Journal of Experimental Botany, Vol. 54, No. 385, pp. 1259–1268, April 2003
DOI: 10.1093/jxb/erg135

Journal of Experimental Botany

RESEARCH PAPER

Characterization and genetic analysis of a lettuce (*Lactuca sativa* L.) mutant, *weary,* that exhibits reduced gravitropic response in hypocotyls and inflorescence stems

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Received 9 September 2002; Accepted 13 January 2003

Abstract

A lettuce (*Lactuca sativa* L.) mutant that exhibits a procumbent growth habit was identified and characterized. In two wild type (WT) genetic backgrounds, segregation patterns revealed that the mutant phenotype was controlled by a recessive allele at a single locus, which was designated *weary*. Hypocotyls and inflorescence stems of plants homozygous for the *weary* allele exhibited reduced gravitropic responses compared with WT plants, but roots exhibited normal gravitropism. Microscopic analysis revealed differences in the radial distribution of amyloplasts in hypocotyl and inflorescence stem cells of *weary* and WT plants. Amyloplasts occurred in a single layer of endodermal cells in WT hypocotyls and inflorescence stems. By contrast, amyloplasts were observed in several layers of cortical cells in *weary* hypocotyls, and *weary* inflorescence stem cells lacked amyloplasts entirely. These results are consistent with the proposed role of sedimenting amyloplasts in shoot gravitropism of higher plants. The phenotype associated with the *weary* mutant is similar to that described for the *Arabidopsis* mutant *sgr1/scr*, which is defective in radial patterning and gravitropism.

Key words: Amyloplast, Asteraceae, endodermis, gravitropic mutant, gravitropism, *Lactuca sativa,* lettuce.

Introduction

The successful growth of higher plants depends on their ability to perceive and respond to an array of environmental stimuli such as light, moisture and gravity. Gravitropism is the process by which plants sense and respond to gravitational forces. For most plants, each organ grows at a consistent angle relative to the gravitational vector; for example, shoots usually grow upward, opposite the gravitational vector, whereas roots usually grow downward. Plants that are reoriented with respect to gravity typically bend and resume growth at the preferred angle. This response involves four steps: (1) detection of gravity by cells in the site of perception, (2) production of a molecular signal, (3) transduction of this signal to the site of bending, and (4) the differential cell elongation that results in bending (Tasaka *et al*., 1999).

According to the widely accepted starch-statolith hypothesis of gravity perception, starch-containing organelles called amyloplasts act as statoliths, providing information about the orientation of the gravity vector as they sediment within cells (statocytes) under the effects of gravity (Kiss, 2000). Amyloplasts are normally found within the sites of gravity perception in both roots (root tip columella cells) and shoots (endodermal cells) and their absence in mutant plant genotypes has been associated with reduced gravitropic sensitivity (Kiss *et al*., 1997; Sack, 1997; Weise and Kiss, 1999). After gravity is perceived, gravitropic bending is thought to be the direct result of differential cell elongation in response to a localized auxin gradient, but the precise nature of the components involved in signal transduction leading to the formation of the auxin gradient following perception are not known (Rosen *et al*., 1999).

Mutants with abolished or reduced gravitropic responses have been identified in several crop plants, including pea, maize, tomato, barley, and rice (Roberts, 1987; Godbolé *et al*., 1999). The affected organs and the pleiotropic effects associated with these mutations vary widely. To develop a more complete understanding of the basic processes underlying gravitropism, a systematic approach

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has been used to generate and characterize mutants of *Arabidopsis thaliana* that are defective in all steps of the gravitropic response pathway (Fukaki *et al*., 1996*a*, *b*; Yamauchi *et al*., 1997). Analyses of over 20 *Arabidopsis* mutants with altered gravitropic responses have provided insights into the mechanisms of gravity perception and response by plants (Fujihara *et al*., 2000; Tasaka *et al*., 1999, 2001). Consistent with the starch-statolith hypothesis, many of the genes identified control starch synthesis, endodermal cell differentiation or amyloplast sedimentation. Other genes appear to be components of signal transduction pathways, or are involved in auxin or ethylene synthesis or responses, suggesting their involvement in the latter steps of gravitropism. In wild-type (WT) *Arabidopsis* plants, the roots, hypocotyls, and inflorescence stems exhibit gravitropic bending. When the loci involved in gravitropism are classified according to the organs in which they act (inflorescence stem only, hypocotyl only, root only, and all possible combinations of the three), six of the seven possible classes have been identified. This indicates that the graviresponse pathways in these organs share some common elements, but that other components are organ-specific.

Lettuce (*Lactuca sativa* L.), like *Arabidopsis*, is a dicot which initially grows as a rosette plant whose main stem elongates and grows vertically during the transition from vegetative to reproductive growth. Lettuce is well suited to hydroponic production due to rapid growth rate, high harvest index, and limited post-harvest processing requirements. Because of its suitability for hydroponic culture, lettuce is considered by the National Aeronautics and Space Administration to be a priority crop for use in advanced life support systems under conditions of microgravity, such as space stations (Henninger, 1998). Despite intensive efforts to identify optimal conditions for hydroponic lettuce production, very little is known about the gravitropic response mechanism(s) of lettuce. Consequently, the degree to which micro- or macrogravity conditions affect lettuce developmental processes, and therefore crop productivity, is poorly understood. A better understanding of the interactions between gravitropism and essential plant developmental processes will undoubtedly allow better prediction and control of plant growth and productivity in microgravity, and in a range of controlled environments on earth.

