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Brassinosteroids, microtubules and cell elongation in *Arabidopsis thaliana*. I. Molecular, cellular and physiological characterization of the *Arabidopsis bul1* mutant, defective in the Δ^7-sterol-C5-desaturation step leading to brassinosteroid biosynthesis

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Abstract. Although cell elongation is a basic function of plant morphogenesis, many of the molecular events involved in this process are still unknown. In this work an extremely dwarf mutant, originally named *bul*, was used to study one of the main processes of plant development, cell elongation. Genetic analyses revealed that the *BUL* locus was linked to the nga172 marker on chromosome 3. Recently, after mapping the new *dwf7* mutation of *Arabidopsis*, which is allelic to *ste1*, it was reported that *dwf7* is also linked to the same marker. Sterol analyses of the *bul1-1* mutant indicated that *bul1-1* is defective in the Δ^7-sterol-C5-desaturation step leading to brassinosteroid biosynthesis. Considering these findings, we designated our *bul* mutant as *bul1-1*/*dwf7-3*/*ste1-4*. The *bul1-1* mutant was characterized by a very dwarf phenotype, with delayed development and reduced fertility. The mutant leaves had a dark-green colour, which was probably due to continuous stomatal closure. The *bul1-1* mutant showed a partially de-etiolated phenotype in the dark. Cellular characterization and rescue experiments with brassinosteroids demonstrated the involvement of the BUL1-1 protein in brassinosteroid-dependent plant growth processes.

Key words: *Arabidopsis* (brassinosteroids) – Brassinosteroid – Cell elongation – Δ^7-Sterol-C5-desaturase

Introduction

Cell growth and development produce an amazing diversity of forms and species, in both plant and animal kingdoms. There are approximately 285,000 different species of flowering plants, all of which share three simple processes at the cellular level: cell division, cell enlargement and cell differentiation. These processes give rise to the formation of different tissues and organs in a plant. Not only is the direction of cell division important in the formation of various organs, but the direction of cell enlargement is also critical. In most plant organs, such as stems, petioles, hypocotyls, and roots, enlargement occurs in a preferential direction, resulting in cell elongation soon after cell emergence from the meristem. Indeed, cell elongation is of major importance in determining plant growth and final plant form. Although there are some physiological data on cell enlargement, (e.g. cell enlargement is largely a matter of water uptake into an enlarging vacuole), cell elongation, leading to plant body pattern, is still poorly understood. Indeed, little is known about the molecular processes governing plant cell elongation and differentiation. A model for the molecular rearrangements occurring within the cell wall during cell elongation has also been proposed (McCann and Roberts 1994).

Genes and phytohormones act on cell shape (Davies 1991), but the mechanisms through which this occurs are largely unknown. Green (1994) underlined that genes do not directly control final shape, but control the production of shape, i.e. the slope of a cell or organ elongation. The signal transduction pathway that determines the elongation axis remains largely unknown. However, there is general agreement that the relationship between the soluble, isotropic factors (gene products and hormones) and the solid, anisotropic responsive three-dimensional structures, is mediated by the cytoskeleton, especially the microtubules (MTs) (Nick and Furuya 1992; Goddard et al. 1994).

To understand how plant morphogenesis, and in particular cell elongation, is controlled by genes, mutants provide a unique tool, and their study has gained increasing interest during recent years (Cove 1993). Recently, several mutants affected in cell elongation have been described for a number of plant species, especially *Arabidopsis* (Estelle and Somerville 1987; Torres-Ruiz and Jürgens 1994; Takahashi et al. 1995;

Abbreviations: BR = brassinosteroid; homoBL; 22(S),23(S)-homobrassinolide; MT = microtubule

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Traas et al. 1995; Desnos et al. 1996; Dubois et al. 1996; Leyser et al. 1996; Li et al. 1996; Kauschmann et al. 1996; Szekeres et al. 1996; Azpiroz et al. 1998; Choe et al. 1999a, b, 2000; Ephritikhine et al. 1999a,b). Despite this broad spectrum and the apparent diversity of such cell-elongation-affected mutants, physiological, cellular and molecular approaches have led to the discovery of a new important class of phytohormones regulating plant morphogenesis, the brassinosteroids (BRs). Among the variety of physiologically important traits influenced by BRs (for review, see Szekeres and Koncz 1998), their important role in the cell elongation process has been pointed out (Clouse 1996; Fujioka and Sakurai 1997; Sasse 1997). Although numerous data are emerging from isolation of new genes involved in BR metabolism, future progress is expected to give insight into the understanding of mechanisms by which BRs control basic functions, such as cell elongation and morphogenesis. These findings emphasize the necessity to isolate BR-deficient mutants, exhibiting a dwarf phenotype, characterization of which might allow us to understand the role of BR in the cell elongation process. We have established a T-DNA insertion library based on an *Agrobacterium*-mediated in vitro transformation protocol in *Arabidopsis* and looked for a dwarf phenotype in F2 plantlets (Sangwan et al. 1991, 1993; Vilcot 1996). One of the mutants identified carried a recessive nuclear mutation, which we named *boule* (*bul*). By characterizing this very dwarf mutant, the involvement of BR in cell elongation process will be even more noticeable. Genetic and sterol analyses, physiological, light and electron-microscopic studies of the *bul1-1* mutant, as well as its response to BRs, are reported here.

Materials and methods

Isolation of the mutant

Using four promoterless vectors: pPCVT-GUS, pPCV6NF-Hygr, pPCV621 and pPCVNF-Lux (Walden et al. 1991), kindly provided by Csaba Koncz (MPI, Cologne, Germany), and employing the *Agrobacterium*-mediated in vitro transformation technique of *Arabidopsis thaliana* (L.) Heynh. (ecotype C24), several hundred T-DNA insertion lines were generated (Sangwan et al. 1991, 1993). After selfing, the S1 seeds were aseptically cultured on a germinating medium under long-day conditions (16 h light, 50–100 μmol m^{-2} s^{-1}) at 21 ± 1 °C (day) and 18 ± 1 °C (night), in the greenhouse. Several morphological dwarf mutants were observed; one of them, which we named *boule* (*bul*; obtained with the vector pPCVT-β-glucuronidase), displayed a severe dwarf phenotype. In the greenhouse, it normally died 1–2 months after reaching a height of 1 cm, producing a few seeds only after hand-pollination. Twenty-five heterozygous plants from the same batch (T-DNA lines) were grown to flower and, after selfing, most of the F2 population segregated to give *bul* mutant plants. Plants from these progenies were used throughout this study.

Mapping at the BUL locus

CAPS (*C*leaved *A*mplified *P*olymorphic *S*equences) and SSLP (*S*imple *S*equence *L*ength *P*olymorphism) mapping were carried out as described by Konieczny and Ausubel (1993) and Bell and Ecker (1994). The *bul* mutant, background C24, was crossed with the wild-type Columbia ecotype. From each of the crosses, F2 individual *bul* plants were harvested and DNA was isolated as in Dellaporta et al. (1983). The CAPS primers were purchased from Research Genetics (Huntsville, Ala., USA), and the SSLP primers were obtained from Eurogentec Bel S.A. (Angers, France).

Allelism tests

Allelism tests between the *bul* mutant and other dwarf mutants, such as *det2*, *cpd*/*cbb3*/*dwf3*, *dim*/*dwf1*/*cbb1*, *cbb2*/*bri1* and *dwf4* (Takahashi et al. 1995; Clouse et al. 1996; Kauschmann et al. 1996; Szekeres et al. 1996; Fujioka et al. 1997; Azpiroz et al. 1998), and including *dwf7* (Choe et al. 1999a) and *ste1* (Gachotte et al. 1995) were performed through crosses of heterozygous *bul* plants with heterozygous or homozygous plants of each other mutant line. The *Arabidopsis* dwarf mutants were obtained from the Nottingham *Arabidopsis* Stock Centre, UK or kindly provided by K.A. Feldmann, University of Arizona, USA.

In vitro culture and phytohormone treatments

Wild-type and mutant plants were grown on a basal germination medium (MS/2) comprising Murashige and Skoog (1962) macro and micro elements and vitamins (half strength), sucrose 15 g/l (pH 5.8) and Difco-bacto agar 7 g/l. The cultures were kept at 21 ± 1 °C/18 ± 1 °C (day/night temperature) under long-day conditions (16 h light, 50–100 μmol m^{-2} s^{-1}). For BR treatment, brassinolide [homoBL, 22(S),23(S)-homobrassinolide] was filter-sterilized and added to the autoclaved medium. The seeds were germinated on the MS/2 medium and transferred, at the cotyledon stage, to medium supplemented with various concentrations of homoBL (0.1–1 μM) as indicated in tables and figures. The plantlets were also further cultured for 4–6 weeks under standard conditions before analysis. For dark-growth cultures, Petri dishes containing plated seeds were wrapped in three layers of aluminium foil and were grown at 22 °C in a growth chamber. All the chemicals used were obtained from Sigma.

