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Simulations of virtual plants reveal a role for *SERRATE* in the response of leaf development to light in *Arabidopsis thaliana*

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Summary

• The *SERRATE* gene (*SE*) was shown to determine leaf organogenesis and morphogenesis patterning in *Arabidopsis thaliana*. The *se-1* mutant was used here to investigate the role of *SE* in leaf development in response to incident light. Virtual plants were modelled to analyse the phenotypes induced by this mutation.

• Plants were grown under various levels of incident light. The amount of light absorbed by the plant was estimated by combining detailed characterizations of the radiative environment and virtual plant simulations.

• Four major changes in leaf development were induced by the *se-1* mutation. Two constitutive leaf growth variables were modified, with a lower initial expansion rate and a higher duration of expansion. Two original responses to a reduced incident light were identified, concerning the leaf-initiation rate and the duration of leaf expansion.

• The *se-1* mutation dramatically affects both changes in the leaf development pattern and the response to reduced incident light. Virtual plants helped to reveal the combined effects of the multiple changes induced by this mutation.

Key words: absorbed radiation, *Arabidopsis thaliana*, leaf development, light intensity, morphogenesis, organogenesis, *SERRATE*, virtual plant.

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Introduction

Higher plants display a high degree of plasticity in developmental responses to the environment. Given the importance of photosynthesis to plant functioning, light is one of the most significant environmental factors. In *Arabidopsis thaliana*, decreases in light intensity induce many changes in organogenesis, morphogenesis and plant architecture for the optimization of light interception (Chenu *et al.*, 2005). Light affects leaf morphogenesis in terms of leaf expansion, blade shape, cell number and size, specific leaf area and petiole length (Pigliucci & Kolodynska, 2002; Chenu *et al.*, 2005; Cookson & Granier, 2006).

The *SERRATE* (*SE*) gene was recently shown to be involved in specializing the leaf surfaces by controlling adaxial cell fate (Grigg *et al.*, 2005). It was suggested that *se* mutants might display unusual light-response phenotypes. The *SE* gene has pleiotropic effects and encodes a zinc-finger protein that may regulate the expression of other genes by controlling chromatin activity (Prigge & Wagner, 2001). *SE* is involved in organogenesis and morphogenesis, and is expressed throughout plant development, from embryonic development to flower production (Prigge & Wagner, 2001). The *se-1* mutant obtained by X-ray mutagenesis has a weak *SE* allele, resulting in unusual phenotypes. This mutation impairs shoot apical meristem activity, affecting leaf emergence rate, leaf number, phyllotaxy and the transitions from juvenile to adult, and vegetative to reproductive phases (Clarke *et al.*, 1999; Serrano-Cartagena *et al.*, 1999). The *se-1* mutant also displays changes in organ morphogenesis, with reduced leaf and root expansion (Groot

Table 1 Environmental conditions (treatments correspond to different levels of incident light)

Expt number	Treatment	Incident PAR (mol m^{-2} d^{-1})	HR_{air} (%)	VPD leaf–air (kPa)	Rosette temperature (day : night) (°C)
1	Standard	9.3	72.3	0.67	21.0 : 19.1
1	Moderate	6.4	72.3	0.54	20.3 : 19.4
1	Severe	3.7	72.3	0.49	20.0 : 19.5
2	Standard	11.2	74.6	0.92	22.1 : 16.5
2	Moderate	5.0	74.6	0.85	19.9 : 16.2
2	Severe	2.7	74.6	0.67	18.0 : 16.1

Means of daily incident photosynthetically active radiation (incident PAR), air humidity (HR_{air}), vapour pressure deficit (VPD) between the leaves and the atmosphere, and rosette temperature were calculated using measurements taken from plant emergence until the end of leaf expansion for all leaves. VPD values were averaged from measurements taken throughout the light phase.

& Meicenheimer, 2000; Prigge & Wagner, 2001), serrated leaf blades and elongated petioles (Serrano-Cartagena *et al.*, 1999). The architecture of *se-1* plants (number of leaves, phyllotaxy, petiole and blade morphology) therefore contrasts strongly with that of wild-type Columbia plants.

Plant morphology, and petiole length, in particular, play a key role in light interception in *A. thaliana* (Chenu *et al.*, 2005). Quantitative three-dimensional models of plant development can be used to account for differences in architecture (Prusinkiewicz, 1998) and to define precisely the basis of leaf developmental plasticity in response to absorbed light. Such quantitative models have recently been constructed for *A. thaliana* (Mündermann *et al.*, 2005) and could be used to improve the understanding of plant developmental physiology and genetics. Chenu *et al.* (2005) successfully used a model of this type for the analysis and better elucidation of leaf development responses to various levels of incident light in *A. thaliana* accession Columbia. This model is used and adapted here for the *se-1* phenotype to analyse the involvement of *SE* in the leaf development response to light, and to determine whether this response resulted from the changes in plant morphology induced by the *SE* gene. Leaf development and rosette architecture were investigated in *se-1* and Columbia plants grown under various light levels, kept constant throughout the vegetative development period. The incident photosynthetically active radiation (PAR) absorbed by the plant was estimated using 3-D virtual plants coupled to a radiative model. Rosette architecture and leaf development were characterized at the organ level. Leaf development was assessed in terms of the date of leaf initiation, the relative leaf-expansion rate and the duration of leaf expansion.