In the present study, a naturally occurring lettuce mutant with a procumbent, or 'weary', growth habit is characterized. The objectives were (1) to determine the inheritance of the weary phenotype, (2) to characterize mutant and WT plants with respect to gravitropic response of hypocotyls, roots and inflorescence stems, and (3) to compare the anatomy of hypocotyl, root and inflorescence stems in mutant and WT plants.

Materials and methods

Plant material

Seeds of all lettuce genotypes were obtained from the US Department of Agriculture–Agricultural Research Service lettuce germplasm collection in Salinas, CA. Both 'Prizehead', a red leaf lettuce cultivar with a light green base colour, and 'Salinas 88', a medium-green iceberg lettuce cultivar, have a long vegetative growth phase prior to the transition to reproductive growth (bolting). 'Salinas 88' has the virus resistance gene *mo*, which is absent in 'Salinas', but is otherwise nearly isogenic to 'Salinas'. PI 251245 is a red-tinged darker green primitive *L. sativa* accession that bolts very quickly in response to long days.

Weary plants, which exhibited a horizontal growth habit after bolting, were first identified among the F_3 progeny from a single F_2 plant from a cross between PI 251245 and 'Prizehead'. F_4 progeny were produced by self-pollinating a single weary and a single WT (erect) plant. Single plants from true-breeding lines were self-pollinated to produce F_5 progeny. Individual plants from two F_5 lines, 00-241 (weary) and 00-235 (erect), were crossed for inheritance studies and were self-pollinated to produce F_6 progeny for studies examining seedling phenotypes. Aside from the weary phenotype, 00-241 and 00-235 were morphologically similar to one another and to the parent genotype PI 251245.

Controlled reciprocal crosses were made between 00-241 and the two WT genotypes 00-235 and 'Salinas 88' as described by Ryder and Johnson (1974) to maximize hybrid seed production. Morphological markers were used to confirm F_1 identity in lettuce, because occasional self-pollination of the female parent occurs. For crosses with 'Salinas 88', F_1 plants were identified using morphological markers (leaf width, leaf colour, number of days to bolting). For crosses between 00-241 and 00-235, similar morphology prevented the identification of F_1 progeny by morphological markers other than the weary phenotype. Putative F_1 plants were self-pollinated, and segregation in progeny populations confirmed the identity of true hybrids.

Cultural conditions

For greenhouse studies, seeds were sown directly into plastic pots containing field soil or into plug trays containing Sunshine Mix No. 5 (Sun Gro Horticulture, Bellevue, WA). Seedlings were transplanted at approximately 3-weeks-old into plastic pots. Greenhouses were located in Salinas, CA, lacked supplemental lighting, and had minimum and maximum average daily temperatures of 18 °C and 35 °C, respectively.

Evaluation of gravitropic response

To evaluate gravitropic responses of inflorescence stems, plants were grown in the greenhouse. After stems had elongated at least 15 cm (approximately 70 d after seeding), plants were placed in a growth chamber in complete darkness at 20 °C and were laid horizontally so that the shoot axis was perpendicular to the gravitational vector. Initial orientation and all subsequent measurements were recorded using an Epson PhotoPC800 digital camera. The final measurement was taken 24 h after gravistimulation. Visible markings on pots permitted alignment of images taken at different time points. Curvatures and lengths of stems were measured from digital images using Adobe Photoshop v5.5 (Adobe Systems, Inc., San Jose, CA) and Canvas v5.0.3 software (Deneba Systems, Miami, FL). To examine the gravitropic response of detached inflorescence stems, the distal 15–20 cm of plants were excised, placed in moistened florists' foam in a darkened growth chamber, and were laid horizontally. Detached stems were examined visually for bending indicative of a gravitropic response at varying times after gravistimulation.

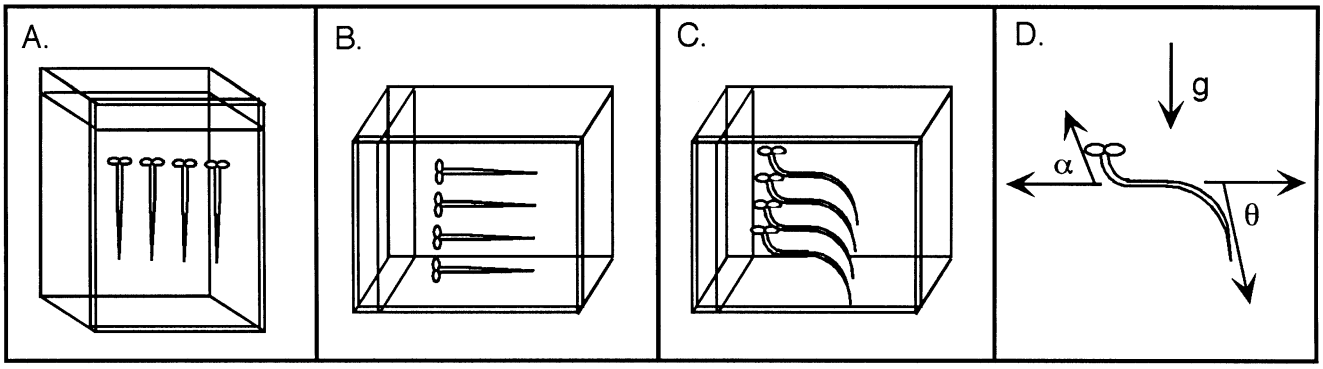

Fig. 1. Method used to evaluate graviresponse in lettuce seedlings. Straight seedlings were placed against the agar-coated inner wall of a Magenta box (A), which was then rotated 90° (B). Responding seedlings reoriented to resume vertical growth (C). Measurements taken included hypocotyl curvature, the angle by which the hypocotyl deviated from horizontal growth, α, and root curvature, the angle by which the root deviated from horizontal growth, θ, as well as hypocotyl and root length. The arrow designated with the symbol 'g' indicates the direction of the gravity vector (D).