Sterol analysis

Lipids from 30 to 300 mg of ground lyophilized material were extracted at 70 °C in dichloromethane:methanol (2:1, v/v). The dried residue was saponified with 6% (w/v) KOH in methanol at 90 °C for 1 h to release the sterol moiety of steryl esters. Sterols were then extracted with 3 volumes of *n*-hexane, and an acetylation reaction was performed on the dried residue for 1 h at 60 °C in toluene with a mixture of pyridine:acetic anhydride (1:1, v/v). Steryl-acetates were resolved by TLC using pre-coated silica plates (60F254, Merck, Darmstadt, Germany), with one run of dichloromethane as a single band at $R_f = 0.5$. Purified steryl-acetates were separated and identified using a gas chromatograph (model 8300; Varian, Les Ulis, France) with a flame-ionization detector and a glass capillary column (wall coated, open and tubular; 30 m long, 0.25 mm internal diameter; coated with DB1; J & W Scientific, Folsom, Calif., USA) using H_2 as a carrier gas (2 ml/min). The temperature program included a fast increase from 60 °C to 230 °C (30 °C/min) and a slow increase from 230 °C to 280 °C (2 °C/min). Data from the detector were monitored with a computer program (Varian Star 4.51). Sterol structures were confirmed by GC-MS (model MD 800 gas chromatograph; Fisons Instruments, Beverly, Mass., USA) equipped with a glass capillary column (WCOT coated with DB5; J & W Scientific) on the basis of reported data (Rahier and Benveniste 1989).

Histological analyses

For quick studies, small pieces (e.g. roots) were mounted in phosphate buffer and directly observed on a light microscope under dark-field illumination. For precise histological studies, wild-type and mutant explants were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 h, then washed three times in cacodylate buffer. After dehydration in a graded ethanol series, the samples were embedded in LR White resin (Polysciences, Warrington, Pa., USA) as previously described (Sangwan et al. 1992). For light microscopy, semi-thin sections (1–5 μm thick), cut with a Leica microtome, were stained using the periodic acid-Schiff's (PAS) reaction for detection of insoluble polysaccharides and naphthol blue black as described by Sangwan et al. (1992). For scanning electron microscopy (SEM), tissues were fixed in 1% glutaraldehyde (0.1 M) cacodylate buffer, and dehydrated in a graded ethanol series. Dehydrated material was dried to critical point in liquid carbon dioxide, and gold-coated specimens were mounted on a scanning electron microscope (Philips 505) at 20 kV acceleration.

Results

Isolation and description of the bul1-1 *mutant*

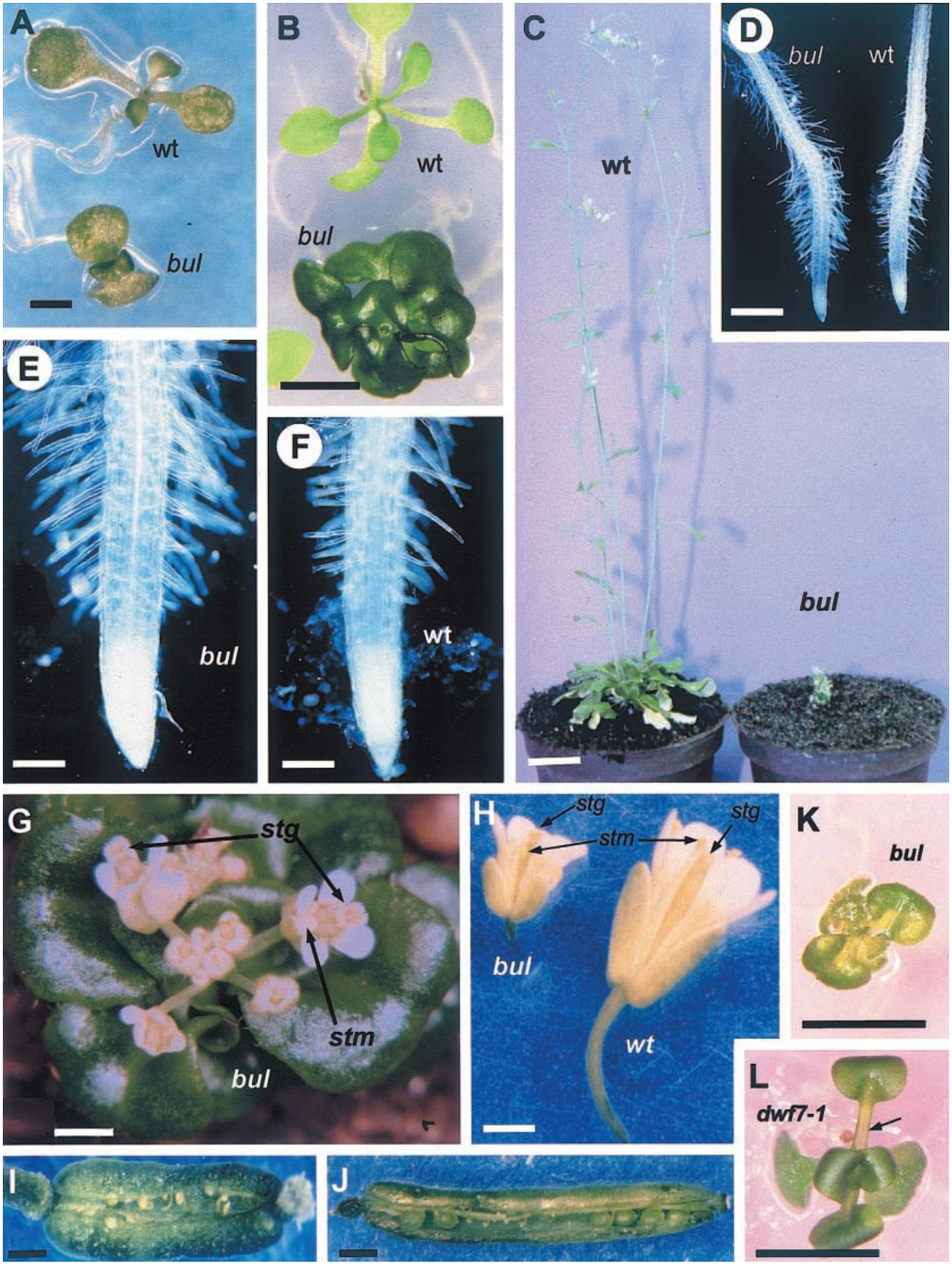
To identify mutants defective in cell elongation, a collection of T-DNA mutants (Sangwan et al. 1993) was screened. One mutant displayed an extreme dwarf phenotype and was named *boule* (*bul1-1*). In contrast to wild-type *Arabidopsis thaliana*, which produced an elongated petiole, stem and hypocotyl, *bul1-1* mutants showed an extreme dwarf phenotype with short stems, petioles and dark-green leaves, after 7 and 15 d of culture (Fig. 1A and 1B respectively). Table 1 summarizes the kinetics of organ elongation of wild-type and *bul1-1* seedlings. In this mutant, no decrease in apical dominance was observed. Both the wild-type and *bul1-1* seedlings began to form true leaves in a spiral phyllotaxis, with the first leaf primordium developed between the cotyledons (Fig. 1A). In a Petri dish, *bul1-1* mutants were easy to identify because they grew slowly, developed 6–8 leaves and reached a height of only 1 cm after a month in culture. The *bul1-1* plants were also transplanted to soil but died off without setting seed. Wild-type and *bul1-1* plants differed in phenotype from the start of seedling development to plant maturity. For example, *bul1-1* aerial organs, such as stems, leaf petioles and inflorescences, formed by cell elongation, were highly reduced and mainly compressed in their proximal-distal axis. This lack of elongation was clearly seen in the petiole that was hidden by the leaf blade, because the elongation zone was very reduced (Fig. 1B, see also Fig. 2C). In *bul1-1* plants, the leaf blade appeared to be directly attached to the stem. Roots of *bul1-1* plants were apparently normal, well ramified and covered with normal-looking root hairs. However, at an early stage of growth (7-day-old), *bul1-1* seedlings showed a reduced primary root. In comparison to wild-type plants, this reduction in *bul1-1* root length appeared less evident in older developmental stages (30-d-old plants; Table 1). Microscopic observations of roots of 5-d-old wild-type and mutant seedlings (Fig. 1D–F) showed that morphology, cell size and organization of *bul1-1* roots were not altered. The mutant root apex displayed the typical structure of those of the wild type, strictly organized into different zones (meristem, root cap and elongation zone) in which cells were arranged in regular files (data not shown). While subtle differences may have gone unnoticed, no major aberrations, especially concerning the arrangement of cell files (during cell elongation), were found in mutant roots. However, *bul1-1* roots did not display the characteristic distribution of root hairs, observed in wild-type plants (Fig. 1D). *bul1-1* mutants developed numerous root hairs which, in contrast to those of the wild type, were located in sub-apical as well as in upper zones.

