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Short communication

Modeling the *Arabidopsis* seed shape by a cardioid: Efficacy of the adjustment with a scale change with factor equal to the Golden Ratio and analysis of seed shape in ethylene mutants

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ABSTRACT

A new model for the description of *Arabidopsis* seed shape based on the comparison of the outline of its longitudinal section with a transformed cardioid is presented. The transformation consists of scaling the horizontal axis by a factor equal to the Golden Ratio. The elongated cardioid approximates the shape of the *Arabidopsis* seed with more accuracy than other figures.

The length to width ratio in wild-type Columbia *Arabidopsis* dry seeds is close to the Golden Ratio and decreases over the course of imbibition. Dry seeds of *etr1-1* mutants presented a reduced length to width ratio. Application of the new model based on the cardioid allows for comparison of shape between wild-type and mutant genotypes, revealing other general alterations in the seeds in ethylene signaling pathway mutants (*etr1-1*).

Introduction

Work in biology today is based on model species. In *Arabidopsis*, accurate morphological descriptions are required to understand changes during development as well as changes that occur in response to the environment. In a previous article, we described the *Arabidopsis* seed as a prolate spheroid, the ellipsoid of revolution obtained when an ellipse is made to rotate around its major axis. The model allowed the description of changes in shape during seed imbibition, revealing new effects of the ethylene signal transduction pathway (Robert et al., 2008). In this report, we present a new and improved model for the description of *Arabidopsis* seed shape. It is based in the comparison of the outline of the longitudinal section of a seed with a transformed cardioid. The cardioid is the trajectory described by a point of a circle that rolls around another fixed circle with the same radius. This heart-shaped figure appears in the cross section of the stems and in the leaves of some plants, such as a *Hydrochoris morsus-ranae* (Gielis and Gerats, 2004). By a simple transformation, the cardioid resembles the shape of *Arabidopsis* seed with more accuracy than other figures, being either more symmetric or having different types of symmetry. The transformation consists in the change of the aspect ratio multiplying the longitudinal axis by a scaling factor equal to the Golden Ratio.

The Golden Ratio, known also as the divine proportion, is a mathematical constant broadly studied because of its frequent appearance in geometry. It is denoted as φ and equals $(1+\sqrt{5})/2 \approx 1.61803399$. Given a rectangle with sides in the ratio $1{:}\varphi$ and removing a square with side coincident with a minor side of the rectangle, the remaining rectangle maintains the ratio $1{:}\varphi$.

In this article, the length to width ratio in *Arabidopsis* wild-type Columbia seeds was analyzed. We show that its value was close to the Golden Ratio and goes through this value during imbibition. In addition, some genotypes in the ethylene signaling pathway presented differences in the length to width ratio values. Application of the new shape model based in the cardioid curve to ethylene signal transduction mutants revealed other differences in the seed shape of *etr1-1* mutants.

Materials and methods

Plant material

Seeds of *Arabidopsis thaliana*, variety Columbia (Col; wild-type), as well as mutants in the ethylene signaling pathway *ctr1-1* (Kieber et al., 1993), *eto1-1* (Chae et al., 2003; Woeste et al., 1999),

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etr1-1 (Chang et al., 1993), *ein2-1* (Guzmán and Ecker, 1990), and triple mutant *ein2-1, ers1-2, etr1-7* (Hall and Bleecker, 2003) were used in this study. Seeds were harvested from plants grown in the greenhouse between the years 2000 and 2007. Twenty-five seeds were observed and measured for each lot before and after 24 h of imbibition. Five lots per genotype were analyzed, resulting in a total of 125 seeds per genotype.

Photography and image analysis

The seeds were observed with a Nikon 'SMZ-2 T' stereo microscope. Photographs of longitudinal views were taken with a Nikon 'Coolpix 950' digital camera and analyzed with Adobe Photoshop (CS3) image software. Lengths, widths, perimeters, and areas of the seed images were obtained directly from the photographs. In this process, graph paper allowed us to convert pixels into μm.

Accuracy of the image adjustments to a cardioid

For a cardioid generated by circles with radius a (plotted in Fig. 1c), the area is given by $A = 6\,\pi\, a^2$ and the perimeter by P=16a. Then A/P^2 is constant and equal to $3\pi/128$. Normalizing this quotient, for any cardioid, the ratio F=128A/3πP^2 is equal to 1. To adjust the cardioid to the figure of a seed, the x-axis was elongated by a factor of φ (plotted in Fig. 1d). For this modified cardioid, the ratio A/P^2 is a constant and equal to 1/14.59. To evaluate the accuracy of the adjustment of the seed images to a modified cardioid, the index

$$G = 14.59\,\frac{A}{P^2}$$

was applied, where A and P are, respectively, the area and the perimeter of each image. Note that for a geometric φ-modified cardioid, G equals 1. Shape comparison among genotypes was done by the statistical comparison of their G index values.

Results and discussion

The mean value of the quotient L/W for dry seeds of the wild type Columbia was 1.66 and decreased over 24 h of imbibition to 1.29. Thus, in the course of imbibition, L/W went through a value equal to the Golden Ratio $\varphi \approx 1.618$ (Table 1).

ANOVA analysis demonstrated that L/W values were smaller in dry seeds of *etr1-1* mutants than in the seeds of other genotypes. In contrast, after 24 h of imbibition, L/W values were higher in both *etr1-1* and in the triple mutants. In both cases, L/W values in *eto1-1* and *ctr1-1* did not differ from the wild-type. In all genotypes, L/W decreased during imbibition to values lower than φ (Table 1).

The bi-dimensional image of an *Arabidopsis* seed section (Fig. 1a) resembles an elongated cardioid (Fig. 1d) more than an ellipsoid (Fig. 1b). To optimize the adjustment, departing from a cardioid (Fig. 1c), the x-axis was scaled by a factor equal to the Golden Ratio (Fig. 1d).