To evaluate the gravitropic response in seedling roots and hypocotyls, seeds were sown between vertically oriented sheets of moist paper towel and placed in a growth chamber for 16 h of light at 16 °C, followed by complete darkness at 25 °C. Straight seedlings were removed from the paper towels after 48 h and placed in Magenta boxes (Magenta Corporation, Chicago, IL) for gravistimulation (Fig. 1). Magenta boxes were prepared by pouring a thin (approximately 1 mm) layer of 1% agar along one inner wall of the box. Seedlings were laid on the agar-coated wall, with cotyledons and roots pointing towards the top and bottom of the box, respectively. Boxes containing seedlings were returned to the growth chamber, and were positioned vertically until most hypocotyls were at least 5 mm long (2–19 h). The initial orientation was recorded by scanning the Magenta boxes using a standard office photocopier. Boxes were returned to the dark chamber and placed horizontally to provide a gravitational stimulus of 90°. Unstimulated controls were replaced in the original upright position. All seedlings were removed and photocopied intermittently, and returned to the chamber. Data are presented separately for two independent experiments, experiments I and II. The final measurement was taken 90 h after germination, which corresponded to 39 h after gravistimulation for experiment I, and 24 h after gravistimulation for experiment II. Curvatures and lengths of roots and hypocotyls of individual seedlings were measured from digital images of photocopies scanned with an Epson scanner as described for inflorescence stems.

Microscopic analysis

Inflorescence stems were harvested from 70-d-old plants. A hand microtome was used to make cross-sections (approximate thickness 25 μm) from the entire stem of 'Prizehead' and 'Salinas' plants, which were still in the rosette stage and had stems less than 2 cm long. The stems of all other genotypes were at least 5 cm long, and cross-sections were taken from two 5-mm-long regions, approximately 15 and 30 mm below the apical meristem. Sections were fixed in 70% (v/v) ethanol at room temperature for at least 24 h. Sections were then mounted on slides and stained for approximately 1 min with iodine potassium iodide (IKI) stock solution (2% (w/v) iodine, 5% (w/v) potassium iodide, and 20% (w/v) chloral hydrate).

For microscopic analysis of roots and hypocotyls, seedlings were grown in a dark growth chamber at 20 °C for 3 d wrapped in moistened paper towels. For whole mounts of root tips and hypocotyls, intact seedlings were fixed in 70% (v/v) ethanol at room temperature for at least 24 h. To examine root tips, whole roots were mounted onto slides and stained for at least 1 min with IKI. To examine hypocotyls and cotyledons, entire seedlings were stained for at least 24 h with diluted IKI (1:5 dilution of stock solution in distilled water), and were then mounted on slides and gently squashed with a coverslip. Whole mounts were examined from at least five plants of each genotype. Freehand cross-sections were made from unfixed hypocotyls. Sections were taken from the region just below the apical hook, and were mounted on slides and stained for 1 min with diluted IKI. All microscopic slides were viewed and photographed using bright-field optics with a Zeiss Standard 16 light microscope and MC63 35 mm photomicrographic camera (Zeiss, West Germany) with Kodak Ektachrome Elite film at ASA 200.

Statistical analyses

For inheritance experiments, data from reciprocal crosses were bulked after chi-squared tests of homogeneity were performed and chi-squared goodness-of-fit tests were used to examine genetic hypotheses. For experiments examining seedling growth and gravitropic response, the number of seedlings measured per genotype varied from 4–17 for unstimulated controls, and from 14–61 for gravistimulated seedlings. Growth rate data from stimulated and unstimulated seedlings were not significantly different and were therefore combined to evaluate differences between genotypes. Curvature measurements for unstimulated controls of different genotypes were not significantly different, and were therefore combined for comparison with gravistimulated plants. Differences among treatments and genotypes were assessed using analysis of variance (ANOVA). Significant differences between treatments were determined with an error rate of α=0.05 using Student's *t*-test or Tukey's HSD test for all pairwise comparisons. All analyses were performed using the JMP v4.0.4 statistical software package (SAS Institute, Cary, NC).

Results

Description of the mutant phenotype

For all genotypes, the age at which plants bolted depended on daylength. In all seasons, however, PI 251245, 00-241 and 00-235 consistently bolted at approximately the same age and earlier than 'Salinas 88' and the parent cultivar 'Prizehead'. Prior to bolting, weary plants were similar in appearance to both the parent accession PI 251245 and the

Fig. 2. Wild-type (WT) and weary lettuce plants and inflorescence stems. WT (PI 251245, 00-235, Prizehead (Pzhd), Salinas) and weary (00-241) lettuce plants were photographed at 64 d (A) and 82 d (B). Inflorescence stems (C) of WT (00-235) and weary (00-241) plants were photographed after being decapitated, inserted horizontally into moist florists' foam blocks, and incubated for 8 h in a dark growth chamber.

WT erect F_5 sibling line 00-235. After bolting, weary and WT plants were readily distinguished because the main stems of weary plants curved whereas all WT lettuce plants exhibited straight upright growth (Fig. 2A). Fully elongated mature greenhouse-grown weary plants were often prostrate (Fig. 2B), but in some cases plants were only moderately bent or leaning towards one direction. Bolted plants of all genotypes bent slightly towards the south during winter months, when the sun remained near the horizon in the south-western sky throughout the day. During these months, weary plants exhibited more pronounced leaning towards the south than was observed for WT genotypes.