After forming a rosette of 8–10 leaves, a wild-type plant bolted and developed a prominent inflorescence with its flowers and lateral buds well separated by internodes (Fig. 1C). The *bul1-1* plants mimicked these developmental events but, in contrast to wild-type plants, adult flowering mutants showed extremely short inflorescences (Fig. 1C,G) with small flowers, and their fertility was severely reduced. Despite their small size, *bul1-1* flowers contained all organs of wild-type flowers, but these organs did not elongate and were compressed in their proximal-distal axis (Fig. 1G,H). A scanning electron microscopic (SEM) study showed that *bul1-1* flowers had six stamens, positioned as in a wild-type flower, but filaments of *bul1-1* stamens did not elongate, causing the anthers to remain below the stigmatic hairs (Fig. 2A,B) and did not allow self-pollination. However, we were successful in obtaining seeds either after pollinating the *bul1-1* (female) plants with the wild-type pollen or by hand-pollinating the *bul1-1* plants. This clearly indicated that some *bul1-1* male gametophytes were functional. Siliques containing seeds also appeared constricted in their longitudinal axes (compare Fig. 1I with 1J). All these observations suggested that elongation in aerial organs was impaired by the *bul1-1* mutation.

Genetic and molecular analysis

The *bul1-1* mutation was isolated from the progeny of a single transformed plant (T1). To determine the genetic basis of the mutation, self-pollination of F2 plants was performed. The results showed that the *bul1-1* mutation affects a single recessive nuclear locus (Table 2). The segregation ratio indicated one insert of T-DNA which seemed to be unlinked to the *bul* mutation (Table 2, segregation type 1). Segregation types 2 and 3 indicated that in these lines the *BUL* locus did not co-segregate with a T-DNA insertion, because no mutant was observed, whereas these lines segregated for hygromycin resistance. The results for segregation types 4 and 5 indicated two inserts in the same plants but none of these inserts co-segregated with the *BUL* locus. The *bul* mutation was then purified by backcrossing to the original C24 followed by selfing and selection of *bul* offspring.

The location of the *BUL* locus on the genome was determined by CAPS (*C*leaved *A*mplified *P*olymorphic


A
wt
bul
B
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C
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D
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E
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F
wt
G
stg
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H
stg
stg
stm
bul
wt
K
bul
L
dwf7-1
I
J

Fig. 1A–L. Morphological description of the *bul1-1* mutant of *Arabidopsis*. **A** Wild-type (*wt*) and *bul1-1* plants after 7 d of culture. **B** Wild-type (*wt*) and *bul1-1* plants after 15 d of culture. **C** 30-d-old wild-type (*wt*) and *bul1-1* plants in the greenhouse. **D–F** Primary roots of 10-d-old wild-type (*wt*) and *bul1-1* plants, showing normal gravitropism and well developed root system with root hairs. **G** Short inflorescence of a 30-d-old *bul1-1* plant. *stm*, stamen; *stg*, stigmas. **H** Flowers of *bul1-1* and wild-type (*wt*) plants. In wild-type flowers, the stamens (stm) and the stigmas (stg) occur at the same height whereas the filaments of the stamens of *bul1-1* flowers are shorter than the styles. **I,J** Note the large difference in size between siliques of the mutant (**I**) and those of the wild type (**J**). **K,L** Comparison of 10-d-old *bul1-1* (**K**) and *dwf7-1* (**L**) mutants. Note the less drastic phenotype of *dwf7-1* (arrow). Bars = 200 μm (**E**,**F**), 500 μm (**D**), 1 mm (**G–J**), 5 mm (**A**,**B**,**K**,**L**) and 2 cm (**C**)

*S*equences; Konieczny and Ausubel 1993) and SSLP (*S*imple *S*equence *L*ength *P*olymorphism; Bell and Ecker 1994) marker analyses. DNA was isolated from individual F2 progeny (*bul*/*bul*) of the crosses between *BUL*/*bul* (background C24) and Columbia. The *bul1-1* phenotype was found to be linked to the SSLP marker nga172 on chromosome III (only one recombinant out of 125 mutant F2 plants examined). The map distance was determined by the Kosambi method, as described by Koornneef and Stam (1992), for four markers (0.41 ± 0.40 cM to nga172; 0.87 ± 0.86 cM to GAPC; 18.48 ± 3.4 cM to nga162 and 1.61 ± 0.93 cM to CA1). Very recently, a new *Arabidopsis* mutant, *dwarf7*, was reported by Choe et al. (1999a). Although the *ste1* mutant has a phenotype similar to that of the wild type and *dwf7* mutants are not as dwarf as *bul1-1* plants (Fig. 1K,L), the new *DWF7*/*STE1* locus was reported to be also located on the same marker (Choe et al. 1999a). Therefore, mutation mapping suggested that our *bul1-1* mutant could be allelic to *dwf7-1*/*ste1-2*, *dwf7-2*/*ste1-3* and *ste1-1* mutants. Consequently, allelism tests between *bul1-1*, *dwf7* and *ste1* were performed and revealed that *bul1-1* is a new allele of *dwf7* and of *ste1* (data not shown). Thus, we designated our mutant as *bul1-1*/*dwf7-3*/*ste1-4*.

Sterol profile of the bul1-1 *mutant*

To confirm the genetic analysis showing that *bul1-1* is a new allele of *dwf7*/*ste1*, we performed a thorough sterol analysis of *bul1-1*. Wild-type, *dwf7* and *bul1* plants were grown in vitro on MS/2 medium as described in *Materials and methods*. Plants were analyzed after 5 (Table 3) or 10 (data not shown) weeks of culture. The most striking feature seen in the overall analysis reported in Table 4 is that *dwf7* and *bul1-1* accumulate substantial levels of Δ^7-sterols, among which Δ^7-sitostenol is predominant, instead of the Δ^5-sterols usually found in wild-type *Arabidopsis*. Indeed, the proportion of sitosterol was reduced from 60% to 2%; moreover, campesterol which is the precursor for brassinosteroid synthesis in *Arabidopsis* decreased from 9% of the sterols from wild-type leaves to barely detectable trace amounts in *bul1-1* or *dwf7*, as already reported in the latter case (Choe et al. 1999a). These results show clearly that, as in the case of the leaky mutant *ste1* (Gachotte et al. 1995; Husselstein et al. 1999) and *dwf7* (Choe et al. 1999a), the *bul1-1 Arabidopsis* mutant is defective in the Δ^7-sterol-C5(6)-desaturation step in the phytosterol pathway (Fig. 3); due to this defect, *bul1* lacks sufficient amounts of sterol precursors, which are required for brassinosteroid synthesis. Additionally, the following points can be made based on the sterol profile of *bul1-1*. Sterol intermediates of the pathway (Fig. 3) bearing a C-7(8) double bond, such as 24-ethylidene lophenol and avenasterol, were detected in low amounts in both wild-type and *bul1*. However, $\Delta^{7,22}$-sterols were detected as minor compounds only in *bul1-1* (and *dwf7*). Likewise, Δ^8-sterols were detected in the mutant lines but not in the wild-type, indicating that the accumulation of Δ^7-sterols might display a feed-back effect at the level of the enzyme Δ^8-sterol-$\Delta^{7(8)}$-isomerase (Taton et al. 1987; for a review, see Benveniste 1986).

Finally, sterol analyses were carried out for 16 F1 individuals (at the rosette stage) out of the progeny of a *bul1* × *ste1* cross. All of the plants had a sterol composition very close to that of the homozygous *bul1* parents

Table 1. Summary of morphological differences between wild-type (WT) and *bul1-1* plants. Values represent the mean ± SE of 30 measurements (n = 30)

	WT	*bul1-1*	Growth and chlorophyll content of *bul1-1* relative to WT (%)
7-d light-grown plants:			
Hypocotyl length (mm)	2.9 (±0.6)	1.2 (±0.2)	41
Root length (mm)	8.6 (±1.4)	4.6 (±0.4)	53
7-d dark-grown plants:			
Hypocotyl length (mm)	16.2 (±1.5)	7.5 (±0.5)	46
Root length	8 (±2.3)	6.5 (±1.9)	81
Hypocotyl reduction in light vs. darkness	18%	16%	
30-d light-grown plants:			
Aerial seedling height (cm)	20 (±5)	1.5 (±0.5)	7
Hypocotyl length (mm)	4.7 (±0.5)	2 (±0.1)	42
Petiole length (mm)	6.8 (±2.5)	1.2	17
Main root length (mm)	47.6 (±6.53)	35 (±2.9)	74
Leaf chlorophyll content of 15-d-old seedlings ($\mu g \cdot g^{-1}$ FW)	780.9	242.2	31

