Journal of Experimental Botany, Vol. 57, No. 9, pp. 2037–2047, 2006
doi:10.1093/jxb/erj154 Advance Access publication 10 May, 2006
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany

RESEARCH PAPER

Genetic and physiological characterization of tomato cv. Micro-Tom

Esmeralda Martí[1], Carmina Gisbert[1,*], Gerard J. Bishop[2], Mark S. Dixon[3] and José L. García-Martínez[1,†]

[1] *Instituto de Biología Molecular y Celular de Plantas (IBMCP), Universidad Politécnica de Valencia-CSIC, Avda de los Naranjos s/n, 46022-Valencia, Spain*

[2] *Division of Biology, Imperial College London, Wye Campus, Ashford, Kent TN25 5AH, UK*

[3] *School of Biological Sciences, University of Southampton, Southampton SO16 7PX, UK*

Received 13 September 2005; Accepted 6 February 2006

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 GA_3 was applied simultaneously with brassinolide. A novel variety, Micro-0 that is fully susceptible to *C. fulvum* and almost as dwarf as Micro-Tom, 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

* Present address: Centro de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Camino de Vera s/n, 460022 Valencia, Spain.
† To whom correspondence should be addressed. E-mail: jlgarcim@ibmcp.upv.es

Abbreviations: BL, brassinolide; BR, brassinosteroid; D, Dwarf; GA, gibberellin; GUS, β-glucuronidase; Ru, rugose; Sh, short; Sm, smooth; SP, Self-pruning; Ta, tall.

tomato shoot, and it has been shown that one base mutation that changes an amino acid in the protein produces a determinate phenotype in line M82 (Pnueli *et al.*, 1998).

The *DWARF* (*D*) gene was isolated by transposon tagging (Bishop *et al.*, 1996) and shown to encode a P450 protein catalysing the C-6 oxidation of 6-deoxocastasterone to castasterone in brassinosteroid (BR) biosynthesis (Bishop *et al.*, 1999). Genes homologous to tomato *D* showing C-6 oxidase activity have also been isolated from *Arabidopsis* (Shimada *et al.*, 2001) and rice (Hong *et al.*, 2002). In tomato, *D* is expressed in all the organs of the plant, mainly in vegetative and reproductive tissues undergoing expansion (Montoya *et al.*, 2005). The *d* mutation has been used for a long time to create dwarf tomato varieties, and different mutant alleles of *D* presenting different degrees of dwarfism, rugose, and dark-green leaf phenotype are known (Nadhzimov *et al.*, 1988; Bishop, 2003). The strong d^x allele of *D* responded more to gibberellin (GA) application (Nadhzimov *et al.*, 1988) than wild type, suggesting that the BR deficiency may affect the response to GAs. Varietal differences in sensitivity to GA independent of the *d* genotype have also been described (Plummer and Tomes, 1958).

Micro-Tom is reported as having resistance against *Fusarium* wilt race 1 and grey leafspot (Scott and Harbaugh, 1989). However, no other published data exist regarding the multitude of pathogens to which this cultivar can be susceptible. Here, resistance of Micro-Tom to tomato leaf mould (*Cladosporium fulvum*) was examined, and a fully susceptible line, almost as dwarf as Micro-Tom, has been isolated. It is demonstrated that Micro-Tom carries mutations in the *SP* and *D* genes. Additionally, evidence is presented supporting that Micro-Tom has at least a third mutation affecting plant height. The dwarf phenotype of Micro-Tom is thus a consequence of these mutations in addition to the lower response to GA, possibly due to the reduced BR content of this cultivar.

Materials and methods

Plant material and growth conditions

Plants of tomato (*Solanum lycopersicum* L.) cultivars of Micro-Tom (seeds provided originally by A Levy, Israel), Ailsa Craig, UC-82, Rutgers, Madrigal, and Severianin (Centro de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia, Spain) were used in the experiments. Micro-Tom was crossed with Severianin, and F_2 seeds were obtained for segregation analysis. Cultivars used in the sequencing of the *d* allele (LA1525 and GCR565) were obtained from the TGRC stock centre and John Maxon Smith, respectively.

The plants were grown in the greenhouse (22 °C day and 18 °C night) or in a growth cabinet (16 h light at 23 °C and 8 h dark at 20 °C), in a mixture of peat–vermiculite (50:50 v:v), using 120 or 600 ml pots for Micro-Tom, and 5.0 l pots for the other cultivars.