Inheritance of the weary phenotype

All F_1 plants generated by crossing 00-241 and 'Salinas 88' were erect and had morphological features intermediate between parent genotypes. Reciprocal crosses were phenotypically indistinguishable. Among eight putative (00-241×00-235) F_1 progeny, three erect plants that were indistinguishable from the male parent were identified. The five weary plants were presumably the result of self-pollination of the female parent 00-241. Since no erect plants were identified among 24 intentionally self-pollinated progeny of 00-241, erect plants were considered F_1 hybrids. All progeny from the reciprocal (00-235×00-241) cross were erect and indistinguishable from the female parent. F_2 populations from reciprocal crosses using both genetic backgrounds were grown in the greenhouse and evaluated for the weary phenotype after initiation of bolting. Chi-squared tests of homogeneity established that segregation ratios in populations resulting from reciprocal crosses did not differ, providing no evidence for maternal inheritance. In both genetic backgrounds, the weary phenotype segregated in ratios consistent with monogenic recessive inheritance (Table 1). Thus, the recessive allele that confers the procumbent phenotype in the mutant lettuce line 00-241 was designated *weary (wry)*.

Gravitropic response of the weary mutant

Inflorescence stems: The growth habits of WT and mutant lettuce plants grown in the greenhouse are shown in Fig. 2A and B. During the 24 h following gravistimulation in the dark, the apical portions of WT (00-235) plants with elongated main stems (15–20 cm) rapidly bent upward, but *wry* (00-241) plants did not exhibit a bending response (Table 2). During this time period, no stem elongation was observed for either genotype. Mutant plants did not reorient even after several days of horizontal stimulus (data not shown). A rapid gravitational response was

Table 1. *Inheritance of the weary mutant phenotype in two genetic backgrounds*

Genotype	No. weary	No. erect	Expected ratio (weary: erect)	$\chi^{2\,a}$	P^a
00-241 (weary)	24	0	–	–	–
00-235 (erect)	0	24	–		
'Salinas 88'	0	24	–	–	–
(00-241×'Salinas 88') F_1	0	1	0:1	–	–
('Salinas 88'×00-241) F_1	0	26	0:1	–	–
(00-241×'Salinas 88') F_2	159	414	1:3	2.31	0.13
(00-241×00-235) F_1	0	3	0:1	-	-
(00-235′00-241) F_1	0	17	0:1	–	–
(00-241′00-235) F_2	159	426	1:3	1.48	0.22

[a]The χ^2 and P values resulting from a Chi-squared test of fit of the data to expected Mendelian ratios for a single recessive gene. F_2 data from reciprocal crosses were pooled after testing for heterogeneity.

Table 2. *Cumulative curvature of WT and weary lettuce inflorescence stems after a 90° gravitational stimulus*[a]

Genotype	Cumulative curvature after gravistimulation (°)		
	4 h	8 h	24 h
00-241 (weary)	–5±3 a*	–2±3 a*	–8±4 a*
00-235 (WT)	29±11 b	73±3 b	86±3 b

[a]Greenhouse-grown 73-d-old plants with elongated inflorescence stems were transferred to a dark growth chamber and turned horizontally (gravistimulated), and were exposed to light only briefly during photodocumentation at 4, 8 and 24 h after stimulation. Twelve plants were measured per genotype, and the mean ± SE from a single representative experiment were presented. At each time point, means followed by the same letter were not significantly different, and an asterisk denotes measurements that were not significantly greater than zero using Student's *t*-test at $\alpha = 0.05$.

observed for stems of WT plants of all stages, i.e. prior to bud formation, after budding but prior to elongation of the lateral branches bearing reproductive structures, and after buds and flowers were present on elongated lateral branches. Detached inflorescence stems of *wry* and WT plants showed responses similar to those of intact stems (Fig. 2C). These observations establish that the gravitropic response of *wry* inflorescence stems was reduced or absent compared with WT lettuce.

Hypocotyls: Following the application of a 90° gravitational stimulus, WT and *wry* hypocotyls showed similar growth kinetics, initially growing at an approximately linear rate that began to decrease 10 h after stimulation (Fig. 3). Significant differences in cumulative hypocotyl growth were detected among WT genotypes (Table 3). Although *wry* hypocotyl growth was significantly less than that of PI 251245, Salinas 88 and 00-235, it was not significantly less than that of Prizehead (Table 3). Despite different growth rates, all WT hypocotyls curved rapidly following gravistimulation. Curvature of 20–40° was measured 2 h after stimulation, and by 10 h, the total curvature had increased to 45–70°, depending on genotype (Fig. 3). Curvature of unstimulated controls was not significantly different from zero for any genotype. At the conclusion of the experiment, *wry* hypocotyls exhibited significantly less total curvature and curvature per unit of growth in response to gravistimulation than any WT genotype (Table 3). In both experiments, *wry* hypocotyls exhibited 12–14° mean curvature 2 h after stimulation; although significantly greater than zero, this value did not increase over time and was not significantly different from the curvature of unstimulated controls. These observations established that the gravitropic response of *wry* hypocotyls was absent or reduced compared to that of WT lettuce.

Roots: The growth kinetics of WT and *wry* roots were similar (Fig. 3). Despite variation in cumulative root growth among WT genotypes, *wry* roots exhibited significantly less growth than all WT genotypes (Table 3). Despite reduced growth, roots of *wry* seedlings exhibited a gravitropic bending response of similar speed and magnitude as WT genotypes (Fig. 3). Although the cumulative mean root curvature was less for *wry* than for some WT genotypes, in most cases the differences were not statistically significant. Furthermore, when their slower growth was taken into account, the curvature per unit growth of *wry* roots was equal to or greater than that of WT genotypes (Table 3). These observations establish that the gravitropic responses of *wry* roots were equivalent to those of normal lettuce seedlings.