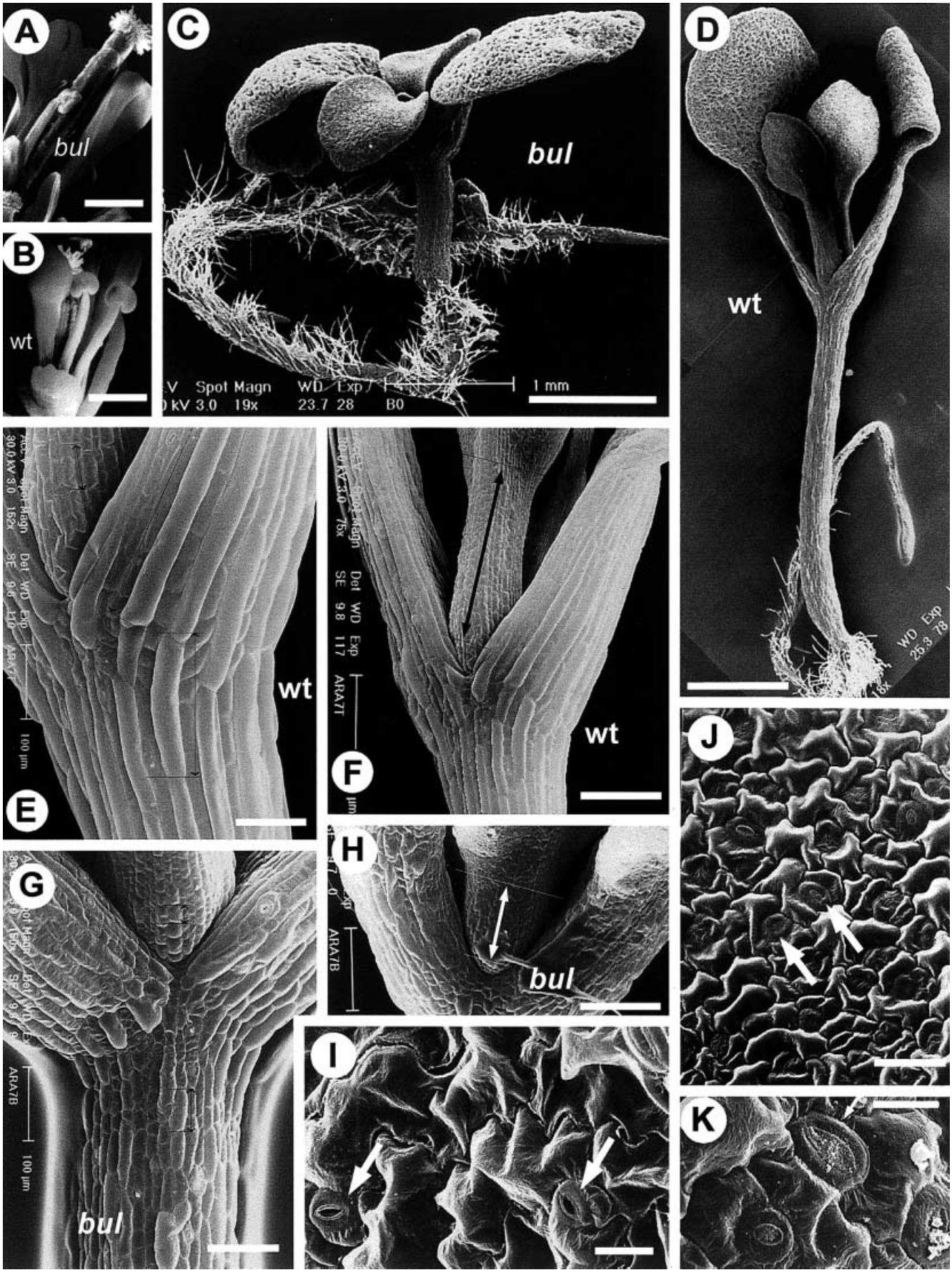
A
bul
B
wt
C
bul
V Spot Magn WD Exp
kV 3.0 19x 23.7 28 B0
1 mm
D
wt
WD Exp
25.3 78
E
wt
100 µm
F
wt
G
bul
100 µm
H
bul
I
J
K

Fig. 2A–K. Scanning elecron microscope study of the *bul1-1* mutant. **A,B** Mutant (**A**) and wild-type (**B**) mature flowers. Note that the filament of the anthers is shorter than the style in the mutant flower. **C,D** Overall views of 10-day-old- (light-grown) mutant (**C**) and wild-type (**D**) plants. **E–H** Higher magnification of wild-type (**E**,**F**) and *bul1-1* (**G**,**H**) organs. Differences in size of epidermal cells of the petiole and hypocotyl of the wild type (**E**) and mutant (**G**) were very obvious. *Double arrows* indicate cell length. Quite obviously, the decrease in cell length is responsible for the reduction in organ (petiole) length (**F**,**H**). *Double arrow* indicates petiole length. **I,J** Epidermal surface of the mature leaves of 20-d-old light-grown wild-type (**I**) and *bul1-1* (**J**) plants. Note that the stomata of the wild type (**I**) were open while they were closed in mutant leaves (**J**, *arrows*). **K** Under higher magnification, closure of stomata is obvious (*arrow*). Bars = 10 μm (**K**), 20 μm (**I**,**J**), 100 μm (**E**,**G**), 200 μm (**F**,**H**), 500 μm (**A**,**B**) and 1 mm (**C**,**D**)

(i.e. accumulation of Δ^7-sterols instead of Δ^5-sterols found in the wild-type) demonstrating therefore the allelic nature of *bul1* and *ste1*.

The cell elongation defect caused dwarfism in the bul1-1 *mutant*

To confirm the idea that a lack or alteration of cell elongation in certain tissues (organs) was mainly responsible for the *bul1-1* phenotype and to understand how BR deficiency can affect plant morphology, a comparative microscopic study of mutant and wild-type plants was undertaken. A scanning electron microscopic (SEM) study of 7-d-old seedlings of *bul1-1* (Fig. 2C) and the wild type (Fig. 2D) confirmed the morphological differences previously observed. Higher magnification indicated that both the petiole and hypocotyl of *bul1-1* seedlings were characterized by reduced (epidermal) cell elongation (Fig. 2E,G), leading to a proportional length decrease in these organs (Fig. 2F,H, double arrows).

SEM observations of *bul1-1* and wild-type leaf blades clearly revealed another major abnormality of the mutant phenotype. Stomata were five to six times more abundant in *bul1-1* with respect to the wild type (Fig. 2I, J, please notice scale bars). Moreover, *bul1-1* stomata were all closed and their size was about 1.5 times smaller than those of the wild type (Fig. 2K), in light-grown plants. Similarly, leaf epidermal cells of *bul1-1* were smaller than those of the wild type.

Table 3. Quantification of endogenous sterols (% of total sterols) from 5-week-old wild-type, *bul1-1* and *dwf7-1* mutants. Major differences between the wild type and mutants are in bold

	Wild-type	*bul1-1*	*dwf7-1*
9β,19-Cyclopropyl sterols			
Cycloartenol	5	tr	tr
24-Methylene cycloartanol	8	1.5	1
Pollinastanol	0	0.5	1
Δ^8-sterols			
Obtusifoliol	2	0	0
Δ^8-Campestenol	0	0.5	0.5
Δ^8-Sitostenol	**0**	**4**	**3**
Δ^7-Sterols			
24-Methylene lophenol	1	0	0
24-Ethylidene lophenol	1	0.5	1
Episterol	0	7	3.5
Δ^7-Avenasterol	1.5	1	1
Δ^7-Cholestenol	0	1	1
Δ^7-Campestenol	**0**	**5**	**7**
Δ^7-Sitostenol	**1**	**70**	**72**
$\Delta^{7,22}$-Cholestadienol	0	tr	tr
$\Delta^{7,22}$-Ergostadienol	0	tr	0.5
$D^{7,22}$-Stigmastadienol	0	5.5	4
Δ^5-Sterols			
Cholesterol	3	1	2
Brassicasterol	2	0	0
Campesterol	**9**	**tr**	**tr**
Sitosterol	**60**	**2**	**2**
Stigmasterol	4	0.5	0.5
24-Methylene cholesterol	0.5	0	0
Isofucosterol	2	0	0

tr, traces detected in GC-MS

In order to observe inner structural modifications induced by the *bul1-1* mutation, we undertook an histological study. Longitudinal sections of shoot apices showed no major differences between *bul1-1* (Fig. 4A,B) and wild-type (Fig. 4C) meristems, which presented the previously well defined tunica-corpus organization (see, e.g. Steeves and Sussex 1989). Only minor differences were observed between wild type and *bul1-1* leaf primordia, e.g. we noted a slight cell size reduction and delayed initiation of procambium in *bul1-1* plants. These observations suggest that primary morphogenetic events of leaf initiation (which involved a shift in polarity of cell expansion) are not affected by the *bul1-1* mutation. Strong differences between the wild type and *bul1-1*, however, occurred only after leaf initiation. Cells