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh. plants, accessions Columbia (Col-0, N907) and *serrate* (*se-1*, CS3257), were grown in plastic containers (0.5 m wide, 0.2 m long and 0.15 m deep) filled with a mixture (1 : 1, v/v) of loamy soil and organic compost, in a growth chamber (Conviron E15, Controlled Environments LTD, Winnipeg, Manitoba, Canada). Seeds were incubated at 4°C for 3 d. They were then suspended in water and sown individually, at one seed cm^{-2}. Plant density was reduced twice a week to ensure the plants did not overlap and to limit neighbour photodetection (Ballaré, 1999). Soil water content was maintained at a constant level, close to soil storage capacity, by daily watering with Hoagland solution (diluted to 1/10 original strength). Light was provided, with a 16-h photoperiod, by a bank of cool-white fluorescent tubes (neon Slimline F72T12CW, OSRAM Sylvania GmbH, Munich, Germany) and halogen bulbs (Halolux, 100 W, OSRAM GmbH, Munich, Germany).

Air temperature and relative air humidity were measured at plant height with a thermohygrometer (HMP35A Vaisala Oy, Helsinki, Finland) shaded from incident radiation. Rosette temperature was measured using microthermocouples (Cooper-Constantan, 0.08 mm in diameter) placed in the soil until the emergence of the first leaves and then positioned against the leaf abaxial surface. Incident light was measured at plant level, using a PAR (400–700 nm) sensor (LI-190SB, Li-Cor, Lincoln, NE, USA). For each treatment, measurements were taken every 20 s and were averaged and stored every 600 s, using a datalogger (CR10X, Campbell Scientific Inc, Shepshed, UK). The corresponding environmental conditions are described in Table 1.

Light treatments

Shading nets (cloth no. 13, Bouillon, Paris, France) were used to vary the level of incident radiation and to ensure incident PAR was homogeneous within each treatment, as described by Chenu *et al.* (2005). Plants were subjected to three light treatments (Table 1): 'standard' treatments, >7.5 mol m^{-2} d^{-1} (130 µmol m^{-2} s^{-1}), corresponding to treatments that did not affect plant leaf expansion (Chenu *et al.*, 2005); 'moderate'

decreases in light intensity, to 4–6.5 mol m^{-2} d^{-1} (70–113 µmol m^{-2} s^{-1}); and 'severe' decreases in light intensity, to <4 mol m^{-2} d^{-1} (70 µmol m^{-2} s^{-1}).

The spectral distribution of incident radiation was determined with a spectroradiometer (LI-800, Li-Cor) at plant level for each treatment. Phytochrome photoequilibrium and P_r/P_{fr} ratio were calculated as described by Sager *et al.* (1988); Smith (1982), respectively. These values were not affected by the shading nets and remained constant for all the treatments at 0.74 and 1.00, respectively. Blue light was defined as the photon flux density between 350 and 500 nm (Gautier *et al.*, 2001) and accounted for 16% of incident PAR in each treatment.

Plant measurements

Samples of six plants were harvested every 2–3 d during the first 10 d after plant emergence, and then every 3–4 d until the end of vegetative development. Plants were dissected under a microscope (Leica wild F8Z stereomicroscope, Leica Wetzlar, Germany) coupled to a video camera (Sony CCD-IRIS/RGB colour video camera, Japan). The blade area of every leaf of the harvested plants was determined with an image analyser (Bioscan-Optimas V4.10, Edmonds, WA, USA). Architectural measurements were performed for construction of a 3-D virtual plant and estimation of the amount of PAR absorbed by the plant. Lengths and widths of blades and petioles were measured at every other sampling. Phyllotaxy and zenithal angles were measured once a week with a digital protractor (Pro 360, Travers, NY, USA).

Leaf development variables

Variables relating to leaf development were expressed as a function of thermal time (*tt*; cumulative degree days, °Cd), making it possible to take into account the slight differences in rosette temperature between treatments (Table 1). Daily thermal time was calculated as the difference between the daily mean rosette temperature and a base temperature of 3°C (Granier *et al.*, 2002).

The number of initiated leaves was determined by counting. Leaves were considered to be initiated when their area reached approx. 0.001 mm^2. A linear fit of the relationship between the natural logarithm of leaf area and thermal time accumulated was used to estimate the time of leaf initiation. Plant emergence was defined as the first leaf initiation, which corresponded approximately to cotyledon unfolding.

Leaf-initiation rate (IR) was estimated by calculating the local slope of the relationship between the number of initiated leaves (N) and thermal time (tt), as follows:

$$IR = \frac{dN}{dtt} \quad \text{Eqn 1}$$

Blade area (A) was calculated over thermal time (tt) for each leaf, as follows:

$$A = \frac{A_f}{1 + \exp\left(4LER_m \frac{tt_m - tt}{A_f}\right)} \quad \text{Eqn 2}$$

where A_f is the final blade area, tt_m is the thermal time at which leaf-expansion rate is maximal, and LER_m is the maximum leaf-expansion rate.

