Journal of Plant Biology, January 2008, 51(1) : 64-73

Spatial Distribution of Leaf form and the Self-thinning Exponent are Affected by the Sensitivity of the Response to Abscisic acid in an *Arabidopsis thaliana* Population

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The spatial distribution of leaves is related to the exponent of the self-thinning relationship in plant populations. In this study, we evaluated the fractal dimension of rosette leaves of wild-type (WT) *Arabidopsis thaliana* and of an abscisic acid (ABA) – insensitive mutant (*abi2-1*) to test a model of the spatial distribution of leaf form in an *Arabidopsis* population based on subdivision of a cube surrounding the leaf into uniform boxes and to investigate ABA's affect on this model of the leaf. The values of the self-thinning exponent were -1.31 and -1.45 for WT and *abi2-1*. The mean dimensions of the box used to model the spatial distribution of leaf form, estimated using our model, were 2.08 and 2.03, respectively. By assuming that the box dimension equals the fractal dimension within the populations, the predicted self-thinning exponent equaled -1.40 for WT and -1.49 for *abi2-1*. When exogenous ABA was applied to both genotypes, the self-thinning exponent became -1.26 and -1.43 for WT and *abi2-1*, and the exponents predicted using the dimensions of the box were -1.37 and -1.46, respectively. The empirically predicted exponent equaled that predicted using the dimensions of the box (95% confidence interval). Empirical prediction of the spatial pattern using the two genotypes with and without ABA showed that ABA influenced the spatial form of the rosette leaves. Therefore, sensitivity to ABA can affect self-thinning through genetically determined changes in leaf form and its spatial distribution.

Key words: ABA, abscisic acid, *Arabidopsis*, fractal dimension, self-thinning rule spatial distribution of leaf form

The "-3/2 power rule" (White and Harper 1970; Yoda K et al. 1963) relates plant size to population density when density-dependent mortality (self-thinning) is occurring. In such a way that plant size increases as population density decreases. These changes can be described by the equation:

$$w = k N^{a} \quad (1)$$

where w is the average mass (g) per plant, N is the plant density (number per m^2), and k and a are regression parameters, with a often taking a value close to -3/2. This relationship is also cited as the "self-thinning rule" (Westoby, 1984). There have been attempts to use the average size and shapes of various plant parts, and their allometric relationships, to derive a (Enquist et al., 1998; Enquist et al., 2003; Long and Smith, 1984; Norberg, 1988; Osawa, 1995; Osawa and Allen, 1993; Weller, 1987a; Weller, 1987b; Weller, 1990; West et al., 1999; White, 1981; Yoda K et al., 1963; Yoda et al., 1963). Metabolic scaling theory predicts that the resource use per unit area is independent of the average mass per individual and that the slope of the log mass-log density relationship should be -4/3 (Enquist et al., 2003; West et al., 1999). Other research has indicated that the self-thinning exponent is usually regulated by abiotic or biological factors (Weller 1987a; Yoda K et al., 1963). Some abiotic factors, such as light, water, nutrient availability, and temperature, can directly affect the self-thinning exponent in plant communities (Callaway et al., 2002; Deng et al., 2006; Morris 1999; Thomas and Weiner, 1989). Gene expression, intracellular signaling, and hormone response have also been shown to indirectly affect the self-thinning exponent in plant communities (Stoll et al., 2002; Zhang et al., 2006; Zhang et al., 2005).

Osawa (1995) reported that the allometric exponent (λ) of the relationship between mean crown volume per tree (v_c) and mean foliage mass per tree (m_L) was a critical parameter:

$$v_c = p.m_L^{\lambda} \quad (2)$$

where p is a real coefficient. By assuming another allometry between mean plant mass (w) and mean crown volume, with real parameters q and ö:

$$w = q.v_c^{\varphi} \quad (3)$$

and by assuming a constant foliage mass per unit of ground area (M_L) in a stand, Osawa (1995) determined that mean plant mass should be related to plant density as follows:

$$w = (p\, q^{\varphi} M_L^{\varphi\lambda}) \bullet N^{-\varphi\lambda} \quad (4)$$

Comparison of Eqs. 1 and 4 suggests:

$$a = -\varphi\lambda \quad (5)$$

Fractal Dimension

Although allometries have provided useful results, they generally have insufficient resolution to account for variations in the spatial distribution of the form of plant parts such as leaves. A more promising approach involves the use of fractal geometry (Mandelbrot, 1983) to model the plant parts. Any spatial dimension of an object, D, whether it is a Euclidean integer dimension or a fractal dimension, can be

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represented by the exponent of the relationship between the number (N_r) of units such as small boxes used to model the dimension inside a cube that contains the whole object, and the linear dimension of the box (r), which represents the length of the sides of the box (Mandelbrot 1983):

$$N_r \propto r^{-D} \quad (6)$$

Zeide and Pfeifer (1991) defined this fractal dimension for the relationship between crown volume and foliage area or mass at the level of individual trees, and quantitatively examined this relationship. Suppose that n_1 and n_2 are the numbers of fixed boxes with side length (dimension of the box) r that cover the convex shape and the foliage of a particular crown, respectively. If we magnify the object μ times, the volume of the convex shape (which defines the crown volume, v_c) and the amount of foliage (e.g., foliage mass, m_L) are proportional to the magnification (μ) raised to the exponents 3 and D (the fractal dimension of the crown), respectively:

$$v_c = n_1 \cdot \mu^3$$
$$m_L = n_2 \cdot \mu^D$$

These relationships exist are derived because the unit of measurement (r) and the initial number of objects contained within the cube are constant under any magnification. Therefore, the relationship between crown volume and foliage mass becomes:

$$v_c = (n_1 \cdot n_2^{-3/D})\, m_L^{3/D}$$

where $(n_1 \cdot n_2^{-3/D})$ is constant (Osawa and Kurachi 2004). Applying this relationship to the means for crown volume (v_c) and foliage mass (m_L) for a study site and comparison with Eq. 2 yields the following relationship:

$$\lambda = 3/D$$

where D is a real number between 2 and 3. Eq. 5 is therefore equivalent to:

$$a = -3\varphi/D \quad (7)$$

Zeide and Pfeifer (1991) and Osawa (1995) proposed that the crown's fractal dimension is a parameter that can be used to describe the patterns of leaf distribution within the three-dimensional space of the plant's canopy. It should be noted that the crown's fractal dimension may correspond to multiple spatial leaf distributions, some of which would include very complex patterns. In reality, D is a positive real number that lies between 2 and 3. The results of this analysis led Osawa and Kurachi (2004) to conclude that the self-thinning exponent was likely to be determined by the value of a tree crown's fractal dimension, D. This conclusion has important ecological implications. The spatial distribution of the leaves in the canopy must be related to leaf physiology and longevity, branching patterns, and the crown architecture, and all of these factors should affect the crown's fractal dimension.

The Role of Abscisic Acid

The plant hormone abscisic acid (ABA) plays a wide range of important roles in plant growth and development, including in embryogenesis, seed maturation, dormancy, root and shoot growth, transpiration, and stress tolerance (Himmelbach et al. 1998). Mutant plants that differ from the wild-type in their ABA sensitivity provide well-defined experimental material that can be used to test the effect of the mutation on resource utilization and other physiological activities (Koornneef et al., 1998; Leung and Giraudat, 1998; McCourt, 1999). The *abi2-1* mutation primarily affects plant vegetative responses, such as gene induction and stomatal closure, after ABA exposure. The wild-type gene product (ABI2) is the protein serine-threonine phosphatase 2C. The *abi2-1* mutation, in which the amino acid Gly is replaced by Asp, is dominant (Leung and Giraudat, 1998; Meyer et al., 1994). ABI2 has been suggested to be a negative regulator of ABA responses (Gosti et al., 1999). *Arabidopsis* ABA-insensitive (*abi*) mutants can be identified by their tolerance of exogenous ABA during germination (Koornneef et al., 1984). The dominant *abi2-1* mutation reduces the responsiveness to ABA of root growth, stomatal closure, and gene induction by osmotic stress (Koornneef et al., 1998; Leung and Giraudat, 1998).

A Potential Relationship Between ABA and the Self-thinning Exponent

In the present study, we hypothesized that the sensitivity of the plant's response to ABA may affect the self-thinning exponent in plant populations. Our logic was as follows: It is likely that (1) the spatial distribution of the leaf form of rosette leaves is closely related to the sensitivity of their response to ABA, and that (2) wild-type and *abi2-1* genotypes would have different self-thinning exponents as a result of differences in the response of their rosette leaf form to ABA. We therefore investigated the effect of ABA sensitivity on the self-thinning exponent and the spatial distribution of rosette leaf form of the wild-type and the *abi2-1* mutant of *Arabidopsis*.

MATERIALS AND METHODS

In this study, we planted the wild-type and the *abi2-1* mutant of *Arabidopsis thaliana* at a range of densities and counted the number of surviving individuals, then determined their dry mass, and estimated the dimension of the boxes used to model the spatial distribution.

Plant Material

The wild-type (Landsberg) and the *abi2-1* mutant of *Arabidopsis thaliana* were chosen as the research material in this study. Unlike the wild-type, *Arabidopsis abi2-1* germinates even in the presence of exogenous ABA (Koornneef et al., 1984). The dominant *abi2-1* mutation reduces the responsiveness to ABA of root growth, stomatal closure, and gene induction by osmotic stress, but there are no significant morphological differences between the mutant and the wild-type in the absence of stress (Koornneef et al., 1998; Leung and Giraudat, 1998). Seeds of the wild-type and of the *abi2-1* mutant were provided by Dr. Z.M. Pei (Duke University, Durham, NC, USA).

Growing Conditions

The seeds were stored in the dark at 4°C for 4 days before sowing on 2 March 2004. Surface sterilization of *Arabidopsis* seeds was accomplished by washing the seeds for 8 minutes in 95% ethanol, and then quickly air-drying the seeds on sterile filter paper. Seeds were sown in 1-cm-deep Petri dishes half-filled with Murashige and Skoog growth medium. The agar concentration was reduced to 0.6% to make it easier to remove the roots (Weigel and Glazebrook, 2002).

One week after germination, we transplanted small seedlings individually into plastic pots (9 cm in diameter, 8 cm in height) filled with a 4:1 mixture of peat moss and perlite. Because the environment of the agar plate is often substantially more humid than soil, the soil mixture was moistened thoroughly and the pots were covered with plastic film for 5 days after transplanting.

Pots that are 2.5 × 2.5 cm are suitable for growing a single plant to maturity (Weigel and Glazebrook, 2002), and this is equivalent to a density of 10 plants per pot for the pots used in the present study. To create varying levels of crowding, we planted five densities (10, 50, 100, 500, and 1000 seedlings per pot) that were equivalent to ca. 1500, 7500, 15 000, 75 000, and 150 000 seedlings per m^2 (henceforth, referred to as density1 to density5, respectively), and provided five replicates for each genotype-density combination. For experiments using exogenous ABA, plants were watered daily with an aqueous solution containing 5 μM ABA (100 mL per pot) after transplanting. The pots were all placed in the growth chamber of the Key Laboratory of Arid and Grassland Ecology of Lanzhou University under the following growth conditions: 25°C and light for 16 hours, followed by 20°C and dark for 8 hours; 70% relative humidity; artificial light with a minimum photon fluency rate of 175 μmol m^{-2} s^{-1} and a maximum of 220 μmol m^{-2} s^{-1}).

Plants were harvested 35 days after sowing, before flowering time. The center 2 × 2 cm area of each pot was used to estimate plant density and avoid edge effects at the three highest densities. First, we measured the leaf area, and the height, length, and thickness of the randomly selected leaf. We then constructed a cubic volume large enough to contain the whole selected leaf. Harvested plants were then oven dried at 105°C for 15 minutes, then at 70°C for 48 hours. The rosette leaves were separated from the rest of the plant, then, the dry mass of these leaves and the total biomass were both measured.