Table 2. Genetic analysis of self offspring from five F2 plants, isolated from the *bul1* segregated line. Resist, hygromycin-resistant seedling; Sens, hygromycin-sensitive seedling; WT, wild-type; χ^2 value for corresponding ratio (Resist:*bul*:Sens)

Resistant		Sensitive	Segregation type (Resist:*bul*:Sens)	χ^2	P^a
bul	WT				
373	135	0	Type 1: (3:1:0)	0.67	$< 0.05^b$
437	0	0	Type 2: (1:0:0)	–	
550	0	177	Type 3: (3:0:1)	0.17	$< 0.05^b$
1,166	0	89	Type 4: (15:0:1)	0.93	$< 0.05^b$
676	193	54	Type 5: (47:13:4)	3.88	$< 0.05^c$

[a] Using χ^2 test, P values < 0.05 indicate segregation type is in accordance with scoring results
[b] 1 *df*
[c] 2 *df*

Table 4. Cytomorphometric analysis of *bul1-1* and wild-type *Arabidopsis* seedlings grown for 15 d on basal medium with (MSBR) or without (MS0) addition of 1 μM homoBL, under light conditions. Data represent mean ± SE of 15 cell size measurements. For each condition, measurements of petiole and hypocotyl cells were performed on 3 and 2 perfect longitudinal and axial sections. Fold increase = ratio of mean cell size on MSBR to mean cell size on MS0

	Wild-type seedlings			*bul1-1* seedlings		
	MS0	MSBR	Fold increase	MS0	MSBR	Fold increase
Hypocotyl epidermal cells						
Length (μm)	220 ± 39[a]	273 ± 100[a]	1.2	81 ± 17.3[b]	202 ± 73[a]	2.5
Width (μm)	22.63 ± 6[a]	32.2 ± 6.2[b]	1.4	26.3 ± 4.8[a]	27.6 ± 3.2[a]	1.05
Petiole epidermal cells						
Length (μm)	200 ± 83[a]	305 ± 110[b]	1.52	36.4 ± 14[c]	405 ± 81[d]	11.25
Width (μm)	16.12 ± 9[a]	29 ± 4.6[b]	1.8	13 ± 2.06[a]	52 ± 8[c]	4

[a–d] For each line, different letters indicate a statistical difference at the $P < 0.01$ level of significance by bilateral Student's t test

of wild-type leaf primordia quickly start to elongate (Fig. 4D) to reach their final size (Fig. 4E) in approximately 5 d whereas epidermal and subepidermal cells of *bul1-1* elongate more slowly and remained five times shorter than those of wild-types. In contrast, radial extension of *bul1-1* epidermal cells appeared unaltered (Fig. 4F). However, in vascular bundles of *bul1-1*, cell elongation was also affected but cell proportions of height to width were maintained. We also noted that differentiation of vessel elements (xylem) and formation of the lignified secondary wall were not affected in *bul1-1* plants (Fig. 4G,H). Cytomorphometric analyses (Table 4) further confirmed that petiolar epidermal cells were significantly (5.5 times) longer in the wild type than in *bul1-1*. Table 4 also shows the results obtained by comparative histological study of *bul1-1* and wild-type hypocotyls. The mean length of wild-type hypocotyl epidermal cells was 220 μm, whereas it was only 81 μm for the *bul1-1* mutant, suggesting that the reduced size of the *bul1-1* hypocotyl was due to a defect in cell elongation (approximately 2.8 times shorter in *bul1-1* than in wild-type). Thus, our histological studies revealed that the morphological defects observed in *bul1-1* were principally due to a reduction in cell size along the longitudinal axis of organs.

Leaf cross-sections from 10 d-old light-grown *bul1-1* and wild-type plants revealed another major modification induced by the *bul1-1* mutation. *bul1-1* leaves displayed the typical histological structure of wild-type leaves, with epidermis, palisade and spongy parenchyma, and vascular bundles, but were obviously thicker (Fig. 4I,J, observe the scale bar). This increased thickness of *bul1-1* leaves resulted from an enlargement of parenchyma cells, more precisely of palisade parenchyma cells (Fig. 4K–M). Despite their larger size, *bul1-1* parenchyma cells were highly cohesive and generated few and reduced intercellular spaces (Fig. 4L,M). Moreover, leaf cross-sections confirmed that wild-type stomata were open whereas those of the mutant were closed (Fig. 4L,M). We also observed that sub-stomatal chambers (constituted from intercellular spaces) were very reduced in *bul1-1* (Fig. 4L,M). Although the mutant leaves appeared dark greener than those of the wild type, the chlorophyll content of the mutant leaves was much less than the wild-type (Table 1). This dark-green colour may be due to the reduction in intracellular spaces and sub-stomatal chambers (i.e. the influence of the gaseous phase and light absorbance).

Effects of BR on bul1-1 *morphology*

In order to visualize the effect of BR treatment on our *bul1-1* mutants, 22(S),23(S)-homobrassinolide (homoBL) was tested on the *bul1-1*mutant. Figure 5 summarizes the effect of BRs on hypocotyls and petioles of wild-type and *bul1-1* light-grown seedlings. As shown in Figs. 5 and 6A, hypocotyl and petiole elongation was observed in the presence of homoBL (between 0 and 0.1 μM). The beneficial effect was best observed in the hypocotyl where approximately a 2-fold elongation was seen at 0.1 μM homoBL. Similarly, at the same concentration, a drastic elongation of *bul1-1* petioles was induced which led to a modification of the mutant phenotype (Figs. 5B, 6B). At higher BR concentrations, mutant petioles continued to elongate (reaching nearly 90% of the wild type grown in the absence of BR), but in a non-ordered way (twisting of the petiole was frequently observed; Fig. 6C,D).

At the cellular level, cytomorphometric analyses (Table 4) further indicated that petiole and hypocotyl developments were due to cell elongation. An effect of homoBL on cell elongation was particularly evident on *bul1-1* petiole cells, i.e. the increase in length caused by homoBL treatment was 11.3-fold. Hypocotyl cells of *bul1-1* were less affected by homoBL treatment since the increase in length reached only 2.5-fold. Therefore, it was clear that hypocotyl and petiole cells reacted differently to homoBL treatment. It was concluded that BR application to *bul1-1* can restore the untreated wild-type phenotype, in light.

The observation that BR treatment gave the mutant leaves the light-green colour found in the wild-type leaf prompted an SEM analysis of stomatal functioning in mutant leaves, after homoBL (0.5–1 μM) application. Interestingly, after this treatment, the stomata of *bul1-1* leaves were found to be open in light, as in light-grown untreated wild-type plants (Fig. 6E). However, the intensity of stomatal opening was clearly dependent on homoBL dose, e.g. at a low concentration (0.5 μM BL)