Application of plant growth substances

Brassinolide (BL; CIDtech Research Inc., Mississauga, Ontario, Canada) and GA_3 (Sigma, St Louis, MO, USA), separately or 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 4*GUS*, 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. U84140) and *D* (accession no. U54770) genes were used for PCR amplification. The pairs 5′-ATGGCTTCCAAAATGTGTGAACC-3′ (sense) and 5′-TCAACGCCTTCTAGCGGCAGT-3′ (antisense) were used as primers to amplify by RT–PCR the entire coding region of *SP*, and 5′-ATGGCCTTCTTCTTAATTTTTCTTTCATCC-3′ (sense) and 5′-TTAGTGAGCTGAAACTCTAATCCGTAGAC-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′-AGTCTATATAAACAGAGCACAC-3′, sense) and DW31 (5′-TGCATCCTACAAATCTCTTCTC-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′-GGGTATATCATTCCTAAAGGATGGAG-3′ (sense), and B=5′-TTAGTGAGCTGAAACTCTAATCCGTAGAC-3′ (antisense)] or by 4% agarose gel electrophoresis [C=5′-GGAACTTGGTGTAGCAGAAATTTCCACATTTC-3′ (sense), and D=5′-TTAGTGAGCTGAAACTCTAATCCGTAGAC-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 45 s/62 °C 45 s/72 °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′-CATCTCTCTCATTGTCTCTTCC-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). $[^2H]GA_1$, $[^2H]GA_8$, $[^2H]GA_{19}$, $[^2H]GA_{20}$, $[^2H]GA_{29}$, and $[^2H]GA_{44}$ (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 C_{18}-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. U84140), 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).

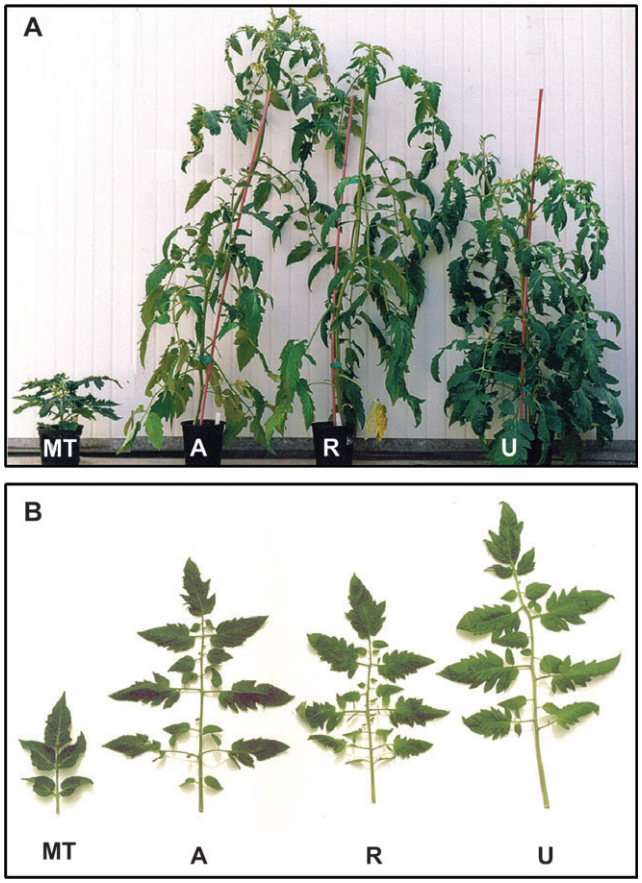

Fig. 1. Plants of Micro-Tom, Ailsa Craig, Rutgers, and UC-82. (A) Entire plants at the time of flowering (~2 months old). (B) Fifth leaf from the base. MT, Micro-Tom; A, Ailsa-Craig; R, Rutgers, U, UC-82.