Dimension of the Boxes Used to Model the Distribution of Leaf Form

The distribution of leaf form in the three-dimensional space of the plant canopy has been expressed by estimating the fractal dimension of the leaves within this space (Zeide 1998). There have been previous attempts to divide the canopy space into many boxes, some of which contain parts of the plant and some of which do not, as a means of describing the spatial distribution of foliage and of the light environment (Kurachi, et al. 1986; Osawa and Kurachi, 1997). In the present study, we adapted box dimension to *Arabidopsis* by considering the rosette as an inverse tree crown, and developed a method of estimating the dimension of the boxes used to model *Arabidopsis* foliage for use in characterizing the rosette leaves of this species. The number of rosette leaves varies with growing conditions. About 35 days after sowing, the size and number of the rosette leave had nearly reached their maximum. In the rosette leaves, a healthy leaf (leaf c in Fig. 1a) was randomly chosen, and we used 25 replicates for each plant type and density. To support our analysis, we constructed a cube containing the whole leaf that preserved the leaf's original spatial form, and divided the cube into a three-dimensional mesh using smaller cubes (hereafter, boxes) whose side length (hereafter, dimension) equaled *r* (Fig. 1b). In other words, we

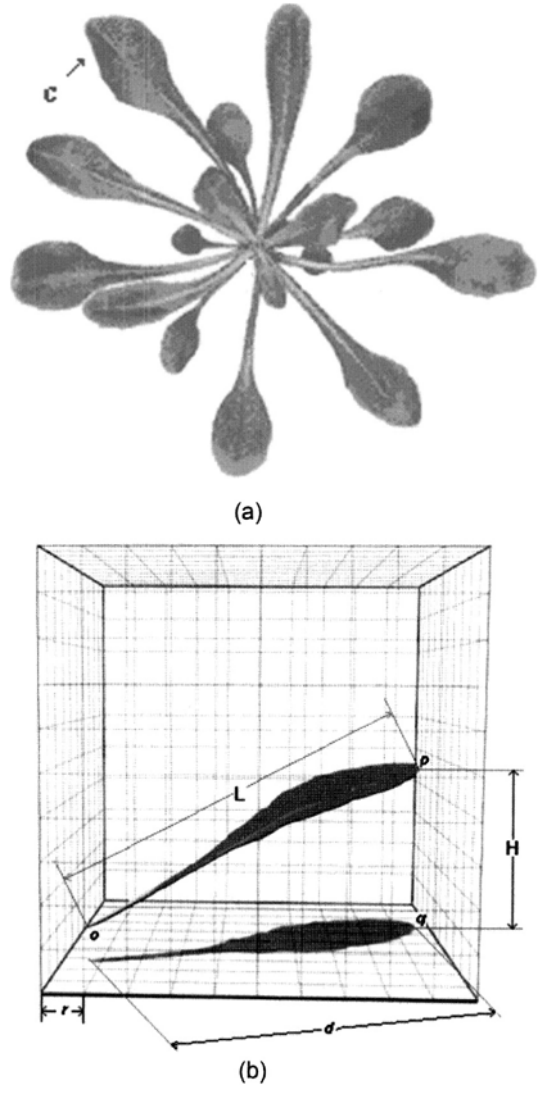

Figure 1. (a) Rosette leaves of *Arabidopsis thaliana*. The number of rosette leaves varies with growth conditions, but one normal leaf ("c") was chosen randomly for the modeling in this study. (b) Illustration of the cube containing the leaf and of the dimensions of the boxes used to model the spatial distribution of leaf "c". The length of the side of each box (i.e., the dimension of the box) is *r*. The length (*L*), height (*H*), and area of each leaf were measured. The length of the shadow cast by the leaf (*d*) was calculated using the values of *L* and *H*. The area of the leaf shadow at the bottom of the cube was calculated using the ratio of *L*/*d*. The number of boxes could then be estimated by dividing the area of the shadow by the area of one face of each box. See the text for details.

Table 1. Summary of stand conditions and leaf parameters for *Arabidopsis thaliana* populations composed of either the wild-type or the *abi2-1* mutant. These parameters were used to estimate the dimension of the box used to model the spatial distribution of the leaves.

Stand no. (target density)	Treatment	Actual stand density (no./m^2)	Mean leaf length (L, mm)	Mean leaf height (H, mm)	Mean leaf thickness (mm)	Total aboveground dry mass (g)
Density1 (1500 / m^2)	Wild-type	1476	50	9.7	0.23	0.4739
	Wild-type+ABA	1432	48	13.9	0.23	0.4505
	abi2-1	1444	48	9	0.21	0.4687
	abi2-1+ABA	1486	47	9.8	0.22	0.4767
Density2 (7500 / m^2)	Wild-type	6877	42	9	0.20	0.2700
	Wild-type+ABA	6642	41	12.5	0.21	0.2683
	abi2-1	6468	41	8.7	0.19	0.2767
	abi2-1+ABA	6380	42	9.5	0.19	0.2654
Density3 (15 000 / m^2)	Wild-type	13160	26	8.7	0.15	0.1736
	Wild-type+ABA	13490	27	10.3	0.14	0.1829
	abi2-1	11460	25.3	7	0.14	0.1253
	abi2-1+ABA	11050	24	7.2	0.13	0.1199
Density4 (75 000 / m^2)	Wild-type	64720	15	8.7	0.12	0.1364
	Wild-type+ABA	69290	16	10.5	0.12	0.1475
	abi2-1	55330	16	6	0.11	0.0844
	abi2-1+ABA	56290	17	7	0.11	0.091
Density5 (150 000 / m^2)	Wild-type	126800	11	9	0.10	0.1128
	Wild-type+ABA	139400	12	9	0.11	0.1382
	abi2-1	110400	9	4.3	0.10	0.0716
	abi2-1+ABA	117000	9	4.5	0.10	0.0737

divided the cube containing the leaf into many smaller boxes of identical size. Among the boxes, some contained part of the leaf's tissue, but many more did not. Every box containing leaf tissue projected a specific square shadow (with side length r) on the bottom of the cube that contains all the boxes (a vertical projection using parallel rays of light coming from above the cube). The number of boxes containing leaf tissue (N_r) was calculated based on the number of the smaller square shadows that formed on the bottom side of the cube, with the condition that there was no vertical overlap in the boxes that contained parts of the leaf. In other words, the number of square shadows equals the number of boxes containing parts of the leaf.