The relative expansion rate (RER) of each leaf at time t was estimated by calculating the local slope of the relationship between the Napierian logarithm of leaf area (A) and thermal time (tt):

$$RER = \frac{1}{A}\frac{dA}{dtt} = \frac{d(\ln A)}{dtt} \quad \text{Eqn 3}$$

For both genotypes, the duration of the light-sensitive period was defined for a given leaf as the period between leaf initiation and the thermal time at which no significant difference ($P = 0.05$) in the RER could be observed for any light treatments within each experiment. Mean relative expansion rates during this light-sensitive period (RER_s) were calculated for each leaf as follows:

$$RER_s = \frac{\ln A_s - \ln A_i}{tt_s} \quad \text{Eqn 4}$$

where A_s is the estimated blade area at the end of the light-sensitive period (calculated using equation 2), A_i is the blade area at leaf initiation (0.001 mm^2), and tt_s is the duration of this period expressed in thermal time.

The total duration of leaf expansion was calculated in thermal time as the period from leaf initiation to the date when this organ reached 97% of this estimated final area (A_f, equation 2).

3-D virtual plants

Three-dimensional virtual plants (Fig. 1) were constructed on a daily basis for each treatment, using AMAPsim software (Barczi *et al.*, 1997; Rey *et al.*, 1998; for a detailed description see http://amap.cirad.fr). The positions, shapes and sizes of the various organs were estimated for average plants, from measurements of blade (length, width and area), petiole (length and width) and organ angles (zenithal and azimuthal angles).

Plant geometry was specific to each genotype. Different symbols were used for the blade, with some serrations in the case of *se-1*. The blade shape varied with the leaf rank and with the experimental treatment. It was simulated based on observed data for the ratio between blade length and width. The phyllotaxy was considered as stable over time and experimental situations. Zenith angles of the different phytomers decreased over time, following the same pattern as observed.

Organ size was simulated differently depending on experimental conditions. Leaves of 0.001 mm^2 were initiated at a thermal time estimated from observed data, as described previously. Leaf blades expanded in response to temperature

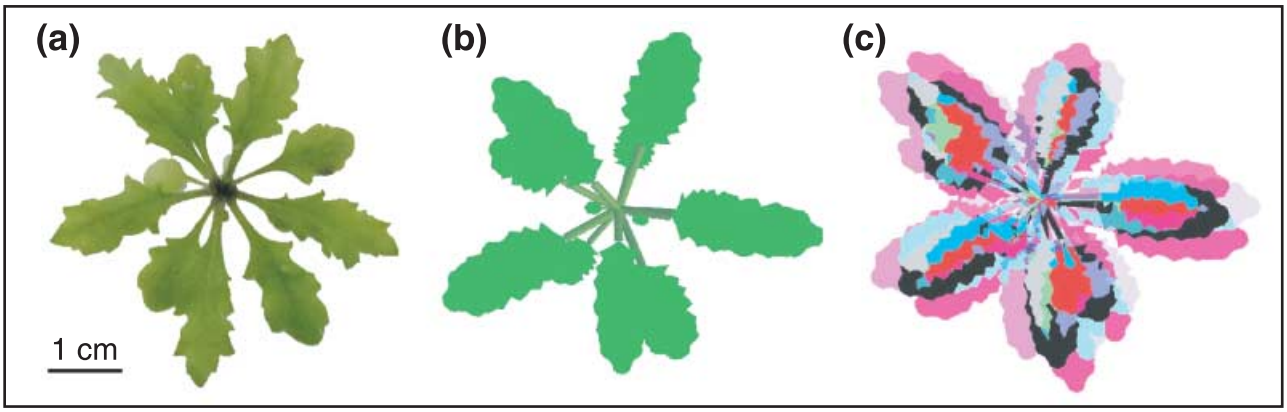

Fig. 1 (a) Example of an observed *se-1 Arabidopsis thaliana* plant under unlimited radiation conditions; (b) 3-D virtual plant corresponding to a mean representation of the observed plants; (c) map of light intercepted by the rosette, taking into account the directional radiative climate. Comparison of the projected areas of 3-D virtual plants with those of the corresponding real plants showed a linear relationship ($y = 1.045x$) with $r^2 = 0.983$ and coefficient of variation of error (CV_{error}) = 0.163.

(equation 2). The petiole length ($L_{petiole}$) and width ($W_{petiole}$) were estimated in relation to the blade area (A) of the leaf considered:

$$L_{petiole} = a + bA^c \quad \text{Eqn 5}$$

$$W_{petiole} = d + e\ln(A) \quad \text{Eqn 6}$$

where a–e were fitted parameters estimated for each experimental situation (data not shown).

Estimation of the amount of PAR absorbed by the plant

The 3-D virtual plants generated were used to estimate the radiative balance of the plants during each light treatment (Dauzat & Eroy, 1997). A light sensor based on the 'Turtle' model of den Dulk (1989) was adapted for measuring the directional components of the radiative climate in the growth chamber. This sensor was made up of six individual PAR sensors positioned to measure light from six directions (one zenith-facing and five azimuth-facing; all inclined to 26.57° from horizontal). The measurements taken during each treatment were then used to calculate the numerical radiative balance. Three different types of software (http://amap.cirad.fr) were used to simulate the radiative transfers within the plant (Dauzat & Eroy, 1997): MIR to estimate the fraction of the incident flux intercepted by the plant organs and the soil; MUSC to estimate the multiple scattered fluxes between plant elements; and RADBAL to combine the results of the MIR and MUSC analysis for the six directional light sources. Scattered fluxes, including transmittance and reflectance measurements, were measured using a spectroradiometer (Fieldspec, ASD Inc., Boulder, CO, USA). Values of 0.23 for the plant components and 0.06 for the soil were used for all the light treatments. The use of the three different programs allowed us to estimate the amount of PAR intercepted and absorbed by the plant on a daily basis.