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

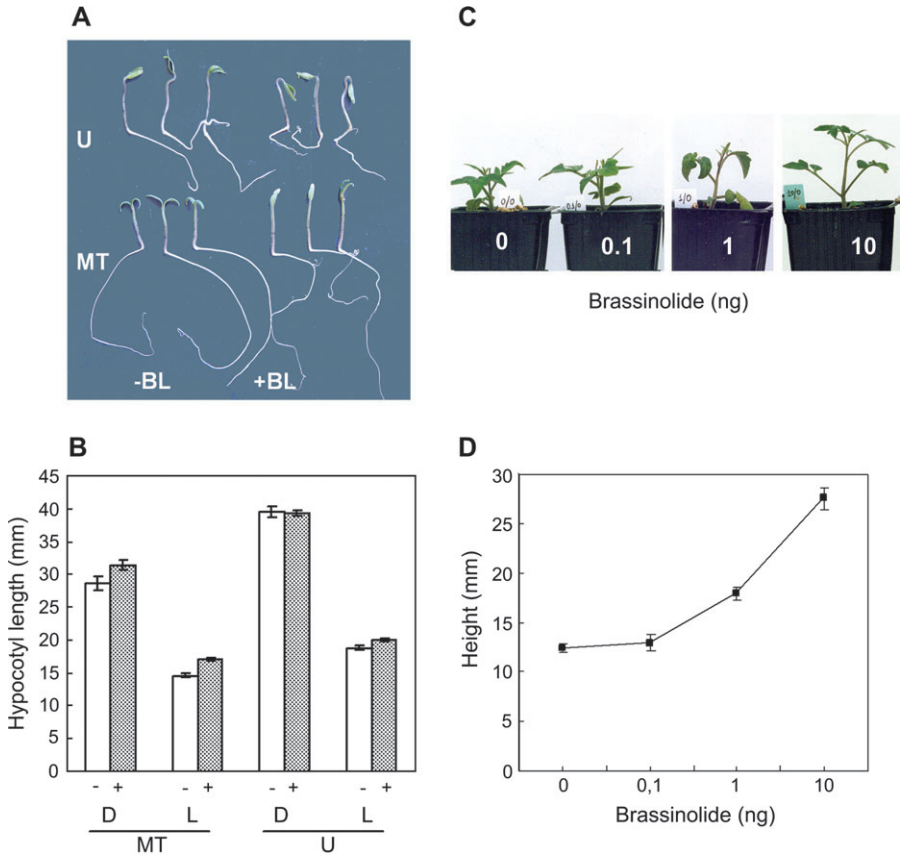

Fig. 2. Effect of brassinolide application on growth of Micro-Tom and UC-82. (A) Phenotypes of representative 5-d-old seedlings grown in the dark and then transferred to light conditions for an additional 3 d. (B) Hypocotyl length of seedlings grown under continous dark (D) or light (L) conditions in the absence (–BL) or the presence (+BL) of 10^{-8} M brassinolide. (C) Phenotypes of representative plants treated with different doses of brassinolide. (D) Length of the first three internodes of light-grown plants treated with different doses of brassinolide. Measurements are the average of 8–15 plants ±SE. MT, Micro-Tom; U, UC-82.

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 F_1 plants were tall and with smooth leaves, similar to Severianin. Plants of the F_2 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 (χ^2, 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

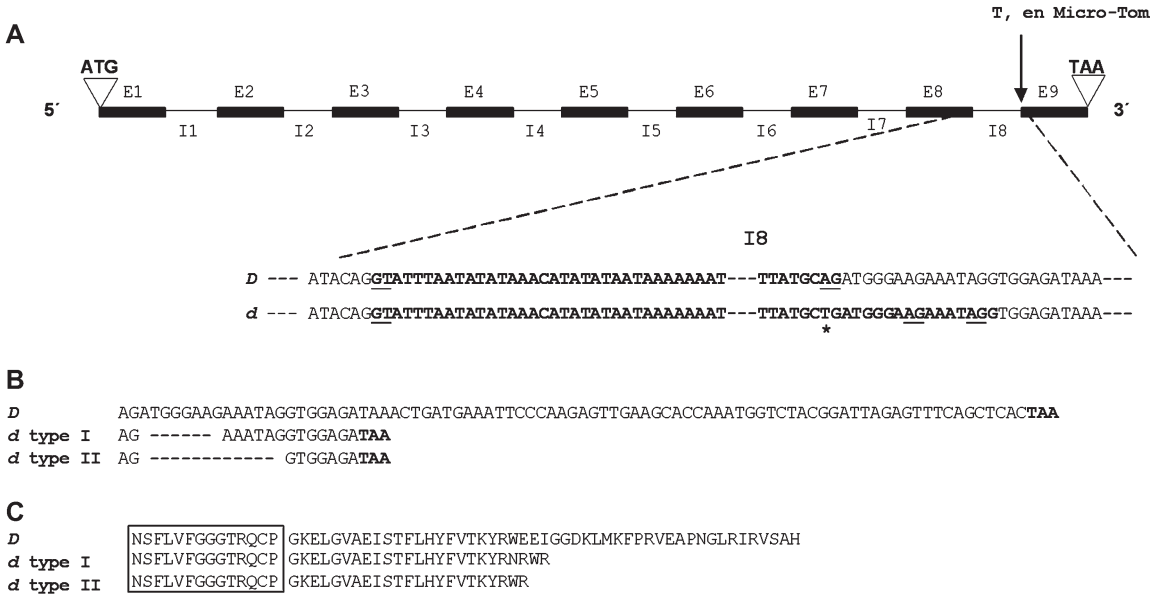

Fig. 3. Genomic and transcript sequences of the *D* gene of Micro-Tom and wild type. (A) Scheme of the genomic structure of *D*. The genomic sequences of I8 (bold letters) and near sequences of E8 and E9 of wild type and Micro-Tom are also given. Intron sequences involved in splicing are underlined. The asterisk marks the base change in *d*. (B) Comparison of *D* and *d* transcript sequences. *d* type I and *d* type II transcript sequences correspond to the two *d* splice variants. (C) Comparison of D and d translated amino acid sequences. The square indicates the iron-binding domain conserved in P450 proteins. Wild-type sequences correspond to those from Moneymaker and Madrigal cultivars.

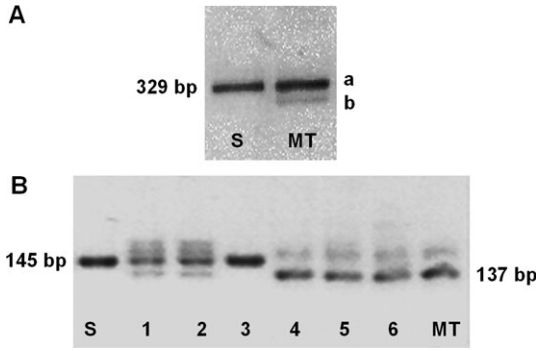

Fig. 4. Co-segregation of the brassinosteroid-deficient phenotype and the presence of two *d* alleles. (A) Separation of PCR products from Severianin (S, left) and Micro-Tom (MT, right) amplified with primers A and B (see Materials and methods) by non-denaturing PAGE. (B) Agarose electrophoresis of PCR products from representative smooth-leaf (lanes 1–3) and rugose-leaf (lanes 4–6) segregating plants amplified with primers C and D (see Materials and methods). S, Severianin; MT, Micro-Tom.