To accurately calculate the number of square shadows, two conditions must be met: (1) there should be no vertical overlap among the boxes. For this reason, we chose a single leaf rather than modeling the whole rosette. (2) If the thickness of the leaf (excluding the petiole) is greater than r, an overlap will be created. Thus, before using different values of r, we must account for the thickness of the leaf. (This will be discussed in more detail in the Results section.) To prepare our leaf samples, we cut at the centre of the rosette leaf blade because the leaf varies in shape between the center of the rosette to the tip of the leaf (Tsuge et al., 1996). We measured the thickness of each leaf to the nearest 0.001 mm under a microscope using an ocular micrometer and a stage micrometer. Because the petiole is thicker than the leaf, we assigned the petiole a thickness twice that of the leaf when it was larger than r, and by doing so, we doubled the number of boxes containing petiole tissue for that part of the leaf. We used the thickness of the leaf (Table 1) to define three grades of r (1, 0.5, and 0.25 mm) for density1, density2, and density3; and four grades of r (1, 0.5, 0.25, and 0.125) for density 4 and density5.

The height of the leaf (H, the vertical distance between points p and q in Fig. 1b) was measured before harvesting the leaf using an acicular ruler, and the surface area of the leaf (including the petiole) and the length of the leaf (L, the distance between the tip of the leaf at point p and the center of rosette at point o in Fig. 1b) were measured after harvesting. The ratio of H and L can be used to express the angle of the leaf with respect to the horizontal plane. The distance, d, between the tip of the leaf shadow at point q and the center of the rosette at point o is:

$$d = (L^2 - H^2)^{1/2}$$

The area of the leaf shadow (S_S) is:

$$S_S = S_L \cdot d/L$$

where S_L is the area of the leaf. By comparing these two equations, S_S becomes:

$$S_S = S_L \cdot (L^2 - H^2)^{1/2} / L^2 \quad (8)$$

We photographed each *Arabidopsis* leaf on paper with a 1-mm grid. To obtain the leaf area, we counted the number of 1-mm^2 squares covered by the leaf (Squares containing only parts of the leaf were also counted). The r in this original photo equaled 1 mm. To improve the accuracy of this count, we used version 9.0 of Adobe Photoshop to separate the leaf from its background and magnified the image to

200% of its original size while keeping the background grid constant. The number of squares covered by the leaf was then counted as the leaf area (using 0.25 mm^2 as the area of each square) for r = 0.5 mm. We then used Eq. 8 to estimate the number of squares occupied by the shadow leaf. This value is N_r (r = 0.5 mm). We calculated N_r for r =1, 0.25, and 0.125 mm using the same procedure.

RESULTS

Self-thinning Relationship

Fig 2 shows the relationship between total aboveground biomass and stand density for the wild-type, the *abi2-1* mutant, and the two genotypes treated with ABA. Reduced-major-axis (RMA) regression indicated a self-thinning slope of -0.31±0.07 (mean±SE, R^2 = 0.86) for the wild-type and -0.45±0.10 (R^2 = 0.85) for *abi2-1*. With the ABA treatment, the exponent was -0.26±0.09 for the wild-type (R^2 = 0.93) and -0.43±0.06 for *abi2-1* (R^2 = 0.91). We analyzed the effect of genotype (wild-type versus *abi2-1*) on total mass (log) and density (log) using one-way ANOVA. At the two lowest densities, there were no significant differences in total mass and density between the wild-type and mutant genotypes (for total mass and density, respectively: density1, F = 0.012, n.s., F = 0.121, n.s.; density2, F = 0.030, n.s., F = 0.911, n.s.). At the medium and higher densities, both total mass and density differed significantly between the wild-type and *abi2-1* genotypes (for total mass and density, respectively: density3, F = 11.826, $p < 0.01$, F = 7.824, $p < 0.05$; density4, F =23.642, $p < 0.001$, F = 6.670, $p < 0.05$; density5, F =12.976, $p < 0.01$, F = 9.224, $p < 0.05$). We also analyzed the effects of exogenous ABA on total aboveground biomass (log) and density (log) for both genotypes, and found no significant differences between them at any of the five density levels.

Constant Leaf Biomass

For both the wild-type and *abi2-1* mutant, leaf mass per plant and stand density were inversely related in the self-thinning stands. Figure 3 shows that the RMA regression slope for this relationship (log-log graph) was -1.09±0.15 for the wild-type and -1.15±0.19 for *abi2-1*, and these values changed to -1.05±0.06 and -1.15±0.13, respectively, after ABA treatment. Our results did not reject the hypothesis that the mean leaf mass and stand density were inversely proportional for both genotypes. Therefore, total leaf biomass per pot remained more or less constant at varying densities of self-thinning.