Microscopic analysis

Inflorescence stems: Light microscopy of inflorescence stem cross-sections of 70-d-old plants revealed several layers of cortical cells between the stele and a clearly defined epidermal layer comprised of two to three cells. The number of cortical cell layers varied widely, both within and among genotypes. The mutant (00-241) and WT (00-235) sibling lines were very similar in appearance and could not be differentiated on the basis of number of cortical cell layers or other gross structural differences. Inflorescence stem cross-sections stained with IKI showed amyloplasts of similar size and frequency in a single layer of cells in the cortex of all WT genotypes, including 00-235 (Fig. 4A). By contrast, amyloplasts were not observed in IKI-stained cross-sections from any *wry* plants (Fig. 4B).

Hypocotyls: For all genotypes, cells in cotyledons and the uppermost region of the hypocotyl, the apical hook, were uniformly darkly stained by IKI solution, confirming the presence of starch in these tissues. Individual darkly stained amyloplasts were also observed below the apical hook (Fig. 4C, D). The vertical distribution of amyloplasts followed a gradient, with their frequency declining such that very few were usually observed in the lower half of the

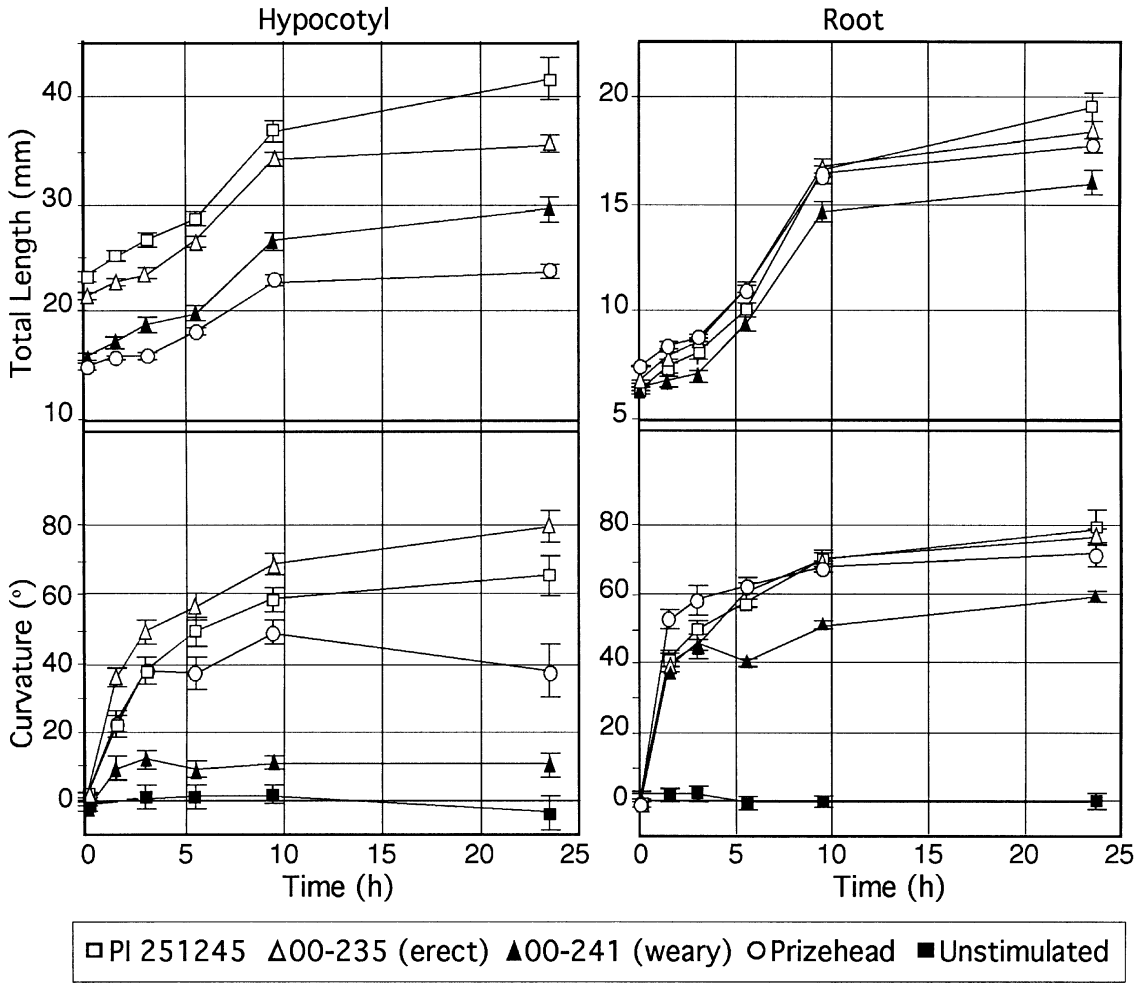

Fig. 3. Growth and reorientation of lettuce seedlings after a 90° gravitational stimulus. Dark-grown 3-d-old straight seedlings were turned horizontally (gravistimulated) or maintained vertically (unstimulated controls), and were exposed to light only during photodocumentation of growth and curvature. Growth and curvature of roots and hypocotyls were measured prior to stimulation and at several time points following stimulation. Mean ±SE of growth and curvature from experiment II are presented for weary (00-241) and three WT genotypes (PI 251245, 00-235, and Prizehead). For growth measurements, data from stimulated and unstimulated controls did not differ and were combined for each genotype. For curvature measurements, data from unstimulated controls of all genotypes were combined.