(Fig. 4A) 5% PAGE. 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. 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.

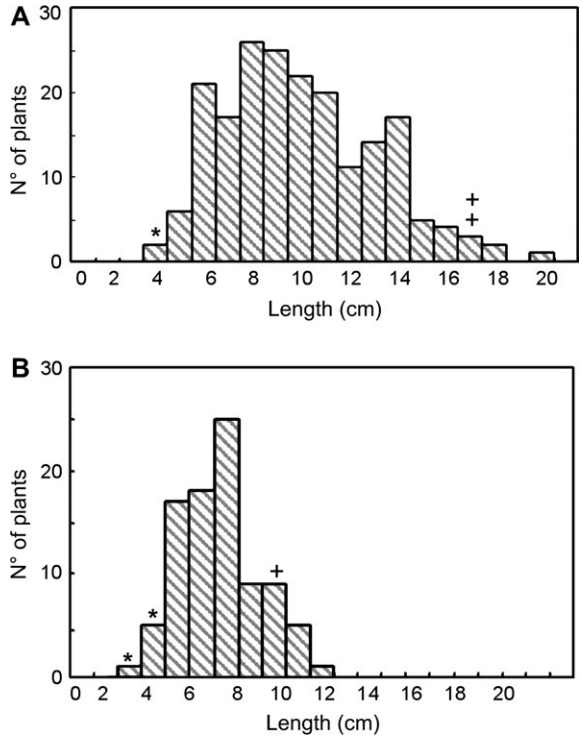

Fig. 5. F_2 height segregation of Micro-Tom×Severianin. (A) Distribution of the F_2 population of smooth-leaf plants. (B) Distribution of the F_2 population of rugose-leaf plants. Height was determined as length of the first five internodes. *, Selected dwarf plants; +, selected tall plants.

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 GA_1 (the active GA) and their immediate precursors (GA_{44}, GA_{19}, and GA_{20}) between Sh and Ta plants in Ru- and Sm-leaf phenotypic background (Table 1). Interestingly, the content of GA_{29} and GA_8, catabolic products of GA_{20} and GA_1, respectively, were 2–4-fold higher in Ru than in Sm plants (Table 1), raising the possibility that BR defficiency may enhance the GA deactivation pathways.

All the segregant plants from the Micro-Tom× Severianin cross elongated in response to GA_3, and their stem length was reduced by paclobutrazol, an effect that could be reversed by GA_3 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, GA_3-treated Sh

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 F_2 Micro-Tom×Severianin cross*

Values are means of 3–6 biological replicates ±SE (except GA_{29} in Rugose-Tall which is the mean of only two replicates). See Fig. 5 for the origin of selected rugose- and smooth-leaf lines.

	GA_{44}	GA_{19}	GA_{20}	GA_{29}	GA_1	GA_8
Rugose-short	0.22±0.09	0.07±0.04	0.33±0.08	4.2±0.9	0.38±0.08	5.0±1.2
Rugose-tall	0.24±0.05	0.08±0.02	0.82±0.08	5.3	0.26±0.05	4.1±1.3
Smooth-short	0.32±0.10	0.16±0.04	0.45±0.16	1.7±0.8	0.23±0.04	1.1±0.4
Smooth-tall	0.28±0.04	0.10±0.05	0.55±0.16	1.5±0.2	0.24±0.04	1.5±0.5

Table 2. *Response of selected Severianin×Micro-Tom F_2 lines to GA_3 and paclobutrazol application*

GA_3 (1 μg in 10 μl of solution) was applied every 3 d to the youngest leaf, and paclobutrazol (10^{-6} M solution) to the roots as water solution. The values (mm) are the lengths of the first three internodes and represent means of 12–24 plants from two similar lines ±SE. ΔGA_3 = (GA_3–control)/control×100. See Fig. 5 for selected rugose- and smooth-leaf lines.