Constant Leaf Number in the Main Rosette

Instead of using the whole rosette to estimate the dimension of the box, we chose to use a single leaf. Thus, it was necessary to test our assumption that measurements of a single leaf could be used to represent the whole rosette. The space occupied by the whole rosette can be modeled as a cylinder with a radius (R) equal to d and a height (h) equal to H. The values of h and R would be the same for both the rosette and the single leaf, so the ratio of leaf volume to rosette volume would be determined by the total number of healthy leaves in the main rosette. We counted the number of leaves in the main rosette, and obtained the following results for the wild-type for density1, 8±2; for density2, 8±2; for density3, 9±1; for density4, 7±1; and for density5, 8±1. For the wild-type with exogenous ABA, the values for density1 were 8±1; for density2, 9±1; for density3, 8±1; for density4, 9±2; and for density5, 9±1. For *abi2-1*, the values for density1 were 9±1; for density2, 8±1; for density3, 9±1; for density4, 9±2; and for density5, 8±1. For *abi2-1* with exogenous ABA treatment, the values for density1 were 8±2; for density2, 9±2; for density3, 9±1; for density4, 8±2; and for density5, 9±1. The mean number of leaves in the main rosette (±SE) thus appears to be 8±1 for the wild-type, 9±1 for the wild-type with ABA treatment, 9±2 for *abi2-1*, and 9±1 for *abi2-1* with ABA treatment. These results suggest that it is safe to consider the number of leaves in the main rosette to be constant among treatments in our study.

Parameter φ

The φ exponent of the allometry for mean plant mass as a function of mean rosette volume (Eq. 3) equaled 0.97±0.08, for the wild-type, and 1.01±0.09 for *abi2-1*. After exogenous ABA was applied, the values for the wild-type changed to 0.97±0.11 and 0.99±0.09 for *abi2-1*, as shown in Fig. 4.

Dimension of the Boxes Used to Model the Leaf Distribution

Table 1 summarizes the stand characteristics and plant parameters for the five stand densities of the wild-type and *abi2-1* genotypes that were used to estimate the fractal dimension of the spatial distribution of leaf form. These stands appear close to the self-thinning boundary (Fig. 2). Figure 5 plots the log-log relationship between N_r and r for the two genotypes. The D values (and ±SE) for the wild-type were 2.04±0.12, 2.05±0.18, 2.07±0.15, 2.13±0.20, and 2.05±0.16 for density1 to density5, respectively (Fig. 5a). For the wild-type with exogenous ABA treatment, the corresponding values were 2.07±0.11, 2.07±0.17, 2.25±0.09, 2.05±0.08, and 2.09±0.14, respectively (Fig. 5c). For *abi2-1*, the corresponding values were 1.98±0.20, 2.08±0.19, 2.01±0.16, 2.03±0.13, and 2.02±0.09, respectively (Fig. 5b). For *abi2-1* with exogenous ABA, the corresponding values were 1.90±0.06, 2.14±0.14, 2.12±0.07, 2.05±0.09, and 2.05±0.11, respectively (Fig.5d). The range in the 95% CI values indicates that the fractal dimensions were similar among most of the stands we examined. The mean fractal dimensions for the wild-type and *abi2-1* genotypes equaled 2.08±0.14 and 2.03±0.16, respectively, versus corresponding values with exogenous ABA treatment of 2.12±0.13 and 2.04±0.11 for the wild-type and *abi2-1*, respectively. The estimated fractal dimensions of the pure stands were nearly identical within the same genotype as long as they were located close to the self-thinning boundary.

Predicted Self-thinning Exponent Based on the Dimensions of the Box

The value of the self-thinning exponent was predicted by

solving Eq. 7 for the two *Arabidopsis* genotypes. The self-thinning exponent equaled -1.40±0.08 for the wild-type and -1.49±0.10 for *abi2-1*. After exogenous ABA treatment, the exponents equaled -1.37±0.09 and -1.46±0.07 for the wild-type and *abi2-1*, respectively. Figure 6 illustrates the mean±SE values for the self-thinning exponent predicted using the observed fractal dimension and using the empirical relationship between mean plant mass and stand density. The ranges of the 95% CI for the predicted and empirical exponents overlap in both the wild-type and the *abi2-1* mutant of *Arabidopsis*. The ranges of the 95% CI also indicate that the self-thinning exponent does not differ significantly from the traditional value of -1.5 in the *abi2-1* treatments, but that the exponents were significantly larger (less negative) than -1.5 in the wild-type.

DISCUSSION

There have been many previous attempts to use the average sizes and shapes of various plant parts and their allometric relationships to derive the self-thinning exponent (a) in Eq. 1. Our results have led us to conclude that the self-thinning exponent can have a value other than -3/2 (e.g., -1/2 for the total biomass *vs.* density relationship) for certain combinations of allometric parameters. In our study, we examined the effect of ABA sensitivity on the self-thinning exponent of wild-type and *abi2-1 Arabidopsis*. The mean self-thinning exponent of *abi2-1* (-0.45) was smaller than that of the wild-type (-0.31), and the lines diverge at higher densities until they no longer overlap (Fig. 2). ABA treatment decreased the steepness of the slope (to -0.26 for the wild-type and -0.43 for *abi2-1*), especially in the wild-type, but the change was not statistically significant. We also used the dimension of the box for the spatial distribution of leaf form estimated for each genotype to predict the self-thinning exponent. We expected to find a relationship between the effect of ABA sensitivity on the self-thinning slope and the effect of the two genotypes on the spatial distribution of leaf form.

Because total aboveground biomass was measured first, and mean biomass was calculated as the total mass divided by the plant density, the analysis in Figure 2 used the total aboveground biomass *vs.* density relationship instead of the mean mass *vs.* density relationship to avoid introducing any statistical bias (Weller 1987b). The empirical self-thinning exponents (exponent a in Eq. 1) based on the mean plant mass *vs.* density relationship equaled -1.31, -1.45, -1.26, and -1.43 for the wild-type, *abi2-1*, wild-type+ABA, and *abi2-1*+ABA treatments, respectively.