hypocotyl, near the interface between root and shoot. The size, abundance, and pattern of vertical distribution of amyloplasts were similar for both mutant and WT genotypes. By contrast, differences in the radial distribution of amyloplasts in mutant and WT hypocotyls were readily apparent in whole mounts as well as cross-sections. In whole mounts of WT seedlings, amyloplasts were observed in a narrow band surrounding the vascular tissue (Fig. 4C), whereas amyloplasts in the mutant were more broadly distributed, spanning nearly the entire width of the seedling (Fig. 4D). Cross-sections of all WT genotypes revealed that amyloplasts occurred only in a distinct single layer of cells, the endodermis, located just outside the stele (Fig. 4A). By contrast, amyloplasts in *wry* hypocotyls were distributed throughout several cell layers situated between the stele and the epidermis (Fig. 4B).

Roots: Using light microscopy of fixed and IKI-stained whole mounts of roots, amyloplasts were observed in the columella cells of seedling root caps of all genotypes examined (data not shown). No differences in distribution, size or number of amyloplasts were observed for the mutant and WT genotypes.

Discussion

The procumbent growth habit in the lettuce line 00-241 was found to be controlled by a recessive allele at a single locus, *weary* (*wry*), and was associated with the loss of some or all gravitropic responses in hypocotyls and inflorescences. Since reorientation following perception of a gravitational stimulus requires differential cell elongation, a reduction in growth alone could account for

Table 3. *Effect of a 90° gravitational stimulus applied to wild-type and weary lettuce seedlings*[a]

Trait measured	Genotype	Hypocotyl		Root	
		Experiment I	Experiment II	Experiment I	Experiment II
Cumulative growth (mm)[b]					
00-241	*wry*	92±6 c	98±3 c	99±9 b	95±4 c
PI 251245	WT	151±7 a	132±6 a	171±10 a	181±14 a
'Salinas 88'	WT	123±5 b	–[c]	166±7 a	–
'Prizehead'	WT	–	109±5 bc	–	132±8 b
00-235	WT	–	110±3 b	–	148±6 ab
Cumulative curvature (°)[d]					
00-241	*wry*	14±3 c	12±3 c	55±4 a	61±3 c
PI 251245	WT	74±4 a	66±6 a	71±5 a	106±8 a
'Salinas 88'	WT	60±3 b	–	61±4 a	–
'Prizehead'	WT	–	40±7 b	–	74±6 bc
00-235	WT	–	81±5 a	–	76±4 b
Unstimulated control	all	–1±5 c*	–2±5 c*	–7±7 b*	–4±4 d*
Cumulative curvature per unit of growth (°/mm)					
00-241	*wry*	17±5 b	10±4 cd	56±5 a	63±3 a
PI 251245	WT	60±6 a	50±7 ab	39±7 ab	58±8 a
'Salinas 88'	WT	49±4 a	–	34±5 b	–
'Prizehead'	WT	–	35±9 bc	–	63±6 a
00-235	WT	–	73±6 a	–	56±4 a
Unstimulated control	all	–1±8 b*	1±6 d*	–5±9 c*	–3±4 b*

[a]Dark-grown 2–3-d-old straight seedlings were turned horizontally (gravistimulated) or maintained vertically (unstimulated controls), and were exposed to light only during photodocumentation of growth and curvature. Cumulative growth and curvature were measured prior to stimulation and either 40 h (experiment I) or 23 h (experiment II) after stimulation. Mean ±SE for curvature, growth, and curvature per unit growth from two independent experiments (I and II) are presented. The number of seedlings measured per genotype varied from 4–17 for unstimulated controls and from 14–61 for gravistimulated seedlings. For each trait within an experiment, means followed by the same letter were not significantly different at the α=0.05 level using Tukey's HSD test. An asterisk denotes values that were not significantly greater than zero.

[b]Within each genotype, cumulative growth data were combined for gravistimulated and unstimulated seedlings.

[c]'–' = not tested.

[d]Curvature measurements of unstimulated controls from different genotypes were combined.

an apparent decrease in gravitropic response, and mutant seedlings did exhibit reduced growth compared with some WT genotypes. Even when reduced growth was taken into account, however, *wry* hypocotyls displayed significantly less curvature than WT hypocotyls. By contrast, the curvature per unit growth of *wry* roots was equal to or greater than that observed for WT roots, indicating that the gravitropic response of *wry* roots is not impaired. To the authors' knowledge, *wry* is the first gravitropic mutant characterized within the Asteraceae, a large and diverse plant family.

The pathways involved in perceiving light and gravity interact to determine plant growth (Correll and Kiss, 2002). Receptors of red and blue light, respectively, perceive the signals that trigger photoperiod responses and phototropism (Lin, 2002). The timing of the transition from vegetative to reproductive growth in lettuce is determined by photoperiod (Waycott, 1995; Ryder, 1988). Similar seasonal variation in time to bolting was observed for the wry mutant and WT PI 251245 and 00-235, suggesting that the photoperiodic sensitivity of *wry* plants is normal. Evidence that *wry* plants also have normal phototropic responses came from the observation that, when exposed to unilateral illumination, the phototropic responses of *wry* seedlings were as strong or stronger than those of WT seedlings (data not shown). Further support came from the observation that the mutant, like WT genotypes, grew towards the south-west in response to the asymmetrical illumination provided by the winter sun.