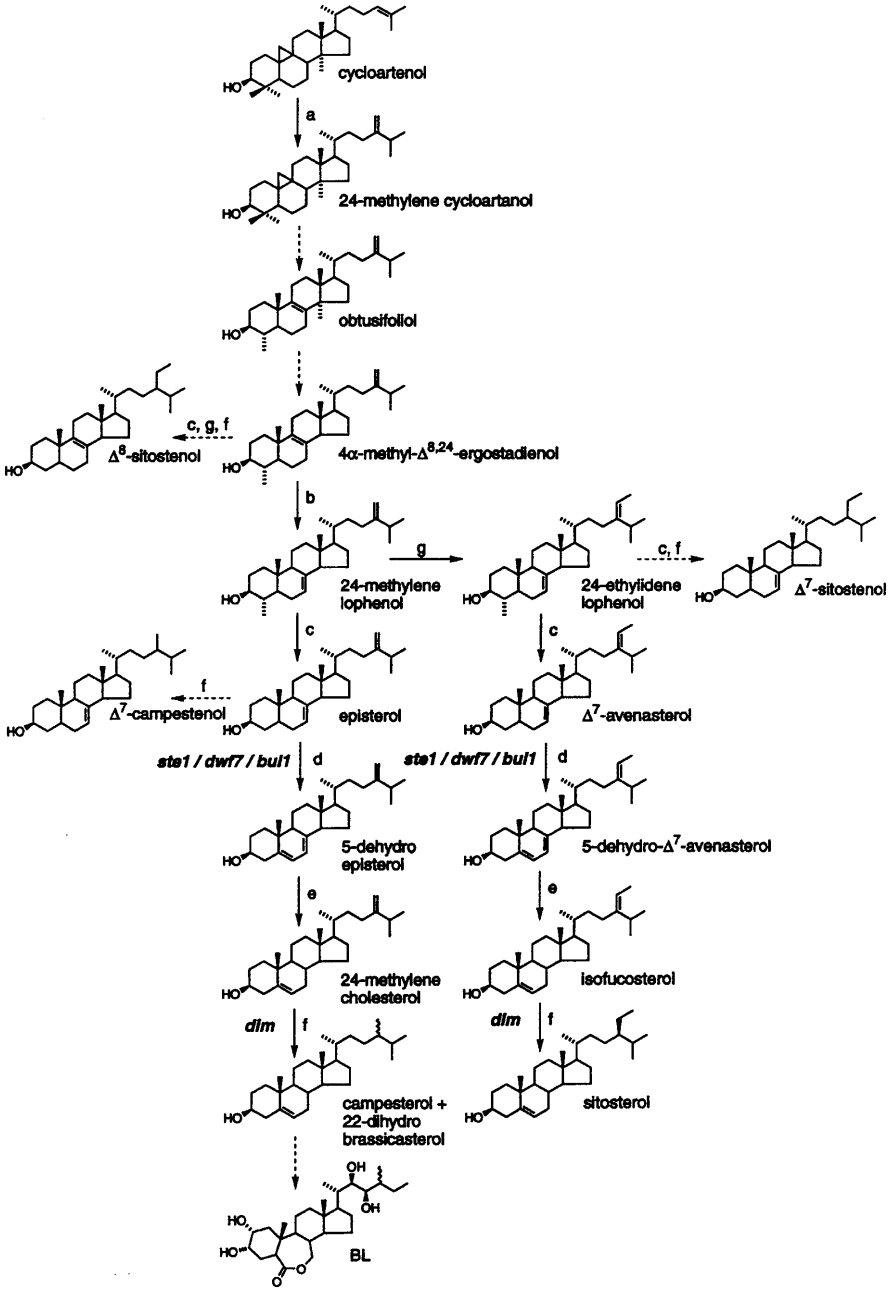

Fig. 3. Simplified biosynthetic pathway of sterols in wild-type and *bul1 Arabidopsis*. The *dashed arrows* indicate more than one biosynthetic step (not shown here). *a*, C-24 methylation; *b*, Δ^8-Δ^7-sterol isomerisation; *c*, C4-demethylation; *d*, C5(6)-desaturation; *e*, Δ^7-reduction; *f*, C24-isomerisation/reduction; *g*, $C24^1$-methylation

only about 18% of *bul1-1* plant stomata were open whereas at a higher concentration (1 μM) nearly 50% of stomata were open.

In order to determine the effects of BR in darkness, *bul1-1* and wild-type plants were cultured for 10 d with or without homoBL . In contrast to the wild type, which exhibited elongated hypocotyls with apical hooks, and closed and undeveloped cotyledons (Fig. 6F), *bul1-1* mutants still had short hypocotyls with apical hooks. The dark-grown *bul1-1* mutant was characterized by reduced hypocotyl elongation, being nearly half that of the wild type, i.e. a 50% reduction in size (see Table 1). Similar to light growth conditions, homoBL treatment permitted an elongation of *bul1-1* hypocotyls that reached nearly 80% of untreated wild-type hypocotyls but only 61% of those of treated wild types (Fig. 6G). Hypocotyls of homoBL-treated wild-type plants did not elongate significantly in comparison to untreated wild types. Furthermore, the twisting of *bul1-1* hypocotyls was also observed as in the case of homoBL-treated petioles of light-grown *bul1-1* mutants.

Discussion

Description of the bul1-1 *mutant*

We have isolated and characterized an extremely dwarf mutant of *Arabidopsis* named *bul1-1*, which was revealed to be allelic to the recently reported mutants *dwf7* (Choe et al. 1999a) and to *ste1* (Gachotte et al. 1995, 1996; Husselstein et al. 1999). The extreme phenotype of the

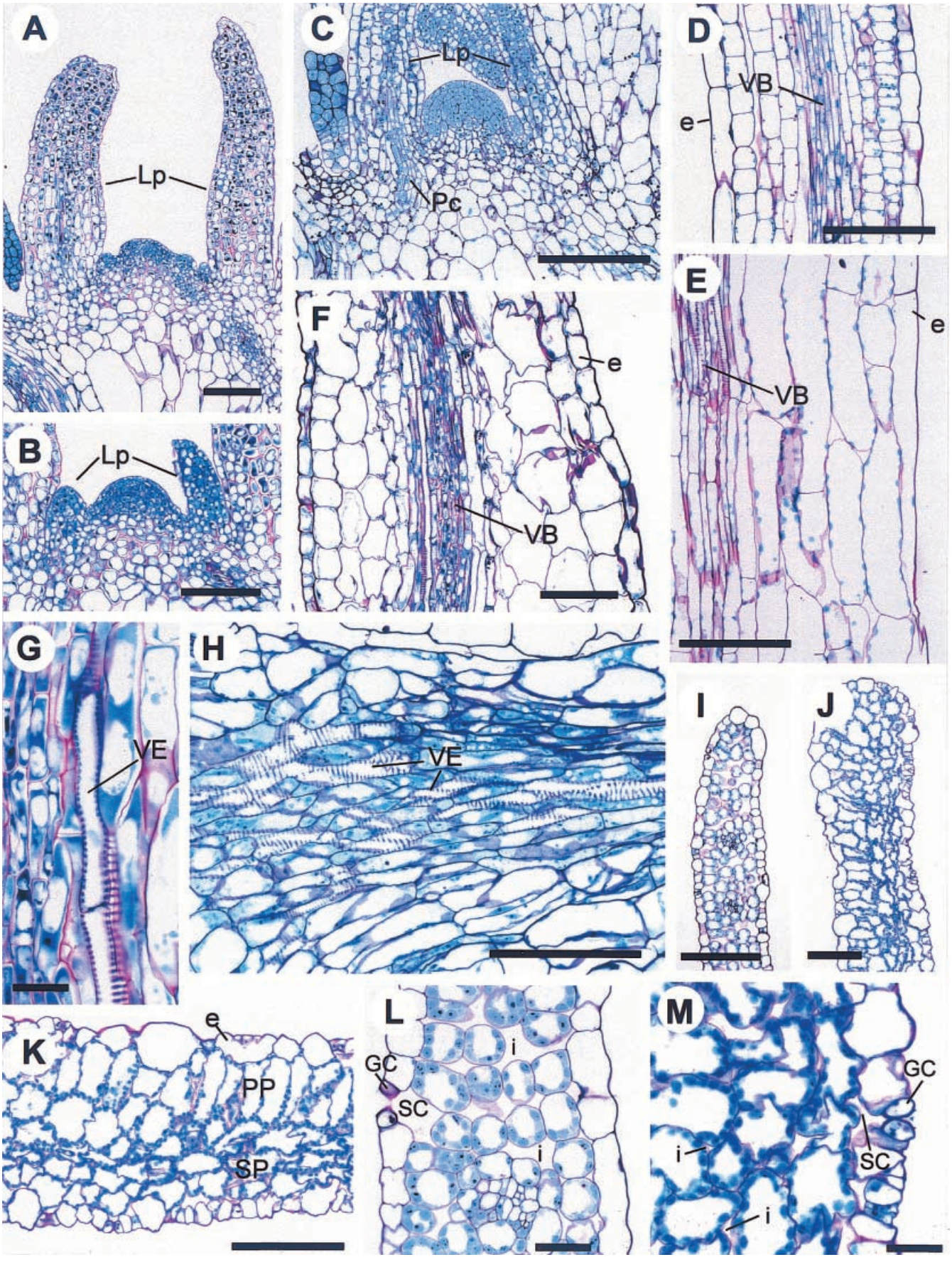
A
Lp
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Fig. 4A–M. Cytological study of *bul1-1* plants, with PAS and naphthol blue black staining. **A–C** Longitudinal sections of *bul1-1* (**A**,**B**) and wild-type (**C**) shoot apical meristems. *Lp* Leaf primordia; *Pc* procambium. **D,E** Longitudinal sections of young (**D**) and mature (**E**) wild-type petiole. *e* epidermis; *VB* vascular bundles. **F** Longitudinal section of mature *bul1-1* petiole *e* epidermis; *VB* vascular bundles. **G,H** Longitudinal sections of *bul1-1* petiole (**G**) and upper hypocotyl (**H**) showing short but normally differentiated (lignified secondary wall) vessel elements (*VE*) of xylem. *I–M* Transverse sections of mature leaves of wild-type (**I**,**L**) and *bul1-1* (**J**,**K**,**M**) 20-d-old light-grown plants. Note the differences in thickness (**I**,**J** and **K**), intercellular space (**I**) and stomatal structure (**L**,**M**) *GC* Guard cell; *SC* substomatal chamber; *e* epidermis; *PP* palisade parenchyma; *SP* spongy parenchyma. Bars = 20 μm (**G**,**L**,**M**), 100 μm (**A–F**, **H–K**)

bul1-1 mutant was due to a recessive nuclear mutation. As is the case of *dwf7* and other BR mutants, *bul1-1* hypocotyls were restored completely to wild-type length with BL treatment, and sterols analysis confirmed that *bul1-1* is defective in the Δ^7-sterol-C5(6) desaturation step of the sterol pathway leading to brassinosteroid synthesis. Consequently, *bul1* is a sterol-defective mutant and also a BR-defective mutant.