The self-thinning coefficients of the wild-type and *abi2-1* differed significantly at medium and higher densities, but not at lower densities. This interesting phenomenon indicated that the effects of ABA sensitivity on the value of the self-thinning exponent increased with increasing density stress. This result was similar to the responses of other plant characteristics to ABA when the plants are exposed to other stresses (Himmelbach et al., 1998; Leung and Giraudat, 1998; McCourt, 1999). Exogenous ABA application produced less-steep self-thinning slopes for both the wild-type and *abi2-1*, although the differences were not significant, especially for the *abi2-1* mutant (Fig. 2). These results may indicate that the change in the self-thinning slope is influenced by the sensitivity of the response to ABA, and not growth limitations for the mutant, and that both endogenous and exogenous ABA were involved in the process, with endogenous ABA having more important effects for the wild-type. Exogenous ABA had little effect on the self-thinning slope in *abi2-1* due to its insensitivity to ABA.

Hypotheses and theories about canopy geometry developed using trees (Osawa, 1995) are based on the spatial distribution of leaves in forest canopies, but our results show that the leaves of *Arabidopsis* can also change angles in response to changes in plant density. There is evidence that

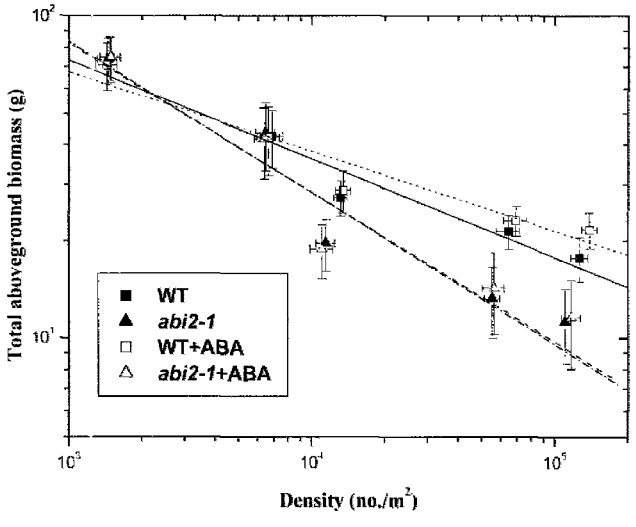

Figure 2. The relationship between total aboveground biomass and density, with the estimated self-thinning boundaries for the wild type (WT) and the *abi2-1* mutant of *Arabidopsis thaliana*. Values are mean±SE (n = 5). The dark solid line represents is the regression for WT; the dotted line represents the regression for WT plus ABA, the dashed line dashed line represents the regression for *abi2-1*, and the dot-and-dash line represents the regression for *abi2-1* plus ABA.

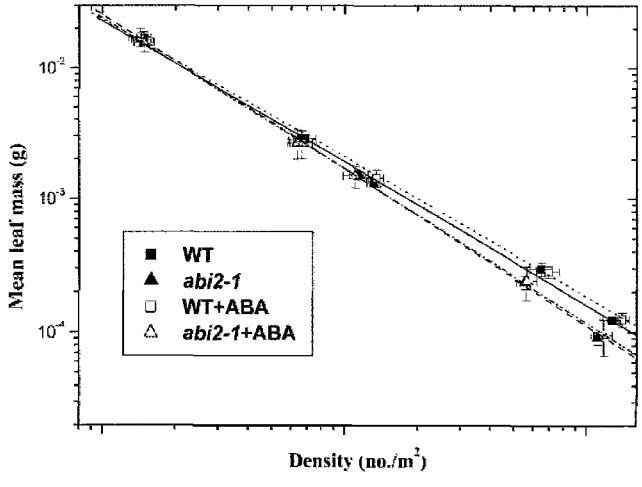

Figure 3. The relationship between mean leaf mass per plant and stand density in self-thinning stands of the wild-type (WT), the *abi2-1* mutant, WT plus exogenous ABA, and *abi2-1* plus exogenous ABA. Values represent the mean±SE (n = 5).

Arabidopsis can regulate the inclination of its leaves in order to avoid shading by its neighbors. ABA is involved in the negative regulation of epinastic leaf growth in *Pittosporum eugenioides* (Dwyer et al., 1995), and ABA plays a similar negative role in submergence-induced hyponastic growth in *Rumex palustris* (Cox et al., 2004). Therefore, we used the models developed for forest crowns in our study to estimate the fractal dimension of the spatial distribution of the leaf form of the main rosette leaves of *Arabidopsis* using an ABA-insensitive mutant.

Many plants that reorient their leaves do so by means of reversible changes in the volume of cells within a structure such as the pulvinus. The molecular mechanisms that control these turgor-driven volume changes are becoming increasingly well understood (Coté, 1995; Koller, 2000). However, many plants, including *Arabidopsis*, lack discrete pulvini, and in these plants, leaf movements are caused by differential growth in the leaves, and the aforementioned research suggests that ABA should be involved in this process. However, there is currently no evidence to show the roles that ABA plays in nastic leaf movements. Moreover, it is likely that other plant hormones will be involved in this process, including ethylene, auxins, and gibberellins. Because mutants with respect to these hormones are easy to obtain, they will represent interesting research subjects in the future.

Flowering can be promoted in response to stresses such as overcrowding, which is perceived as changes in the quality of light (Simpson. and Dean, 2002), and early-flowering plants produce fewer leaves before flowering. For this reason, we harvested our plants before they flowered (35 days after sowing), and the number of main rosette leaves in each treatment group was roughly (Crone. and McDaniel, 1997) constant in our study.

Our results do not exclude the possibility of a numerical relationship between the spatial distribution of leaves in the rosette and the self-thinning exponent (Eq. 7). The box dimension of the rosette leaves was measured by means of simulation using a cubical space broken into a series of identical boxes (Fig. 1b). Our argument was based on the parameter φ (Eq. 3), and the φ of the allometry for the mean plant mass *vs.* mean rosette volume allometry is shown in Figure 4. Osawa ((Osawa 1995; Osawa and Kurachi, 2004) assumed that the exponent ö equaled 1 during self-thinning for *Pinus banksiana* and *Populus tremuloides*, two species of forest tree. However, we applied the value of the allometric exponent for the relationship between mean plant mass and mean rosette volume (Fig. 4) directly in our study to improve the prediction. The results indicated that the dimensions of the box for the spatial distribution of leaf form of rosette leaves in the wild-type and *abi2-1* genotypes are related to the values of the self-thinning exponent.