	Control	GA_3	PCB	GA_3 + PCB	ΔGA_3
Rugose-leaf plants, short	19±1.5	38±3	9±0.5	32±3	100
Rugose-leaf plants, tall	34±2	72±2	11±1	59±3	112
Smooth-leaf plants, short	20±1	64±3	9±0.5	66±4	220
Smooth-leaf plants, tall	46±3	101	16±1	79±5	120

plants did not attain the height of GA_3-treated Ta plants, further supporting the idea that the short phenotype produced by *d* and putative *mnt* was not due to GA defficiency. While the response of RuSh and RuTa plants to GA_3 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 GA_3 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 GA_3 fully counteracted

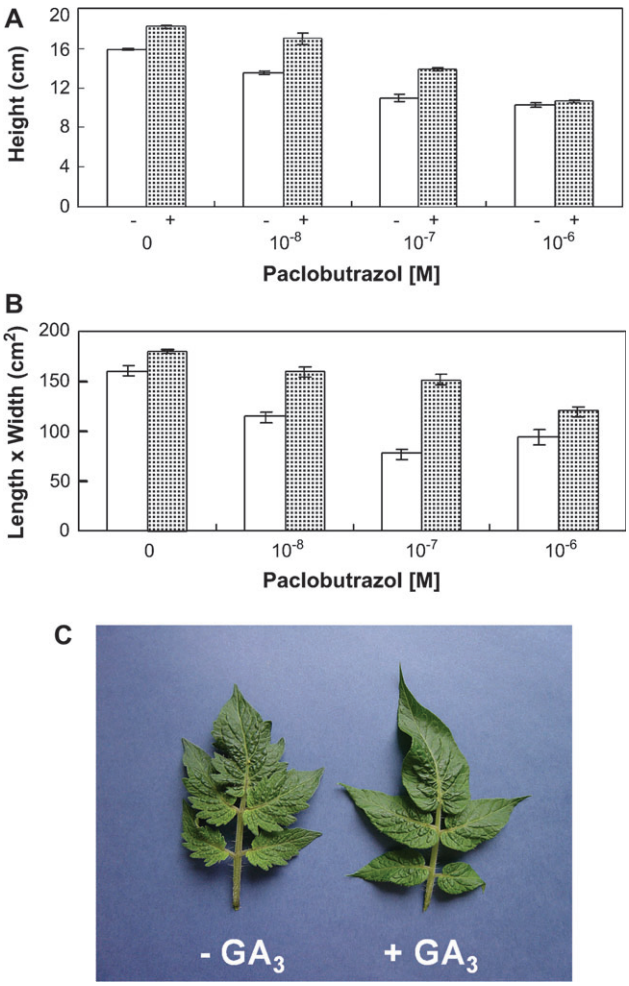

Fig. 6. Effect of GA_3 and paclobutrazol application on Micro-Tom. (A) Inhibition of plant height (first five internodes) by different doses of paclobutrazol and its reversal by GA_3. Data are means of 12 plants ±SE. (B) Inhibition of leaf size (estimated as the product of maximal length by maximal width) at position five from the base by paclobutrazaol and its reversal by GA_3. Data are means of 12 leaves ±SE. (C) Effect of GA_3 application on leaf margin phenotype. –, no GA_3; +, 1 μg of GA_3.

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 GA_3 (Fig. 6B). Also, the application of GA_3 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 GA_1, GA_{19}, GA_{20}, and GA_{29} (Table 3). The level of GA_8 (a GA_1 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 GA_3 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 GA_3 and BL applications, although less to the former (~10 mm with 1 μg of GA_3) than to the latter (~18 mm

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

Values are means of three biological replicates ±SE.

	GA_{19}	GA_{20}	GA_{29}	GA_1	GA_8
Micro-Tom	0.7±0.1	1.1±0.3	1.6±1.0	0.5±0.3	4.6±0.8
Ailsa Craig	0.4±0.05	0.9±0.05	n.a.[a]	0.2±0.1	1.2±0.4
Rutgers	0.6±0.3	1.2±0.9	1.4±0.8	0.2±0.1	0.6±0.5
UC-82	0.7±0.3	0.9±0.2	0.8±0.3	0.2±0.05	n.a.

[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 GA_3 application*

Values (mm) are lengths of the three first internodes and are means of 12 replicates ±SE. GA_3 was applied three times every 3 d in 10 μl of 10% 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.

Cultivar	GA_3 (ng $plant^{-1}$ per application)				
	0	0.2	2	20	200
Micro-Tom	11.4±0.3	11.0±0.7	13.0±0.8	17.0±1.5	23.8±0.8
UC-82	20.8±1.3	20.5±1.6	28.4±2.3	35.2±1.4	59.0±4.1
UC-82/Micro-Tom	–	–	4.8	2.6	3.1

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).