Statistical analysis

Linear and nonlinear adjustments were performed using TABLECURVE 2D (Systat Software Inc., Richmond, CA, USA).

The ANOVA/MANOVA procedure of STATISTICA 6.0 (Statsoft, Tulsa, OK, USA) was used to test for significant differences between means. Differences between the regressions for data sets were assessed by comparing $\Sigma\,SS_i$ (sum of the residual sums of squares for individual fits to each data set) with SS_c (residual sum of squares for a common fit to the whole data set) as follows:

$$F = \frac{\left| SS_c - \sum_{i=1}^{n} SS_i \right| \Big/ ((n-1)k)}{\sum_{i=1}^{n} SS_i \Big/ (N_{data} - k)} \quad \text{Eqn 7}$$

where N_{data} is the total number of data points, n is the number of individual regressions and k is the number of parameters fitted for each regression. The F function follows Fisher's law with $(n-1)k$ and $(N_{data} - k)$ degrees of freedom.

Results

Plant leaf area

Under standard light conditions, Columbia (Col) and *serrate* (*se-1*) differed in terms of plant leaf area (Fig. 2). Plant leaf area was significantly smaller in *se-1* than in Col. This

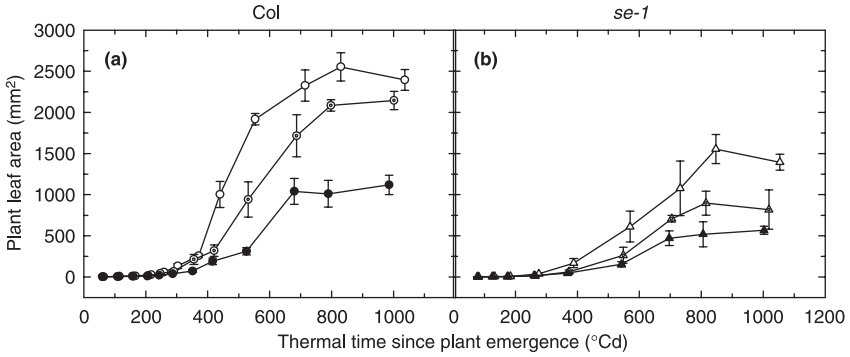

Fig. 2 Changes in *Arabidopsis thaliana* plant leaf area with thermal time since plant emergence for (a) Col plants; (b) *se-1* plants. Data from Expt 1. Open symbols, standard treatment; dotted symbols, moderate treatment (moderate shading); closed symbols, severe treatment (severe shading). Error bars indicate confidence limits at $P = 0.05$.