Allelism tests and sterol analysis revealed that *bul1* is allelic to *ste1* and *dwf7*. We have shown that the phenotypes of *bul1-1* and *dwf7* are different. Despite being a null mutation (Choe et al. 1999a), the *dwf7* mutant displays a weaker dwarf phenotype than *bul1-1* mutants. Therefore, the drastic *bul1-1* phenotype suggested that *bul1-1* is also a null mutation of the *DWF7*/*STE1* gene. Recently, we found in GenBank a new gene coding for a putative Δ^7-sterol-C5(6) desaturase revealed by sequencing the *Arabidopsis* chromosome III BAC F16B3 (AC021640) (Lin et al. 2000). This new gene was also closely linked to the nga172 marker and located just behind the Δ^7-sterol-C5(6) desaturase gene isolated from *ste1* (Fig. 7A). The sequence alignment, shown in Fig. 7B, indicates that this new protein is 80% identical (90% similarity) in amino acid sequence with the STE1 Δ^7-sterol-C5(6) desaturase (AF105034). Choe et al. (1999a) reported that the location of the mutations seems to be related to the phenotypic severity of mutant alleles. Based on their hypothesis and the existence of a second Δ^7-sterol-C5(6) desaturase gene closely linked to the *STE1*/*DWF7* gene, we suggested that *the bul1-1*

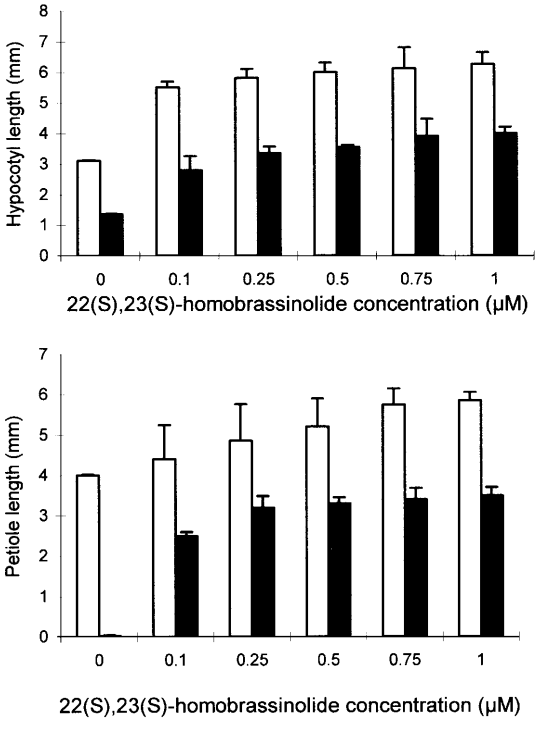


Fig. 5A,B. Responses to brassinolides. Measurements of light-grown hypocotyls (**A**) and petioles (**B**) were performed as described in *Materials and methods*. *Open bars* indicate the wildtype, *filled black bars* indicate *bul1-1*. The 4-d-old light-grown seedlings were grown for 12 d on medium supplemented with homoBL (0.1–1 μM). Data represent the mean of 20 measurements ± SE

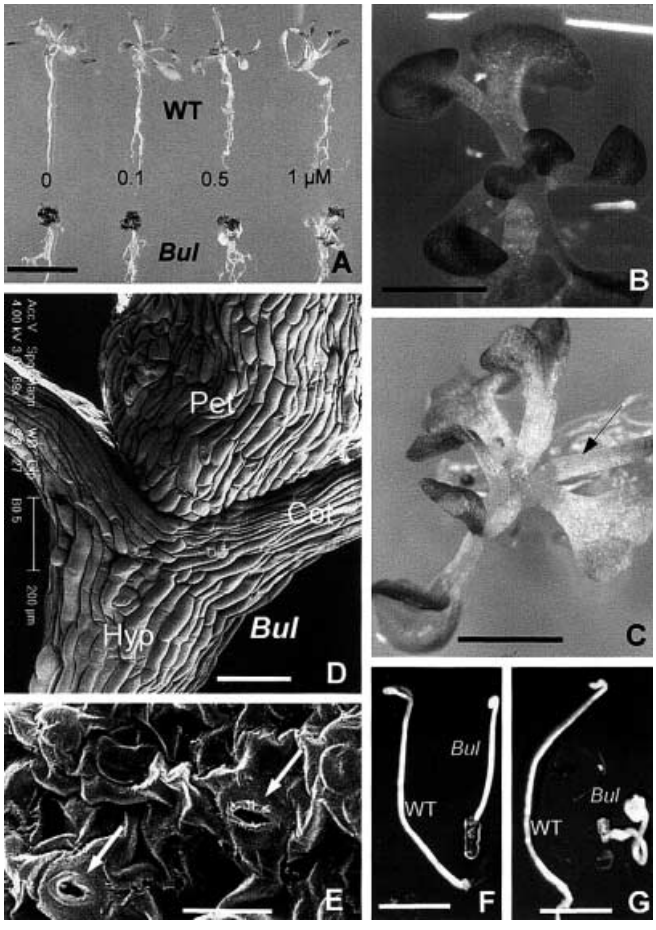


Fig. 6A–G. Effects of homoBL on the *bul1-1* mutant. **A** 15-d-old light-grown wild-type (*wt*) and *bul1-1* plants treated or not treated with homoBL (0.1–1 μM). Note the rescue of the light-grown phenotype of *bul1-1* seedlings with 1 μM of homoBL. **B,C** Rescue of the light-grown phenotype of *bul1-1* seedlings with 1 μM of homoBL. *bul1-1* seedlings were cultured for 10 d on medium supplemented with homoBL but seedlings were transferred at 2 d old (**B**) or 10 d old (**C**) after germinating on MS/2. Note the elongation and the twisting of the petiole (pale-whitish zones, *arrow*). **D** Cell elongation and twisting in BR-treated *bul1-1* plants. **E** *bul1-1* plants treated with 1 μM homoBL. Note the opening of the stomata. **F,G** 10-d-old dark-grown mutant and wild-type (*wt*) plantlets, not treated (**F**) or treated with (**G**) 1 μM homoBL. Note the elongation and the twisting of *bul1-1* hypocotyl. Bars = 10 μm (**E**), 200 μm (**D**), 5 mm (**B**,**C**,**F**,**G**), 2 cm (**A**,**B**)

mutant could be affected in both Δ^7-sterol-C5(6) desaturase genes. Probably, this new gene corresponds to the *HDF7* gene (*HOMOLOG OF DWF7*, *HDF7*) reported by Choe et al. (1999a). Only the isolation and sequencing of the *bul1* gene would clarify this possibility (the experiments are in progress).

The *bul1-1* mutant showed an extreme dwarf phenotype with short hypocotyl and petioles, dark-green and small rounded leaves. *bul1-1* leaves were organized in the correct phyllotaxis. In the greenhouse, the mutant reached a height of about 1 cm after one month of growth, whereas the height of the *dwf7-1* mutant was 5.8 ($\pm$0.5) cm and 12.6 ($\pm$1.5) cm for *ste1-1*, at the same age in our culture conditions (data not shown). The flowers of *bul1-1* were reduced in size. Self-pollination could not occur spontaneously due to a defect in stamen filament elongation. As a consequence, pollen could not reach the stigmatic hairs, as already observed in other dwarf mutants, *dwf4* (Azpiroz et al. 1998) and *dwf7* (Choe et al. 1999a). Only hand-pollination provided seeds. The *bul1-1* mutant, like its allelic counterparts, was principally affected in cell elongation. Macroscopically and at the cellular level, *bul1-1* exhibited a rather extreme dwarf phenotype. The reduction in cell elongation was already obvious in light-grown plants but it became more pronounced in dark-grown seedlings. In contrast to the other BR-defective mutants, which were mainly analyzed at the biochemical and molecular levels, the *bul1-1* mutant was studied at the physiological and, in particular, cellular levels to determine the involvement of BR in the cell elongation process and to elucidate the interactions with BRs and other signalling pathways.