The dimension of the box has mostly been used to estimate the characteristics of the crown of forest trees, and in this study, we adapted it to *Arabidopsis* by considering the rosette as an inverse tree crown. We estimated the dimension of the box with reference to the area of the rosette leaf (data not shown) and its spatial distribution (leaf length and height), as shown in Table 1. Based on this approach, the fractal dimension of the wild-type *Arabidopsis* had a mean of 2.08, whereas *abi2-1* had a mean of 2.03. After exogenous ABA application, the corresponding values changed to 2.12 and 2.04, respectively. However, the new method can only be used to estimate values for a single leaf without any leaf overlap, because it cannot accurately estimate the number of boxes containing part of the leaf when leaves overlap. The size of the leaves of *Arabidopsis* exhibits considerable plasticity and is influenced by environmental and physiological conditions. Heteroblasty is attributable to changes in leaf index (namely, the ratio of leaf length to leaf width) in many cases, and particularly when heteroblasty is induced as an environmental adaptation (Tsukaya, 2002). Thus, a single leaf randomly chosen from among the rosette leaves cannot exactly represent all the rosette leaves, but despite this drawback, the method can describe the changes in the spatial distribution of leaf form at the population level even with a relatively small sample size. As a result, our method offers a new way to study the spatial distribution of leaf form of plants with a smaller rosette, such as *Arabidopsis*.

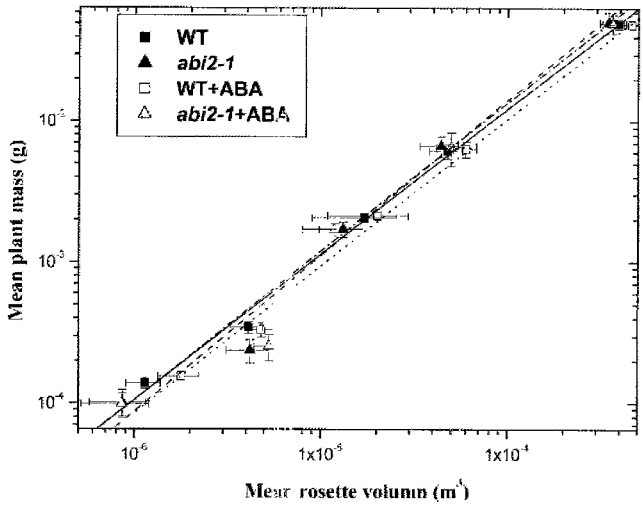

Figure 4. Patterns of the allometry for mean plant mass as a function of mean rosette volume for self-thinning stands of the wild-type (WT), *abi2-1*, WT plus exogenous ABA, and *abi2-1* plus exogenous ABA. Values represent mean±SE (n = 5).

The rosette volume of *Arabidopsis* is related to the leaf length and height, and both indices respond to the plant density (Table 1). Leaf length decreased with increasing density in both the wild-type and *abi2-1*, but height remained almost constant and leaf angle apparently increased with increasing density in the wild-type. ABA caused the leaf angle to increase with increasing density in the wild-type (data not shown), which would have improved the plant's ability to utilize the available light. Zhang et al., (2005) claimed that differences in the self-thinning exponents of various *Arabidopsis* mutants resulted from their different utilization of resources such as light and water in response to density stress. When plants are more sensitive to ABA, they are more responsive to changes in resource utilization under density stress, which results in a larger self-thinning exponent. Exogenous ABA did not significantly affect leaf length and height for both the wild-type and *abi2-1*.

Using the dimensions of the box (Fig. 6), the self-thinning exponent for the wild-type was predicted as -1.40 versus values of -1.49 for *abi2-1*, -1.37 for the wild-type plus ABA,

