0.8  17.0±1.5  23.8±0.8</td>
</tr>
<tr>
<td>UC-82</td>
<td>20.8±1.3  20.5±1.6  28.4±2.3  35.2±1.4  59.0±4.1</td>
</tr>
<tr>
<td>UC-82/Micro-Tom</td>
<td>–          4.8      2.6      3.1</td>
</tr>
</tbody>
</table>

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**Table 3. Gibberellin content (ng g\(^{-1}\) fresh weight) of Micro-Tom, Ailsa Craig, Rutgers, and UC-82 cultivars**

<table>
<thead>
<tr>
<th>GA19</th>
<th>GA20</th>
<th>GA29</th>
<th>GA1</th>
<th>GA8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-Tom</td>
<td>0.7±0.1</td>
<td>1.1±0.3</td>
<td>1.6±1.0</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>Ailsa Craig</td>
<td>0.4±0.05</td>
<td>0.9±0.05</td>
<td>n.a.(^a)</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Rutgers</td>
<td>0.6±0.3</td>
<td>1.2±0.9</td>
<td>1.4±0.8</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>UC-82</td>
<td>0.7±0.3</td>
<td>0.9±0.2</td>
<td>0.8±0.3</td>
<td>0.2±0.05</td>
</tr>
</tbody>
</table>

\(^a\) n.a., Not analysed because the internal standard could not be detected.

**Table 4. Stem elongation of Micro-Tom and UC-82 in response to GA3 application**

Values (mm) are lengths of the three first internodes and are means of 12 replicates ± SE. GA3 was applied three times every 3 d in 10\(^{-6}\)M of 10\(^{6}\) methanol solution to the uppermost growing leaf, starting from the first true leaf. UC-82/Micro-Tom means the ratio of elongations versus dose 0.
with 10 ng of BL) (Fig. 7A, B). However, the elongation response to all doses of GA$_3$ (0.01–1 µg) increased with the simultaneous application of BL, an effect that was proportional to the amount of BL (between 0.1 and 10 ng). For instance, the response to 1 µg of GA$_3$ was ~6-fold higher in the presence of 10 ng of BL (Fig. 5B). This indicates that the response of tomato to GA depends on BR. Conversely, the response to BL was also enhanced by GA$_3$, although not as dramatically (e.g. the response to 10 ng of BL was enhanced about three times by the simultaneous application of 1 µg of GA$_3$) (Fig. 7B).

**Resistance against tomato leaf mould**

Micro-Tom was analysed for resistance to *C. fulvum* and, 14 d post-inoculation with *C. fulvum* race 4GUS, heavy sporulation was macroscopically visible on the lower leaf surface of Cf0 (susceptible) plants, while no sporulation was observed on either Cf5 (resistant) or Micro-Tom plants (Table 5). For a more accurate determination of the resistance present in Micro-Tom, each inoculated plant was assessed for GUS reporter enzyme activity. It has been shown previously that GUS enzyme activity correlates with the level of *C. fulvum* biomass accumulation and therefore indicates the degree of susceptibility (Oliver *et al.*, 1993). Inoculated Micro-Tom contained average GUS levels intermediate to those of Cf0 and Cf5, ~5-fold higher than resistant Cf5 plants, but 10-fold lower than susceptible Cf0 plants (data not shown). These data suggest that the gene(s) conferring resistance in Micro-Tom provide a weaker degree of protection than Cf-5.

To determine the number of resistance genes in Micro-Tom, crosses were made to the susceptible Cf0 line to generate an F$_2$ population segregating for resistance. Due to the relatively weak nature of the resistance in Micro-Tom, an initial screen was performed where young F$_2$ plants were inoculated as previously described and scored for resistance by determination of GUS reporter enzyme activity. Only a single fully susceptible plant was recovered from 20 F$_2$ plants, suggesting that resistance is the consequence of at least two independently segregating genes (Table 5). A further 492 F$_2$ plants were visually screened for susceptibility, and the resulting ratio supports the initial data where GUS reporter activity was assessed. DNA marker analysis on Micro-Tom indicates that neither of these resistance genes is Cf-2, Cf-4, Cf-5, or Cf-9 (data not shown). Further screening of Cf0×Micro-Tom F$_2$ plants allowed the identification of a fully susceptible plant, almost as dwarf as Micro-Tom, leading to the development of a line designated Micro-0. Micro-0 represents a valuable resource for future analysis of *Cf* resistance genes through breeding or transformation.