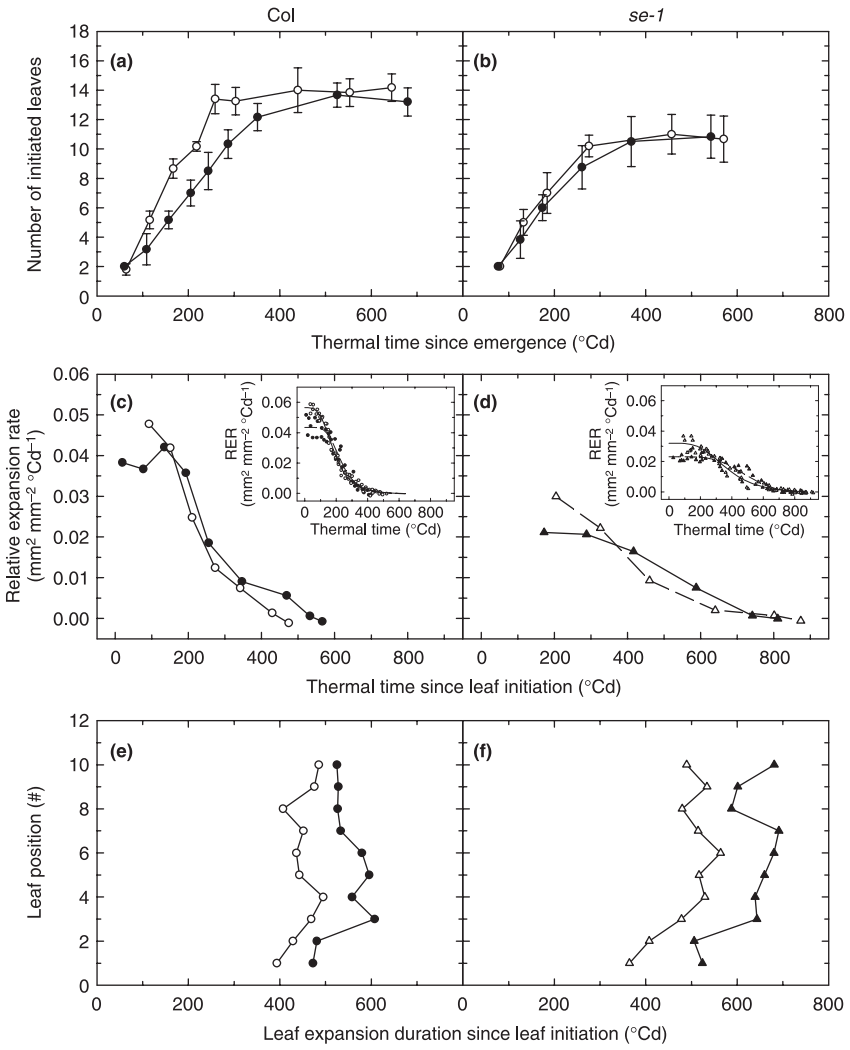

Fig. 3 Characteristics of leaf development in Col (left) and *se-1* (right) *Arabidopsis thaliana* plants. (a,b) Number of initiated leaves over thermal time since plant emergence; (c,d) change in relative expansion rate (RER) of leaf 6 with thermal time since leaf initiation; (e,f) total duration of leaf expansion vs leaf position on the stem for the first 10 leaves. Insets, change in RER of individual leaves (up to leaf 10) with thermal time since leaf initiation. Data from Expt 1. For clarity, only two light treatments are shown. Open symbols, standard treatment; closed symbols, severe treatment (moderate shading). Error bars indicate confidence limits at $P = 0.05$.

difference was observed from the early stages of plant development (300°Cd following plant emergence) and increased during plant development. Differences in final plant area were partly caused by there being fewer leaves in *se-1* compared with Col (Fig. 3a,b).

Lowering the level of incident light significantly decreased final plant leaf area ($P < 0.05$) in both Col and *se-1* from early stages (Fig. 2). In each reduced light treatment, all individual leaves were reduced in size (data not shown). At the lowest level of incident light, plant leaf area was

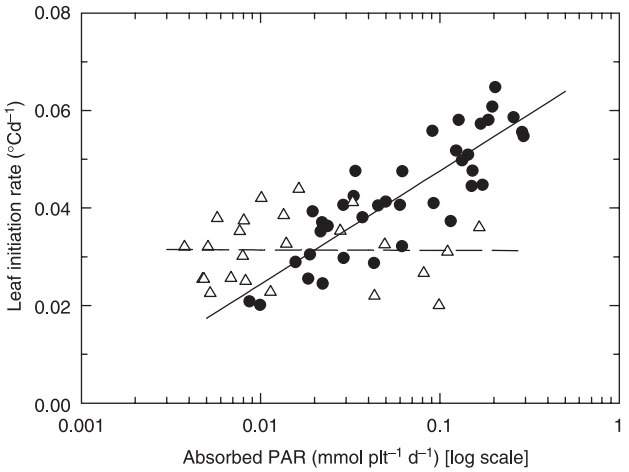

Fig. 4 Leaf-initiation rate vs amount of light absorbed by Col and *se-1 Arabidopsis thaliana* plants. Data from all experiments have been used. Data correspond to means of measurements of individual leaves from position 3 upwards. Circles, Col; triangles, *se-1*. Fit: $y = a \times 10^{-3} \log(x) + b \times 10^{-3}$; Col, $a = 23.3 \pm 6.0$, $b = 70.9 \pm 4.7$, $r^2 = 0.739$, coefficient of variation of error (CV_{error}) = 0.14; *se-1*, $a = -0.1 \pm 6.2$, $b = 31.2 \pm 11.5$, $r^2 = 0.000$, $CV_{error} = 0.22$.

decreased in Col and *se-1* – 56 and 63%, respectively, of the standard treatment.

Comparison of the patterns of leaf development in Columbia and *serrate*

Leaf-initiation rate was lower in the *se-1* mutant than in Col under standard light treatment (Fig. 3a,b). On average, in the different experiments, Col produced a leaf every 20 ± 2°Cd, whereas the *se-1* mutant produced a leaf every 29 ± 3°Cd. Decreases in incident light delayed the initiation of successive leaves from leaf 3 upwards in Col (Fig. 3a). The thermal time period required to produce the last leaves was >40% longer in the lowest light treatment compared with the standard treatment. Interestingly, leaf initiation was not significantly affected by a large decrease of incident light level in *se-1* plants (Fig. 3b).

After initiation, each leaf went through a quasi-exponential phase of expansion, with an almost constant RER. This first phase of exponential expansion was followed by a longer phase in which RER decreased (Fig. 3c,d). During the first phase of expansion, *se-1* plants had a lower RER than Col plants under standard treatment. However, this first phase of expansion was longer in *se-1* than in Col. The second phase of expansion was also longer in *se-1* than in Col, with higher RER values in *se-1* than in Col.

Reducing incident radiation level decreased RER during this almost exponential phase of expansion, and increased the duration of this phase, in both Col and *se-1* plants. Early in leaf development, when significant decreases in RER ($P < 0.05$) were observed in response to decreases in incident light levels, RER decreased by 24 and 27% in Col and *se-1* plants, respectively, for the most severe treatment. During the second phase of leaf development, RER values were similar for the various light intensities tested in Col, whereas they were significantly higher when plants were shaded in *se-1*.