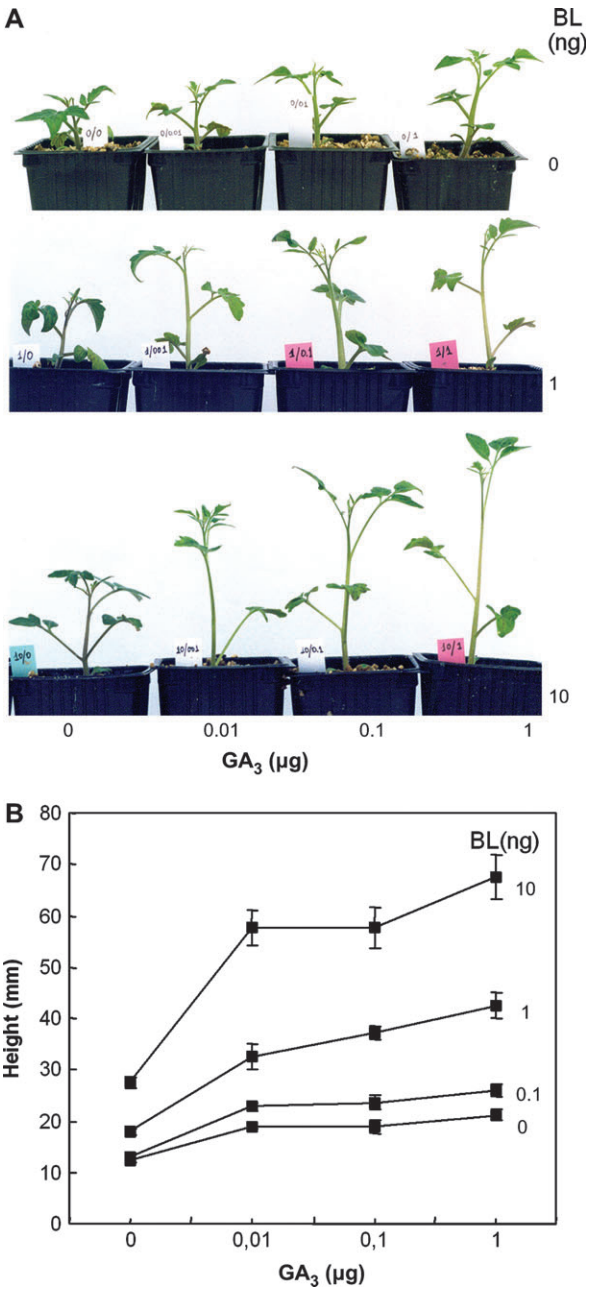

Fig. 7. Effect of separated and combined application of brassinolide and GA_3. (A) Phenotypes of representative plants for the different treatments. (B) Length of the first three internodes. Measurements are means of eight plants ±SE.

Resistance against tomato leaf mould

Micro-Tom was analysed for resistance to *C. fulvum* and, 14 d post-inoculation with *C. fulvum* race 4*GUS*, 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*

R denotes resistance, S denotes susceptibility, + indicates that all plants have this phenotype. $P = 0.05$ for a χ^2 with one degree of freedom = 3.84. Susceptibility assessed by determination of the GUS reporter enzyme activity.

Genotype	R	S	χ^2
Cf0	–	+	–
Cf5	+	–	–
Micro-Tom	+	–	–
Cf0×MT F_2	19	1	0.053

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 defficiency 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 23 and 26 fewer amino acids, respectively. It is likely, however, that, since the three domains conserved in P450 proteins (Bishop *et al.*, 1996) are not affected, reduced enzyme function and only a relatively weak phenotype is generated. Several lines of evidence strongly support the fact that Micro-Tom is a BR-deficient mutant: (i) Micro-Tom but not wild-type hypocotyls respond to low concentrations of BL; (ii) adult Micro-Tom plants present the characteristic BR-deficient phenotype; (iii) the characteristics of BR deficiency co-segregate with the presence of *d* in the F_2 population of a Micro-Tom×Severianin cross; and (iv) *d* is a known allele of the d^x mutation that has been shown to be BR deficient (Bishop *et al*., 1996, 1999). Additionally, Micro-Tom seedlings in the dark present a slight photomorphogenic phenotype and de-etiolate more rapidly than wild-type seedlings upon white light irradiation. The role of BR on photomorphogenesis repression, however, is the subject of current strong controversy (Jager *et al.*, 2005).

When Micro-Tom (dwarf) and Severianin (tall), both of them determinate cultivars, were crossed, the plants with BR-deficient phenotype (bearing rugose and dark-green leaves) were a quarter of the F_2 population (3:1 segregation), as expected for a recessive mutation. The mean height of Ru- (BR-deficient) leaf (6.1 ± 0.2 cm) plants was significantly lower than that of Sm- (non-BR-deficient) leaf plants (9.3 ± 0.2 cm), in agreement with the dwarfing effect of BR deficiency linked to *d*. The height distribution of the Sm-leaf plant population (Fig. 5A), however, suggested the presence of at least another mutation (putative *mnt*) affecting height. Selected Sh lines from the Ru- and Sm-leaf populations, purportedly carrying this mutation, elongated in response to GA_3 application. However, the comparison of Sh and Ta plant levels of endogenous GAs (Table 1) and the response to GA_3 (Table 2) allow us to conclude that the putative *mnt* mutation does not reduce stem length by altering the content of active GA. Although it is not possible to decide yet about the nature of this mutation, it seems to be different from the three previously described mutations in genes of GA metabolism in tomato (*gib-1*, *-2*, and *-3*) (Bensen and Zeevaart, 1990).