Based on existing models describing the mechanisms of gravitropism, the first step in this complex process is the sedimentation of amyloplasts within statocytes under the effects of gravity. The sedimented amyloplast presumably interacts with some component (s) of the statocyte, generating a signal to trigger the bending response. Signal transduction then leads to the establishment of an auxin gradient, and differential growth, i.e. bending, occurs. The majority of gravitropic mutants of *Arabidopsis* either lack amyloplasts in one or more organs, or have amyloplasts which are incapable of normal sedimentation. Five *Arabidopsis* mutants (*shoot gravitropism-1 (sgr1), sgr2, sgr4, sgr7*, and *amyloplastless-1 (eal1)*) have phenotypes similar to *wry* in that they exhibit normal root gravitropism, but abnormal gravitropic responses in both inflorescence stems and hypocotyls (Fukaki *et al*., 1996*b,* 1998; Yamauchi *et al*., 1997). Two of these, *sgr2* and *sgr4*, contain amyloplasts in endodermal cells, but the amyloplasts do not sediment normally (Kato *et al*., 2002). The *eal1* mutant appears to have a normal endodermis, but lacks amyloplasts in all shoot tissues (Fujihara *et al*., 2000). Both *sgr1* and *sgr7*, which are allelic to the radial patterning mutants *scarecrow* (*scr*) (Scheres *et al*., 1995; Di Laurenzio *et al*., 1996) and *short-root* (*shr*) (Scheres

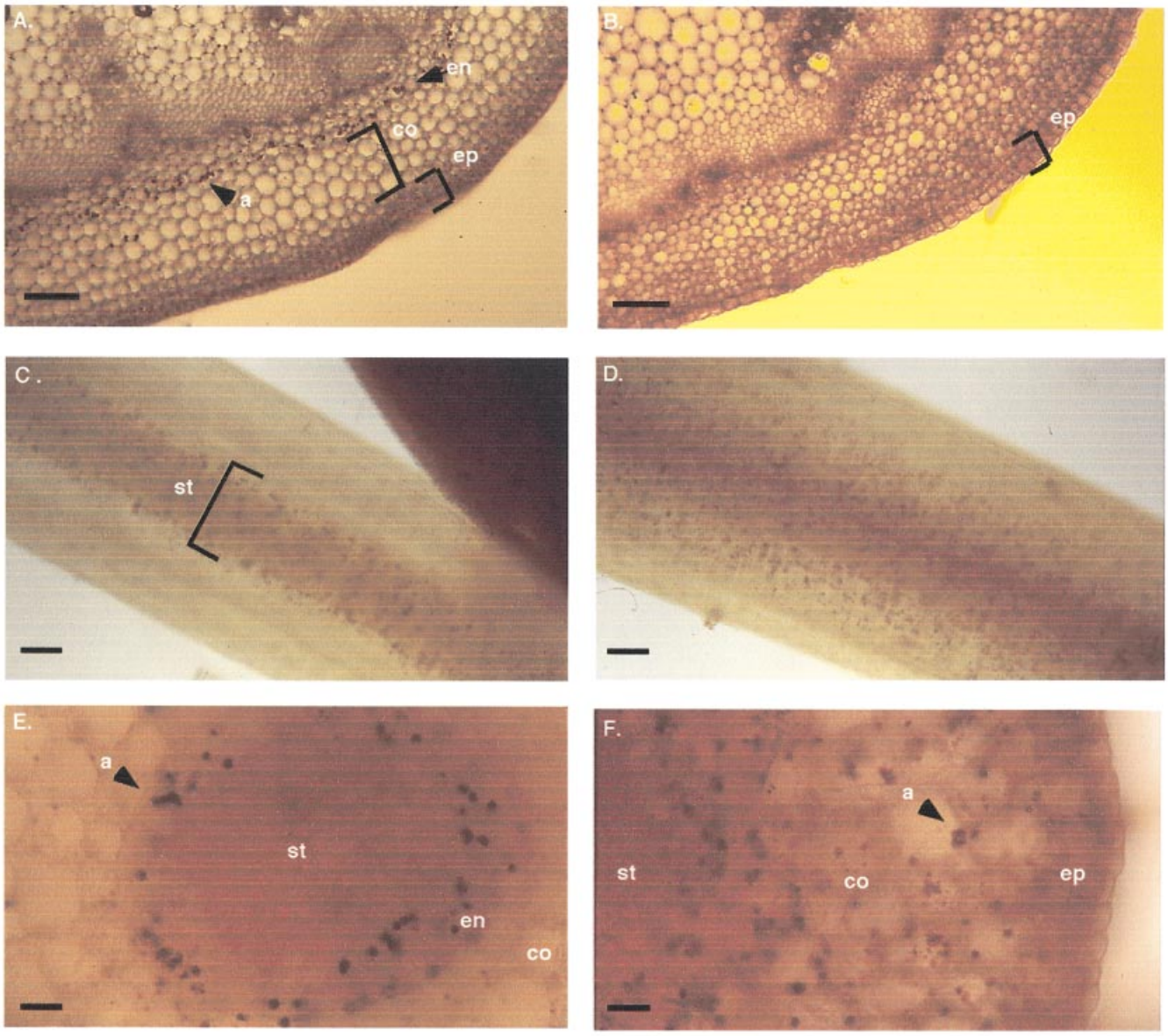

Fig. 4. Whole mounts and cross-sections of wild-type (A, C, E) and *weary* (B, D, F) inflorescences and hypocotyls stained with IKI and viewed with a light microscope. Cross-sections of inflorescences (A, B) were taken from 70-d-old plants and hypocotyl whole mounts (C, D) and cross-sections (E, F) were taken from 3-d-old seedlings. Wild-type genotypes pictured are 00-235 (A) and PI 251245 (C, E). The scale bar denotes 25 μm (A, B) or 100 μm (C, D, E, F). Abbreviations are: a, amyloplasts; co, cortex; en, endodermis; ep, epidermis; st, stele.