Leaf expansion continued for longer in *se-1* than in Col plants under standard treatment (Fig. 3e,f). The duration of expansion was 458 ± 20 and 513 ± 21°Cd for Col and *se-1* plants, respectively, for leaves formed after leaf 2. Lowering the light level increased the duration of leaf expansion in both Col and *se-1* plants. The duration of expansion was 21.6 and 30.2% higher for the severe treatment than for the standard treatment in Col and *se-1* plants, respectively.

Leaf initiation in response to absorbed light

3-D virtual plants coupled with a radiative balance model were used to estimate the amount of light absorbed by the plant. The calculation of this variable makes it possible to establish consistent quantitative relationships (Figs 4, 5), improving our understanding of plant responses to the radiative environment.

For Col, leaf-initiation rate was found to be linearly and positively related to the amount of light absorbed by the plant, plotted on a logarithmic scale (Fig. 4). The amount of light absorbed varied with plant age and incident light level, resulting in a wide range of values corresponding to variation by a factor of 100 during the period of leaf initiation (from 0.0038 to 0.34 mmol per plant d^{-1}). The relationship held for all leaves from position 3 upwards, for all plant leaf areas studied and all light treatments tested.

By contrast, the leaf-initiation rate of *se-1* plants appeared to be insensitive to the amount of light absorbed by the plant. The mean rate of leaf production in *se-1* plants was 0.0312 leaves $°Cd^{-1}$, corresponding to the production of one leaf every 2 d at 19°C. The slope of this relationship was not significantly different from 0. The data scattering observed for each genotype resulted mainly from destructive sampling and the inherent differences between selected plants. It led to variability in estimates of the date of leaf initiation. However, despite this variability, highly significant differences in the slope of the response lines ($P < 0.001$) were found between Col and *se-1* plants.

Leaf-expansion rate in response to absorbed light

For both genotypes, lowering the level of incident light significantly decreased RER during the initial stages of leaf development (Fig. 3c,d). This light-sensitive period corresponded to the first 200 and 250°Cd of leaf development for Col and *se-1* plants, respectively. Relative expansion rate during the light-sensitive period (RER_s) was linearly related to the amount of light absorbed by the plant, plotted on a logarithmic scale, for both Col and *se-1* plants (Fig. 5). This

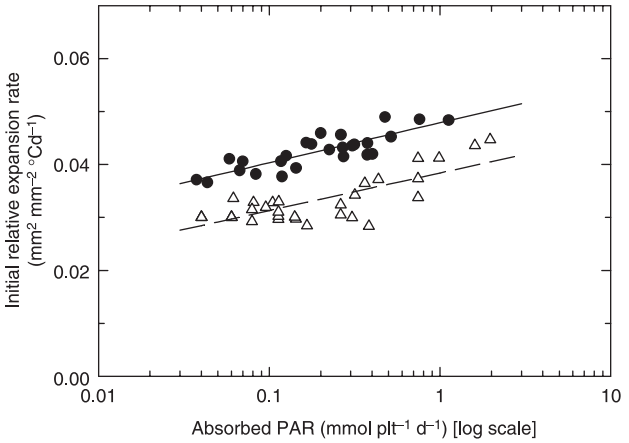

Fig. 5 Leaf relative expansion rates during the light-sensitive period vs the amount of light absorbed by Col and *se-1 Arabidopsis thaliana* plants. Data from all experiments have been used. Data correspond to the means of measurements of individual leaves from positions 3–6. Circles, Col; triangles, *se-1*. Fit: $y = a \times 10^{-3} \log(x) + b \times 10^{-3}$; Col, $a = 7.56 \pm 2.07$, $b = 47.9 \pm 1.7$, $r^2 = 0.703$, coefficient of variation of error (CV_{error}) = 0.04; *se-1*, $a = 7.11 \pm 2.27$, $b = 38.4 \pm 2.1$, $r^2 = 0.567$, $CV_{error} = 0.09$.

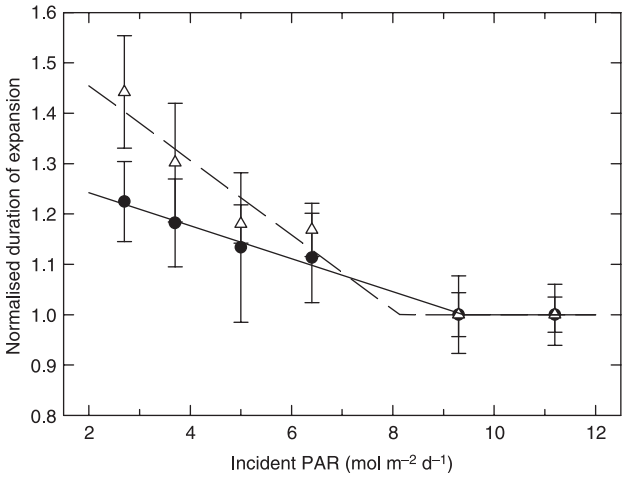

Fig. 6 Duration of leaf expansion vs incident light in Col and *se-1 Arabidopsis thaliana* plants. Data from all experiments have been used. Data correspond to the means of measurements of individual leaves and are expressed as the ratio of the recorded expansion duration to that for the standard treatment. Error bars indicate confidence limits at $P = 0.05$. Circles, Col; triangles, *se-1*. Fit: Col, when $x < 9.4$, $y = a \times 10^{-3}x + b$, else $y = c$; $a = -32.8 \pm 7.0$, $b = 1.31 \pm 0.04$, $c = 1.00 \pm 0.04$, $r^2 = 0.991$, coefficient of variation of error (CV_{error}) = 0.008; *se-1*, when $x < 8.1$, $y = a \times 10^{-3} x + b$, else $y = c$; $a = -74.2 \pm 53.6$, $b = 1.60 \pm 0.25$, $c = 1.00 \pm 0.11$, $r^2 = 0.956$, $CV_{error} = 0.031$.

relationship applied to all leaves in positions 3–6 in the rosette, for all the plant leaf areas and light treatments tested.