**Table 5. Cf resistance genes in Micro-Tom**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>R</th>
<th>S</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cf0</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cf5</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Micro-Tom</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cf0×MT F$_2$</td>
<td>19</td>
<td>1</td>
<td>0.053</td>
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</tbody>
</table>
Discussion

Micro-Tom displays a determinate phenotype, and it has now been shown to carry a base change in the SP gene identical to that found previously to produce a determinate phenotype in tomato. The same sp mutation was also found in Severianin, a tall Russian determinate cultivar similar to Rutgers and UC-82, source of the facultative parthenocarpic gene pat-2 (Fos et al., 2000), used in crossing experiments with Micro-Tom in this work. Growth determination is a phenotypic characteristic incorporated in most of the tomato cultivars used at present. However, no other alleles of SP have been described previously. The fact that the same allele is present in three unrelated cultivars [Micro-Tom and Severianin (this work), and M82 (Pnueli et al., 1998)] suggests that the determinate phenotype was introduced following a unique mutation event.

The dwarf phenotype of Micro-Tom is certainly not due to the sp mutation, as seen in Fig. 1 by comparing Micro-Tom with UC-82 and Rutgers, all determinate cultivars. The phenotype of Micro-Tom (short internodes and small, rugose, and dark-green leaves) is similar to that of BR-deficient tomato mutants (Bishop et al., 1996, 1999), suggesting that this cultivar contains a deficiency of BR biosynthesis. It was found that Micro-Tom carries a mutation in the D gene, known to code for a BR-6-oxidase. The mutation (d) consists of a change of a base at the 3' AG consensus region of intron 8 that leads to mis-splicing and production of transcripts eight (the most abundant) and 14 bases shorter than wild type. These transcripts have premature stop codons that may produce peptides with higher response of the Ddx mutant to GA3 compared with wild-type plants described by Nadhzimov et al. (1988). This difference may be due to the severe phenotype of the d mutant and/or to the different method of GA3 application (Nadhzimov et al. applied GA3 when the plants were 3-weeks-old and measured the final plant height, rather than specific internodes). Interestingly, the GA content of Micro-Tom was similar to those of Ailsa Craig, UC-82, and Rutgers, all of them tall cultivars (Table 3). Although it is difficult to compare GA levels between cultivars with such different genetic backgrounds, the data are in agreement with the absence of significant differences of active GA levels between the Micro-Tom×Severianin tall and short segregants (Table 1), confirming that Micro-Tom is not a GA-deficient cultivar. It also supports the fact that BRs do not regulate GA biosynthesis in tomato. However, the higher content of GA29 and/or GA8 in Ru- versus Sm-leaf plants (Table 1) and in Micro-Tom versus Ailsa Craig and Rutgers cultivars (Table 3) suggests that BR may regulate GA inactivation. These results are in agreement with lkb and lka pea, BR-deficient and BR-response mutants, respectively, which do not have a reduction in GA1 but an increase in GA8 and/or GA29 content (Jager et al., 2005), indicating that in pea, BRs do not promote elongation by altering GA1 levels. This seems to be in contrast to Arabidopsis, where BR treatment increased transcript levels of a GA20-oxidase gene (Bouquin et al., 2001), suggesting that BR might have a direct and positive effect on GA levels (although no GA measurements were carried out). Therefore, the dwarf habit of Micro-Tom, in
addition to BR deficiency, might be due to an altered GA response. It is known that the DELLA proteins, encoded by GAI and GAI-like genes, are repressors of cell elongation that are inactivated by GAs (Olszewski et al., 2002; Sun and Gubler, 2004). In some mutants, their GA-insensitive dwarf phenotype is the result of constitutive activation of DELLA proteins, associated with higher levels of active GAs (Olszewski et al., 2002). However, this does not seem to be the case in Micro-Tom since, in addition to not presenting an accumulation of GAs (Table 3), it was found that the sequence of LeGAI (the only gene known to encode DELLA proteins in tomato; Bassel et al., 2004) was identical to that of the wild type. Interestingly, the response of internode elongation to GA₃ application was much higher (up to 6-fold) in the presence of BL. This effect was dose dependent (Fig. 7) and shows that there is an interaction between these two groups of hormones in vegetative growth of tomato. This conclusion is also supported by the higher response to GA₃ of SmSh (carrying D) than RuSh (carrying d in homozygous) segregating plants (Table 2). A synergistic effect between GA₃ and BL on hypocotyl elongation has also been found in wild-type Arabidopsis grown in the light (Tanaka et al., 2003) and in det-2 mutant Arabidopsis grown in the dark (D Alabadí, personal communication). Azpiroz et al. (1998) obtained indirect evidence from the phenotypic analysis of the dwf4 BR-deficient Arabidopsis mutant that a fully active BR pathway is necessary for a full response to GA, auxin, and darkness. In the BR-deficient mutant dwf1, there is higher expression of GAS4, a GA-regulated gene (Müssig et al., 2002). Also, the fact that BR restores wild-type sensitivity to auxin, abscisic acid, and GA₃ in axl1 seedlings (Ephritikhine et al., 1999) strongly demonstrates that three different signalling pathways controlling cell elongation are under the control of a fourth pathway involving BR. Interestingly, in rice, two XET genes are up-regulated by BRs and GAs, suggesting that BRs are essential for GA sensitivity (Uozu et al., 2000). Therefore, there is substantial evidence supporting that there is cross-talk between BR and GA signalling, and that the dwarf habit of Micro-Tom is also probably due to a reduced GA response as a result of BR deficiency. By contrast, in mung bean epicotyls, it was reported that the application of GA₃ (10⁻⁷ M) has an additive effect with low concentrations of BL (10⁻⁵–10⁻⁸ M) (Gregory and Mandava, 1981), suggesting that in this species the two hormones act independently at the cellular level.