A defect in cell elongation: the origin of the bul1-1 *dwarf phenotype*

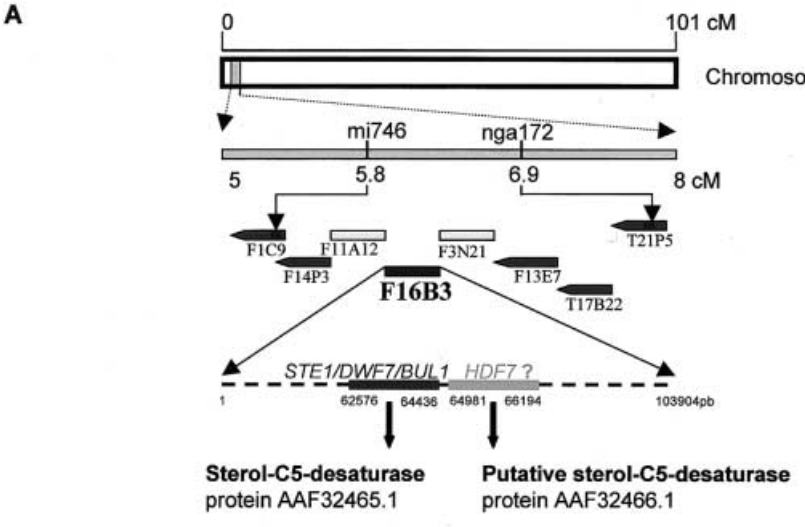
A comparative microscopic study of light-grown *bul1-1* and wild-type plants indicated that a reduction in cell elongation in every organ (hypocotyl, petioles, and stamen filaments) was the origin of the aberrant mutant morphology. However, this reduction was more dramatic, and hence easier to study in hypocotyls and petioles. In longitudinal sections of *bul1-1* hypocotyls and petioles, epidermal and sub-epidermal cells were respectively 3-fold and 5-fold shorter than those of wild-type seedlings (Table 4). This reduction in epidermal cell length is more drastic in *bul1-1* than in *dwf7* (epidermal cell length was $<$18% of wild-type for *bul1-1* and $<$30% of wild-type for *dwf7*). Moreover, no difference in the number of cells per epidermal cell file was observed (Fig. 2). In transverse sections, no major anatomical differences were noticed, indicating that the reduced organ (hypocotyls and petioles) size was due to a reduction in longitudinal cell elongation, and not to a problem in cell division or an increase in transverse cell expansion. Another cellular abnormality was revealed in *bul1-1* leaf blades and leaf epidermal cells, where stomata were closed and smaller than those of wild-type plants (Figs. 2, 4). Thus, our results indicate that the *bul1-1* mutation not only affects shoot elongation but also stomatal functioning, production and spacing. Therefore, the *bul1-1* dwarf phenotype is a result of a reduction in cell elongation, as reported previously for other dwarf mutants (Takahashi et al. 1995; Clouse et al. 1996; Kauschmann et al. 1996; Szekeres et al. 1996; Fujioka et al. 1997; Azpiroz et al. 1998; Choe et al. 1999a,b, 2000; Ephritikhine et al. 1999a,b).

Brassinosteroid and light-signalling pathways in the bul1-1 *mutant*

As is the case for *dwf7*, a restoration of *bul1-1* plants to a phenotype similar to the wild type, when grown under the same conditions, was achieved by addition of BR to the germination medium (Figs. 5, 6). Hypocotyl and petiole elongation were restored, thereby resulting in a wild-type phenocopy of the mutant. At the cellular level, cytometric analyses revealed that hypocotyl cells of BR-treated *bul1-1* plants increased longitudinally in size and their shapes were similar to longitudinally expanded cells of BR treated wild-type plants (Table 4).

Like *dwf7* mutants, the *bul1-1* phenotype was not de-etiolated after 10 d of dark-growth. Its phenotype was similar to that of an etiolated wild-type plant with an apical hook, closed cotyledons and an elongated hypocotyl. However, the *bul1-1* hypocotyl remained shorter than that of the wild type. A transmission electron microscopy study of cotyledons of dark-grown wild-type and *bul1-1* seedlings revealed that both contained normal etioplasts with the characteristic prolamellar body and the absence of thylakoid organization (data not shown). Moreover, our results are similar to those reported by Azpiroz et al. (1998) concerning a BR dependent mutant, *dwf4*, which was partially de-etiolated in the dark. The conclusion about the abnormal skotomorphogenesis of the *dwf4* mutant was that its de-etiolated phenotype was a consequence of the in vitro growth culture conditions. Contrary to Mandava (1988), who reported that BR inhibited de-etiolation of many plant species in the dark, *dwf4* and our *bul1-1* mutant deficient in BR biosynthesis were not blocked in the etiolation response in the dark. In the same way, de-etiolated mutants like *cop* and *fus*, which are not deficient in BR biosynthesis, also became elongated in the dark in the presence of BRs, but not in the light (Szekeres et al. 1996). Such experiments indicate that BRs can overcome the defect of certain regulatory functions required for cell elongation in darkness, but fail to suppress these effects in the light due to a possible antagonistic interaction between BRs and the light-signalling pathway. Thus, our results suggest that the product of the considered gene seems to have no direct effect on the light-signalling pathways. Therefore, we conclude that the *bul1-1* mutant is not a light-regulatory BR-deficient mutant such as *det2* (Chory et al. 1991; Li et al. 1996) and *cpd*/*cbb3*/*dwf3* (Kauschmann et al. 1996; Szekeres et al. 1996).

Based on these findings, we conclude that the *bul1-1*/*dwf7-3*/*ste1-4* mutant represents a new mutant defective in Δ^7-sterol-C5-desaturase leading to BR deficiency. The biochemical and molecular characterization of this



B

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STE1/DWF7      1 MAADNAYLMQFVDETSFYNRIVLSHLLPANLWEPLPHFLQTWLRNYLAGTLLYFISGFLW
AAF32466       1 MAATMAYNDQIVNETSFYNRMVLSHLLPVNLWEPLPHFLQTWLRNYLAGNILYFISGFLW
                 ***  **  * * ******* ******* ********************  *********

STE1/DWF7     61 CFYIYYLKINVYLPKDAIPTIKAMRLQMFVAMKAMPWYTLLPTVSESMIERGWTKCFASI
AAF32466      61 CFYIYYLKLNVYVPKESIPTRKAMLLQIYVAMKAMPWYTLLPAVSEYMIEHGWTKCYSTL
                 ******** *** **  *** *** **  ************* *** *** *****

STE1/DWF7    121 GEFGWILYFVYIAIYLVFVEFGIYWMHRELHDIKPLYKYLHATHHIYNKQNTLSPFAGLA
AAF32466     121 DHFNWFLCFLYIALYLVLVEFMIYWVHKELHDIKFLYKHLHATHHMYNKQNTLSPFAGLA
                   * * * * *** *** *** *** * ****** *** ****** **************

STE1/DWF7    181 FHPVDGILQAVPHVIALFIVPIHFTTHIGLLFMEAIWTANIHDCIHGNIWPVMGAGYHTI
AAF32466     181 FHPLDGILQAIPHVIALFIVPIHLITHLSLLFLEGIWTASIHDCIHGNIWPIMGAGYHTI
                 *** ****** *************  **  *** * **** *********** ********

STE1/DWF7    241 HHTTYKHNYGHYTIWMDWMFGSLRDPLLEEDDNKDSFK
AAF32466     241 HHTTYKHNYGHYTIWMDWMFGSLMVPLAEKDSFKEKEK
                 ************************  ** * *  *   *
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Fig. 7A,B. Schematic view of the duplicate genes of *Arabidopsis*, coding the Δ^7 sterol C-5 desaturase, and amino acid sequence alignment. **A** Both genes are mapped on the F16B3 BAC of chromosome III. The first gene, *STE1/DWF7*, was isolated by Gachotte et al. (1996) and Choe et al. (1999a). Sequencing of the *Arabidopsis* chromosome III revealed another gene with the same function, located just behind the *STE1/DWF7* gene. **B** Sequence alignment of STE1/DWF7 with this putative Δ^7 sterol C-5 desaturase (AAF32466) indicates that this new protein is 80% identical (90% similarity) in amino acid sequence to the STE1/DWF7. To explain the weaker phenotype of their *dwf7-1* mutant, Choe et al. (1999a) reported the existence of a second Δ^7 sterol C-5 desaturase gene, named *HDF7* (*HOMOLOG OF DWF7*). This *HDF7* gene may be this new gene identified in GenBank. Sterol analyses and an allelism test demonstrate the allelic nature of *bul1*, *ste1* and *dwf7*. The severity of the *bul1-1* mutant phenotype therefore suggests a complete null mutation in the *STE1/DWF7* gene function. To explain the extreme dwarf phenotype of *bul1-1*, we suppose that this mutation is affecting both duplicate genes. GenBank accession numbers for the STE1/DWF7 sequence are AAF324465, AAD12944 or AAD38120. *Asterisks* below amino acids indicate identity in both proteins. Sequence alignment was done using binary alignment from Expasy Proteomics Tools

mutant further contribute to the understanding of BR influence on a variety of physiologically important traits, such as cell elongation. However, the physiological significance of BR metabolism is still poorly understood, the elucidation of BR-regulated genes and factors that mediate the cellular response (for example, tubulin gene and microtubules) could be useful.

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