et al., 1995), respectively, lack a normal endodermal cell layer (Fukaki *et al*., 1998). Analyses of *scr*, *shr*, and *eal1* have established that sedimenting amyloplasts, which are normally found in the shoot endodermis, are required for full gravitropic sensitivity and that the endodermis itself is essential for shoot gravitropism in *Arabidopsis* (Fujihara *et al*., 2000; Fukaki *et al*., 1998; Kato *et al*., 2002). Specifically, analysis of *sgr2* and *sgr4* revealed that the vacuoles within endodermal cells play an important role, which may involve amyloplast distribution, gravitropic signalling, or both (Morita *et al*., 2002).

Starch and amyloplasts are synthesized in both roots and shoots of the *wry* mutant. Without endodermis-specific molecular markers or defining anatomical features, the presence or absence of a distinct endodermal cell layer in *wry* could not be conclusively determined. The differences in the radial distribution of amyloplasts in mutant and WT hypocotyls (Fig. 4E, F) and the absence of amyloplasts in *wry* inflorescence stems (Fig. 4A, B) suggest the absence of a normal endodermis and support the hypothesis that the WT gene product of the *weary* locus is required for normal development or differentiation of the endodermis. The

phenotype of *wry* is most similar to those of the *Arabidopsis* mutants *sgr1/scr* and *sgr7/shr*, whose WT gene products encode putative transcription factors and are essential for determining endodermal cell fate. Where WT *Arabidopsis* shoots have three cell layers (one endodermis and two cortex), *scr* and *shr* mutants have two irregular cell layers that have both endodermal and cortical features. Although *shr* hypocotyls lack amyloplasts, *scr* hypocotyls occasionally contain abnormally small and non-sedimenting amyloplasts within the inner irregular cell layer (Fukaki *et al*., 1998). Since *wry* hypocotyls contain amyloplasts, *wry* is more similar to *scr*, but unlike *scr*, *wry* amyloplasts are similar in size to WT amyloplasts. Whether or not these amyloplasts sediment normally is not known.

In *wry* inflorescence stems, the apparent lack of gravitropic response could be due to the absence of amyloplasts, preventing the earliest steps in gravitropic response. In hypocotyls, however, the presence of a large number of normal-sized amyloplasts suggests a different or additional defect in gravitropism. One possibility is that amyloplasts do not sediment normally, as with *sgr2* and *sgr4*. Alternatively, *wry* may have a new type of defect, where signal transduction or the generation of signal via interactions between the amyloplast and the statocyte are prevented. For example, the ability of the statocyte to generate or transmit the gravitropic signal properly may be a specific feature of the endodermis that is lacking in *wry* mutant cell layers. Alternatively, the amyloplast-containing cells of *wry* hypocotyls may perceive gravity and generate the necessary signals in sufficient quantity, but fail to produce a normal gravitropic response due to the altered location or distribution of cells producing the signals.

Hypocotyls of *wry* may have partial gravitropic sensitivity. Although dark-grown *wry* seedlings were as straight as WT seedlings prior to gravitropic stimulation, they exhibited positive mean curvature after stimulation (Fig. 3). Although not significantly different from unstimulated controls (Table 3), their response was significantly greater than zero in both experiments, suggesting at least a minimal ability to respond to gravity. Endodermal cells lacking amyloplasts may act as statocytes with reduced sensitivity, and have been proposed to account for the partial sensitivity of *eal1* in *Arabidopsis* and the restoration of gravitropic sensitivity in starch-deficient mutants grown in abnormally strong gravitational fields (Fitzelle and Kiss, 2001; Fujihara *et al*., 2000). Consistent with this hypothesis, mutants that lack the endodermis entirely (e.g. *scr* and *shr*) do not exhibit partial sensitivity under normal conditions (Fukaki *et al*., 1998). Whether the altered cell layers in *wry*, *scr* and *shr* are capable of serving as statocytes under conditions of hypergravity, effectively restoring gravitropic responses, is not known. Understanding the mechanism of partial gravitropic sensitivity in *wry*, if it exists, may be informative about the specific interactions that take place between the amyloplast and the statocyte. Little is currently understood about the nature of these interactions, which are essential for both the perception of gravity and the production of the signal that triggers the bending response.

In conclusion, this study suggests that the gene product of the dominant WT allele at the *weary* locus is likely to be involved in correctly determining endodermal identity or amyloplast distribution in lettuce. The reported observations are consistent with the proposed role of sedimenting amyloplasts in gravitropic sensing in higher plants. The phenotype of *wry* is very similar to that observed for *sgr1/scr* in *Arabidopsis*, and it is possible that the two loci are homologous. If this is the case, studies of the *wry* mutant may also provide information about the degree to which fundamental plant processes such as specification of organ and tissue identity are conserved within the plant kingdom. There may also be applied benefits to better understanding the gravitropic response mechanism(s) of lettuce, a plant that is targeted for use in hydroponic production systems in environments lacking gravity.

Acknowledgements

The authors thank R Aburomia, C Barr, C Bull, J Epting, D Milligan, E Radwanski, B Robinson, K Schlueter, and J Tanaka for materials, technical assistance and valuable discussions. The authors also thank R Aburomia, E Radwanski, E van der Knapp, and W Waycott for critical review of this manuscript, and express gratitude to the California Lettuce Research Board and to the US Department of Agriculture, Agricultural Research Service for financial support of this work.

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