Micro-Tom is resistant to C. fulvum. However, these data show that resistance does not correspond to the previously characterized Cf-2, Cf-4, Cf-5, or Cf-9 resistance genes (Jones et al., 1994; Dixon et al., 1996, 1998; Thomas et al., 1997). Indeed this is supported by the intermediate level of resistance observed in Micro-Tom when compared with the Cf5 resistant line. Crosses suggest that two independently segregating genes are present and that the amount of resistance that is conferred by each gene must be relatively weak. The effect of Cf resistance genes is known to be additive in a dose-dependent manner (Hammond-Kosack et al., 1994). The resistance genes present in Micro-Tom must also be relatively unlinked to the major genes controlling dwarfism, as a small susceptible line designated Micro-0 was easily isolated from a cross with Cf0 (a susceptible, normal height plant). Many Cf-like genes are known to exist in tomato (Krujkt et al., 2005), but the exact nature of these resistance genes in Micro-Tom remains elusive. The Micro-Tom and Micro-0 lines offer an extremely useful resource for studying disease resistance in mature plants.

In summary, the phenotype of Micro-Tom plants is due to at least three mutations. The characterization of two of them, sp (producing a determinate phenotype) and d (reducing internode length and producing smaller, rugose, and dark-green leaves as a result of reduced BR content), has been carried out in this work. In addition, Micro-Tom has another mutation (putative mnt), still uncharacterized and that does not affect GA metabolism but which is likely to be associated with GA signalling. In addition, an effect of a synergistic interaction between BR and GAs on vegetative growth has also been observed. These findings, and noting the presence of additional Cf resistance genes, highlights that some caution is required when obtaining data using this variety. However, in spite of these mutations altering the architecture of the plant so dramatically, pollinated ovaries develop normally, and unpollinated ovaries also grow in response to auxin and GA₃ application (data not presented), indicating that Micro-Tom remains a useful system to investigate fruit set and development.

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References