For the absorption of a given amount of light by the plant, *se-1* plants had significantly lower initial RER than Col plants ($P < 0.01$) (Fig. 5). However, Col and *se-1* plants had similar RER_s responses to the amount of light absorbed by the plant, as the slopes of the two regression lines did not differ significantly. A 10-fold increase in the amount of light absorbed by the plant led to an increase of 0.007 $mm^2\ mm^{-2}\ °Cd^{-1}$, corresponding to a 15% increase in RER_s. This increase in RER_s has a dramatic effect on leaf area. With no other change in leaf development, this change in RER_s would lead to an increase in final leaf area of >300% for a Col plant grown in standard light conditions.

Leaf expansion duration in response to incident light

The increase in the duration of leaf expansion was related to the intensity of incident light in both Col and *se-1* plants (Fig. 6). Leaf expansion duration responded to changes in light intensity in two phases. Above a threshold of incident light intensity, the duration of leaf expansion was constant. This threshold was 9.4 ± 0.9 and 8.1 ± 1.7 mol $m^{-2}\ d^{-1}$ in Col and *se-1*, respectively. As light intensity decreased below this incident light threshold, increases in the duration of leaf expansion were observed. When normalized with the value obtained in standard conditions, the duration of expansion was significantly different in response to decreases in light intensity in Col and *se-1* leaves ($P < 0.0001$) (Fig. 6). For a 1 mol $m^{-2}\ d^{-1}$ decrease in incident light levels, the duration of leaf expansion increased by 3.3 and 7.4% in Col and *se-1* plants, respectively, indicating that *se-1* was more sensitive than Col to decreases in light intensity.

Discussion

Involvement of *SERRATE* in leaf-expansion pattern

SE plays a key role in early leaf development. Kinematic analysis revealed that the decrease in leaf emergence rate in *se-1*, observed previously by Clarke *et al.* (1999), resulted from changes in two developmental processes. First, the initiation of leaf primordia at the apex was slower in *se-1* than in Col plants (Fig. 3a,b). Second, the initial expansion rate was lower in *se-1* than in Col plants, whereas the exponential phase of expansion lasted longer in *se-1* (Fig. 3c,d). *SE* appears to be involved in various processes associated with early leaf development, occurring during and after leaf initiation. Consistently, the *SE* gene is expressed in both the shoot apical meristem and the adaxial leaf domain of emerging leaf primordia (Prigge & Wagner, 2001). Grigg *et al.* (2005) recently showed that *SE* controls the competence of shoot tissue to respond to *KNOX* activity, which is required for meristem function; and the expression of *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*), which are required to commit cells to an adaxial cell fate (McConnell & Barton, 1998; Fleming, 2005). *SE* therefore regulates both the

meristem activity that leads to primordium initiation, and the leaf axial patterning that may drive leaf expansion. Several studies have shown that juxtaposition of the adaxial and abaxial tissues triggers lateral growth of the leaf to form a flattened lamina (Waites & Hudson, 1995; Waites *et al.*, 1998; Bowman *et al.*, 2002). This work suggests that the differentiation of tissues into adaxial and abaxial domains may play a role in determining leaf-expansion rate in the early stages of leaf development.

The total duration of leaf expansion was greater in *se-1* than in Col (Fig. 3e,f). The smaller final leaf area of *se-1* than of Col plants thus resulted from the antagonistic effects of decreases in the initial rate of expansion and increases in the duration of expansion. We suppose that the late effects of the *se-1* mutation on leaf expansion could be driven by early events. The results of several previous studies are consistent with this hypothesis. For example, cell division and expansion are temporally and spatially coordinated (Granier & Tardieu, 1998; Donnelly *et al.*, 1999), and leaf shape and final size seem to be determined in the early stages of leaf development. A negative correlation between early and late expansion has been reported in many genotypes of *A. thaliana* and for different environmental conditions (Chenu *et al.*, 2005; Cookson *et al.*, 2005; Granier *et al.*, 2006; Cookson *et al.*, 2007). The difference in duration of leaf expansion between *se-1* and Col plants may thus result from early differences in leaf development. However, the expression of *SE* in the later phase of leaf development has not been well characterized, and it is still possible that *SE* plays an additional role in the duration of leaf expansion.