The height of Micro-Tom was reduced even further by paclobutrazol (an inhibitor of GA biosynthesis), and this effect was restored by GA_3 application (Fig. 6). This indicates that internode elongation of Micro-Tom depends on GAs, as also shown with F_2 segregants of the Micro-Tom×Severin cross (Table 2). However, the response of Micro-Tom to GA_3 was several fold lower than the response of UC-82 (Table 4). This is in contrast to the higher response of the d^x mutant to GA_3 compared with wild-type plants described by Nadhzimov *et al.* (1988). This difference may be due to the severe phenotype of the d^x mutation and/or to the different method of GA_3 application (Nadhzimov *et al.* applied GA_3 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 GA_{29} and/or GA_8 in Ru- versus Sm-leaf plants (Table 1) and in Micro-Tom versus Ailsa Craig and Rugers 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 GA_1 but an increase in GA_8 and/or GA_{29} content (Jager *et al.*, 2005), indicating that in pea, BRs do not promote elongation by altering GA_1 levels. This seems to be in contrast to *Arabidopsis*, where BR treatment increased transcript levels of a GA_{20}-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 GA_3 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 GA_3 of SmSh (carrying *D*) than RuSh (carrying *d* in homozygous) segregating plants (Table 2). A synergistic effect between GA_3 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 *GASA4*, a GA-regulated gene (Müssig *et al.*, 2002). Also, the fact that BR restores wild-type sensitivity to auxin, abscisic acid, and GA_3 in *sax1* 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 (Uozo *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 GA_3 (10^{-7} M) has an additive effect with low concentrations of BL (10^{-9}–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 (Kruijt *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 GA_3 application (data not presented), indicating that Micro-Tom remains a useful system to investigate fruit set and development.

Acknowledgements

The work was supported by the Spanish Ministry of Science and Technology (grant BIO2003-00151 to JLG-M) and by the Consellería de Agricultura de la Generalitat Valenciana (fellowship to EM). We thank Professor Avraham A Levy for providing seeds of Micro-Tom, Professor F Nuez for seeds of different tomato cultivars, Ms Teresa Sabater for help with GA analyses, and Drs Isabel López-Díaz and David Alabadí for critical review of the manuscript. GJB thanks J Jones and Y Kamiya for support.

References

Altmann T. 1998. A tale of dwarfs and drugs: brassinosteroids to the rescue. *Trends in Genetics* **14,** 490–495.

Azpiroz R, Wu Y, LoCascio JC, Feldmann KA. 1998. An Arabidopsis brassinosteroid-dependent mutant is blocked in cell elongation. *The Plant Cell* **10,** 219–230.

Bassel GW, Zielinska E, Mullen RT, Bewley JD. 2004. Down-regulation of *DELLA* genes is not essential for germination of tomato, soybean, and arabidopsis seeds. *Plant Physiology* **136,** 2782–2789.

Bensen RJ, Zeevaart JAD. 1990. Comparison of *ent*-kaurene synthetase A and B activities in cell-free extracts from young tomato fruits of wild-type and *gib-1*, *gib-2*, and *gib-3* tomato plants. *Jounal of Plant Growth Regulation* **9,** 237–242.

Bishop GJ. 2003. Brassinosteroid mutants of crops. *Journal of Plant Growth Regulation* **22,** 325–335

Bishop GJ, Harrison K, Jones JDG. 1996. The tomato *Dwarf* gene isolated by heterologous transposon tagging encodes the first member of a new cytochrome P450 family. *The Plant Cell* **8,** 959–969.

Bishop GJ, Nomura T, Yokota T, Harrison K, Noguchi T, Fujioka S, Takatsuto S, Jones JDG, Kamiya Y. 1999. The tomato *DWARF* enzyme catalyses C-6 oxidation in brassinosteroid biosynthesis. *Proceedings of the National Academy of Sciences, USA* **96,** 1761–1766.

Bouquin T, Meier C, Foster R, Nielsen ME, Mundy J. 2001. Control of specific gene expression by gibberellin and brassinosteroid. *Plant Physiology* **127,** 450–458.

Dellaporta SL, Wood J, Hicks JB. 1983. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter* **1,** 19–23.

Dickinson MJ, Jones DA, Jones JDG. 1993. Close linkage between the *Cf-2/Cf-5* and *Mi* resistance loci in tomato. *Molecular Plant–Microbe Interaction* **6,** 341–347.

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG. 1996. The tomato *Cf-2* disease resistance gene comprises two functional genes encoding leucine-rich repeat proteins. *Cell* **84,** 451–459.

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG. 1998. The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *The Plant Cell* **10,** 1915–1925.

Ephritikhine G, Fellner M, Vannini C, Lapous D, Barbier-Brygoo H. 1999. The *sax1* dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, giberellins and ethylene and is partially rescued by exogenous brassinosteroid. *The Plant Journal* **18,** 303–314.

Eyal E, Levy AA. 2002. Tomato mutants as tools for functional genomics. *Current Opinion in Plant Biology* **5,** 112–117.

Fos M, Nuez F, García-Martínez JL. 2000. The gene *pat-2*, which induces natural parthenocarpy, alters the gibberellin content in unpollinated tomato ovaries. *Plant Physiology* **122,** 471–479.

Gregory LE, Mandava NB. 1981. The activity and interaction of brassinolide and gibberellic acid in mung bean epicotyls. *Physiologia Plantarum* **54,** 239–243.