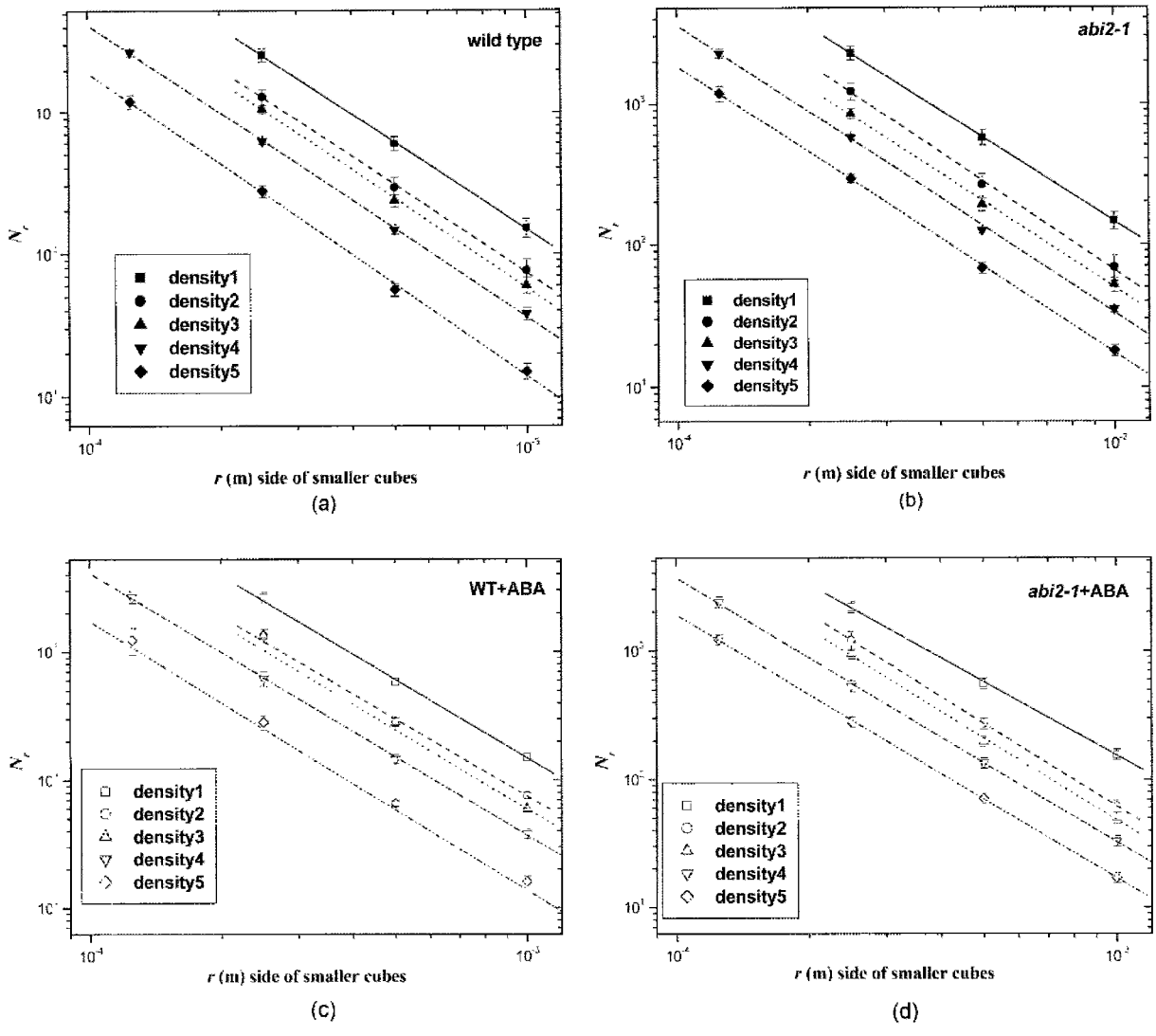

Figure 5. The relationship between the number of boxes (N_r) and the size of the boxes (r) for (a) the wild-type of *Arabidopsis thaliana* (WT), (b) the *abi2-1* mutant, (c) WT plus exogenous ABA, and (d) *abi2-1* plus exogenous ABA. Values represent mean±SE (n = 25). Density1 through Density5 represent densities of 1500, 7500, 15 000, 75 000, and 150 000 plants per m^2, respectively. These data were used to calculate the fractal dimension. The fit linear represent the following plots: density1 (solid); density2 (dash); density3 (dot); density4 (dash dot); and density5 (dash dot dot).

and -1.46 for *abi2-1* plus ABA. The four predicted exponents are all smaller (more negative) than the empirically predicted exponents (-1.31, -1.45, -1.26, and -1.43 for the wild-type, *abi2-1*, wild-type+ABA, and *abi2-1*+ABA, respectively), and the differences were not statistically significant. We directly applied the value of the allometric exponent ö for the relationship between mean plant mass and mean rosette volume in our study to improve the accuracy of the prediction. Nonetheless, there was a similar trend in the change between the empirically predicted value and the value predicted using the dimension of the box. The results indicated that: (1) The dimensions of the box used to model the spatial distribution of rosette leaf form in the wild-type and *abi2-1* were related to the sensitivity of their response to ABA under density stress, even though the difference was not significant (Fig. 6). (2) The slightly different self-thinning exponents of the two genotypes resulted from their different spatial distributions of rosette leaf form in the *Arabidopsis* populations that we examined. (3) Exogenous ABA also

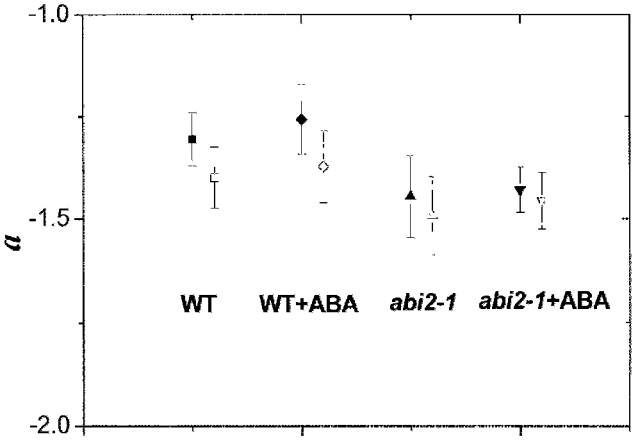

Figure 6. The means and 95% confidence intervals of the self-thinning exponents predicted from the dimensions of the boxes used to model the spatial distribution of leaf forms and estimated from the empirical relationship between mean plant mass and plant density

affected the self-thinning exponent through its effect on the dimension of the box, especially for the wild-type. Therefore, we conclude that sensitivity to ABA is likely to affect the value of the self-thinning exponent as a result of close relationships in which (1) the spatial distribution of rosette leaf form is closely related to the value of the self-thinning exponent and (2) the sensitivity to ABA is closely related to the spatial distribution of the rosette leaf form. On this basis, sensitivity to ABA can affect self-thinning through genetic changes in leaf form and its spatial distribution.

As we described earlier, the crown's fractal dimension is a measure of the population of leaves, not the measure of an individual or of a single leaf. In our approach, any leaf overlap would make the results inaccurate, so it was necessary to analyze a single leaf at a time, and use the results as a sample that represented the rest of the rosette. The relatively high number of randomly selected replicates (n = 25) probably made our results closer to the reality. If estimation of the crown's fractal dimension is constructed at the population level, prediction of the self-thinning exponent will be improved. Therefore, it will be useful to search for methods capable of overcoming the problem of overlapping leaves, thereby permitting studies using this method at the population level.

ACKNOWLEDGEMENTS

We thank Dong-Liang Cheng (Lanzhou University) for his valuable help with our literature review, and Dong-Hui Wang (Arid Regions Environmental and Engineering Research Institute) for help in creating Figure 1b. This study was supported by the Natural Science Foundation of China (30730020) and the Hi-tech Research and Development Program of China (863 program, 2006AA100202).

Received October 22, 2007; accepted December 24, 2007.

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