Involvement of *SERRATE* in leaf plasticity in response to light

SE had a marked effect on the response of leaf initiation to light. The leaf-initiation rate of *se-1* plants, unlike that of Col plants, was not affected by incident light level over the ranges tested. The consistent relationship between leaf-initiation rate and the amount of light absorbed in Col plants (Fig. 4) suggests that carbon metabolism may be involved in leaf initiation by the meristem (see discussion of Chenu *et al.*, 2005). Consistent with this hypothesis, the spatial distribution of carbohydrate metabolism regulation within the meristem is correlated with the parts of the meristem destined to form leaves, suggesting that carbohydrate metabolism is involved in organogenesis (Pien *et al.*, 2001). As the formation of leaf primordia in *se-1* plants was not affected by the amount of light absorbed, we hypothesize that the *SE* gene could affect the organogenesis by involvement in sugar-sensing systems. Interestingly, *SE* is implicated in the timing of transition between the juvenile and adult phases (Clarke *et al.*, 1999), and this transition phase is thought to be regulated by sugar balances (Gibson, 2005).

Leaf expansion in *se-1* plants was affected by shading, with a decrease in the initial rate of leaf expansion, an increase in the duration of leaf expansion, and a decrease in final leaf area. However, there was no genotype–environment interaction for the process of initial expansion rate as affected by light absorption, as the regressions were parallel for the *se-1* and Col plants (Fig. 5). The mutation of *SE* did not affect the initial expansion response to absorbed light.

Conversely, the duration of expansion was slightly more sensitive to shading in *se-1* than in Col plants (Fig. 6). This difference in sensitivity could possibly result from differences occurring during the early stage of leaf development, as discussed previously. The initial RER of a leaf was proportionally more affected by the shading in *se-1* than in Col. Further investigations are needed to understand this regulation of the duration.

The overall consequences of the *se-1* mutation on leaf development responses to incident light result in a decreased impact of reduced incident light on the plant leaf area. As the plant leaf area is widely used as a fitness indicator by environmentalists (e.g. Gaudet & Keddy, 1988), the *se-1* mutation of the *SERRATE* gene could confer an advantage to *A. thaliana* plants in shaded environments.

Use of a virtual plant to identify a novel phenotype induced by a single mutation

Virtual plants were used to estimate the absorbed light covariable and thus to quantify plant responses through consistent quantitative relationships. In the past, light interception by the plant was estimated at the canopy level, by measuring light levels above and below the canopy or by calculations based on leaf area index (LAI, leaf area per unit area of soil). Advances in the 3-D modelling of plant architecture (Room *et al.*, 1996; Prusinkiewicz, 2004) and in radiative models have made it possible to take interactions between the plant and its radiative environment into account, and thus to evaluate the light microclimate at organ level (Chelle & Andrieu, 1999; Chelle, 2005). Greater knowledge of the plant microclimate provides a better understanding of plant–environment interactions. A combination of architectural and 'phylloclimatic' modelling (Chelle, 2005) has already been used for plant studies in ecology and plant physiology. Such studies have provided insights into the impact of individual architectural traits on light interception (Falster & Westoby, 2003; Chenu *et al.*, 2005, 2007; Pearcy *et al.*, 2005) or plant response to light interception (Fournier & Andrieu, 1999; Gautier *et al.*, 2000; Chenu *et al.*, 2005). Virtual plants have been also used in ecology to compare the strategies of different species for coping with their environment (Falster & Westoby, 2003; Pearcy *et al.*, 2004).

Approaches using 3-D virtual plants start to deal with genetic variability within species. Recently, Buck-Sorlin *et al.* (2005) used them to simulate the effect of single genes on plant architecture. The present study reports another use of virtual plants to phenotype the effect of a single gene mutation. 3-D virtual plants allowed a detailed characterization of the

plant–environment interactions for an ecotype and its mutant, and led to the identification of a previously unknown phenotype. The approach developed here is particularly well designed to analyse the genetic variability of processes such as leaf development, which depend on the complex interactions between environmental conditions, plant architecture and plant physiological responses. For instance, variables such as leaf-initiation rate and initial leaf-expansion rate depend on absorbed light (Figs 4, 5; Chenu *et al.*, 2005) and thus on plant architecture through organ morphology, size and spatial distribution. The use of 3-D virtual plants made it possible to distinguish physiological responses from structural changes in the integrative response of plants to their environment.

Four major changes resulting from the *se-1* mutation have been identified. Two constitutive characteristics were affected: the initial rate of leaf expansion, and the duration of leaf expansion. Two light responses were also modified: in *se-1*: the leaf-initiation rate was insensitive to incident and absorbed light, and the increase in the duration of leaf expansion in response to decreasing light intensity was larger than that in Col. If the insensibility of leaf initiation to light intensity in *se-1* might have been deduced from direct measurements (Fig. 3a,b), the use of virtual plants allowed us to formalize and quantify this response. The *SE* gene was involved in the response of leaf initiation not to incident light intensity, but to the amount of light absorbed by the plant. The use of 3-D virtual plants allowed the establishment of stable response curves, thus enabling the distinction between genotypic and environmental effects in generation of the phenotype. The response curves obtained correspond to new phenotypic plant characteristics. They were stable in a broad range of environmental conditions and were strong enough to identify differences resulting from a monogenic mutation. The approach developed was also useful for proposing some hypotheses concerning the mechanisms involved.

This approach illustrates the relevance of modelling tools in integrative biology. It could be extended to a broader range of genotypes to facilitate the identification of other genes involved in the response of leaf development to light. The present study could also be used to build a dynamic model of leaf development in response to light, using virtual plants to simulate the effect of single genes (Hoogenboom *et al.*, 2004; Buck-Sorlin *et al.*, 2005).

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