Hammond-Kosack KE, Jones JDG. 1994. Incomplete dominance of tomato *Cf* genes for resistance to *Cladosporium fulvum*. *Molecular Plant-Microbe Interaction* **7,** 58–70.

Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M. 2002. The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. *Current Biology* **12,** 1557–1565.

Hong Z, Ueguchi-Tanaka M, Shimizu-Sato S, *et al.* 2002. Loss-of-function of a rice brassinosteroid biosynthetic enzyme, C-6 oxidase, prevents the organized arrangement and polar elongation of cells in the leaves and stem. *The Plant Journal* **32,** 495–508.

Jager CE, Symons GM, Ross JJ, Smith JJ, Reid JB. 2005. The brassinosteroid growth response in pea is not mediated by changes in gibberellin content. *Planta* **221,** 141–148.

Jefferson RA, Burgess SM, Hirsch D. 1986. β-Glucuronidase from *Escherichia coli* as a gene fusion marker. *Proceedings of the National Academy of Sciences, USA* **83,** 8447–8451.

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JDG. 1994. Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* **266,** 789–793.

Kruijt M, De Kock MJD, De Wit PJGM. 2005. Receptor-like proteins involved in plant disease resistance. *Molecular Plant Pathology* **6,** 85–97.

Lima JE, Carvalho RF, Neto AT, Figueira A, Peres LEP. 2004. Micro-MsK: a tomato genotype with miniature size, short life cycle, and improved *in vitro* shoot regeneration. *Plant Science* **167,** 753–757.

Meissner R, Jacobson Y, Melamed S, Levyatuv S, Shalev G, Ashri A, Elkind Y, Levy AA. 1997. A new model system for tomato genetics. *The Plant Journal* **12,** 1465–1472.

Montoya T, Nomura T, Yokota T, Farrar K, Harrison K, Jones JGD, Kaneta T, Kamiya Y, Szekeres M, Bishop GJ. 2005. Patterns of *Dwarf* expression and brassinosteroid accumulation in tomato reveal the importance of brassinosteroid synthesis during fruit development. *The Plant Journal* **42,** 262–269.

Müssig C, Fisher S, Altmann T. 2002. Brassinosteroid-regulated gene expression. *Plant Physiology* **129,** 1241–1251.

Nadhzimov UK, Jupe SC, Jones MG, Scott IM. 1988. Growth and gibberellin relations of the extreme dwarf d^x tomato mutant. *Physiologia Plantarum* **73,** 252–256.

Oliver RP, Farman ML, Jones JDG, Hammond-Kosack KE. 1993. Use of fungal transformants expressing beta-glucuronidase activity to detect infection and measure hyphal biomass in infected-plant tissues. *Molecular Plant-Microbe Interaction* **6,** 521–525.

Olszewski N, Sun T-P, Gubler F. 2002. Gibberellin signaling: biosynthesis, catabolism, and response pathways. *The Plant Cell (Supplement)*, S61–S80.

Plummer TH, Tomes ML. 1958. Effects of indoleacetic acid and giberellic acid on normal and dwarf tomatoes. *Botanical Gazette* **119,** 197–200.

Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Alvarez J, Ganal M, Zamir D, Lifschitz E. 1998. The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. *Development* **125,** 1979–1989.

Pnueli L, Gufinger T, Hareven D, Ben-Naim O, Ron N, Adir N, Lifschitz E. 2001. Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. *The Plant Cell* **13,** 2687–2702.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning. A laboratory manual*, 2nd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Scott JW, Harbaugh BK. 1989. Micro-Tom. A miniature dwarf tomato. *Florida Agricultural Experimental Station* **Circular S-370,** 1–6.

Shimada Y, Fujioka S, Miyauchi N, Kushiro M, Takatsuto S, Nomura T, Yokota T, Kamiya, Bishop GJ, Yoshida S. 2001. Brassinosteroid-6-oxidases from Arabidopsis and tomato catalyze multiple C-6 oxidations in brassinosteroid biosynthesis. *Plant Physiology* **126,** 770–779.

Sun T-P, Gubler F. 2004. Molecular mechanism of gibberellin signaling in plants. *Annual Review of Plant Biology* **55,** 197–223.

Tanaka K, Nakamura Y, Asami T, Yoshida S, Matsuo T, Okamoto S. 2003. Physiological roles of brassinosteroids in early growth of *Arabidopsis*: brassinosteroids have a synergistic relationship with giberellin as well as auxin in light-grown hypocotyl elongation. *Journal of Plant Growth Regulation* **23,** 259–271.

Thomas CM, Jones DA, English JJ, Carroll BJ, Bennetzen JL, Harrison K, Burbidge A, Bishop GJ, Jones JDG. 1994. Analysis of the chromosomal distribution of transposon-carrying T-DNAs in tomato using the inverse polymerase chain-reaction. *Molecular and General Genetics* **242,** 573–585.

Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JDG. 1997. Characterisation of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. *The Plant Cell* **9,** 2209–2224.

Uozo S, Tanaka-Ueguchi M, Kitano H, Hattori K, Matsuoka M. 2000. Characterization of *XET*-related genes of rice. *Plant Physiology* **122,** 853–860.