The index G (see section "Materials and methods") serves the purpose of evaluating the validity of the model (i.e. the efficacy of the adjustment) and performing shape comparisons between genotypes under different physiological conditions or seed developmental stages. Statistical comparisons were performed to test for differences between dry and imbibed seeds, and confirmed the differences observed. The shape of imbibed seeds (24 h, mean G=0.96) adjusted better to this model than did dry seeds (mean G=0.90; Table 1).

The G index showed lower values in dry seeds of the *etr1-1* mutant genotype than in *eto1-1* or Columbia; after imbibition, G values were lower in *etr1-1* than in the other genotypes (Table 1).

The shape of organisms is often seen as the result of multiple processes submitted to complex regulation. The ethylene signal transduction pathway in plants affects various aspects of development, such as cytoskeleton organization, the cell cycle and multiple aspects of metabolism (Baluska et al., 1993; Abeles, et al., 1992; Fig. 2). As such a general regulator, the ethylene signal transduction pathway must possess efficient mechanisms to modulate its function in response to environmental changes.

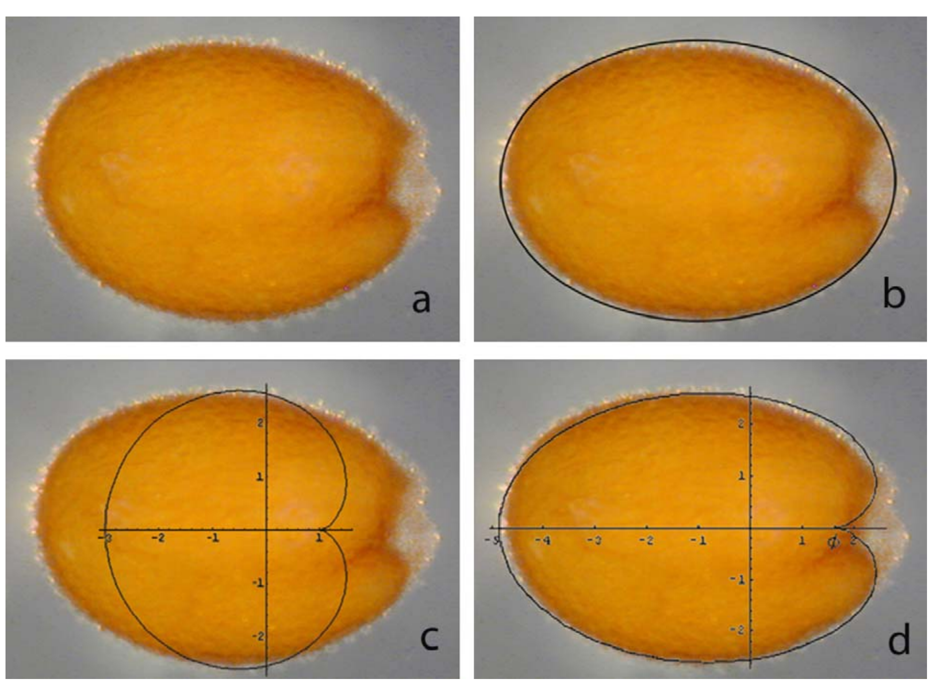

Fig. 1. Adjustment of the bi-dimensional image of an *Arabidopsis* seed (Fig. 1a) to a cardioid. Departing from a cardioid (Fig. 1c), the x-axis was scaled by a factor of φ (Fig. 1d). This results in a better adjustment than an ellipsoid (Fig. 1b).

Table 1
Summary of results. Top: Length to width ratio in dry seeds and after 24 h of imbibition for five genotypes. Bottom: Values of G Index. Letters indicate groups whose members do not differ statistically.

	Dry seeds						24 h of imbibition					
	WTcol	ctr	eto	etr	triple	mean	WTcol	ctr	eto	etr	triple	mean
Length to width ratio	1.66^{A}	1.66^{A}	1.65^{A}	1.53^{B}	1.67^{A}	1.63	1.29^{A}	1.30^{A}	1.29^{A}	1.39^{B}	1.33^{B}	1.32
G index	0.906^{A}	$0.895^{A,\ B}$	0.904^{A}	0.891^{B}	$0.895^{A,\ B}$	0.90	0.965^{A}	0.958^{A}	0.967^{A}	0.932^{B}	0.959^{A}	0.96

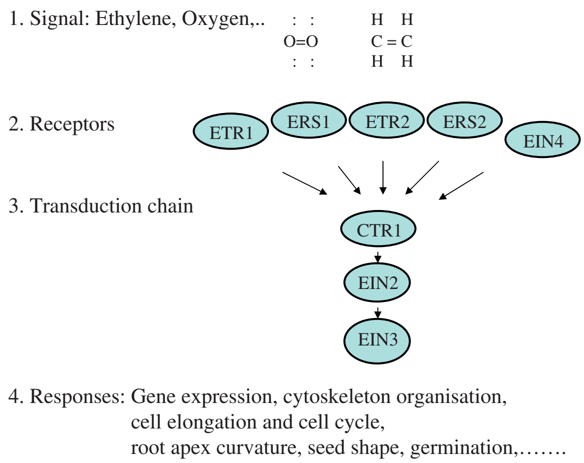

Fig. 2. Schematic representation of the ethylene signal transduction pathway in *Arabidopsis*.

The possibility that the ethylene signaling pathway is related not only to ethylene sensing but also to other, more general control mechanisms (Cervantes, 1998) is supported by experimental evidence from multiple sources. First, ethylene binding to its receptor may result in negative control over the activity of this protein (ETR1; Bleecker and Schaller, 1996). Thus, the mutation *etr1-1*, dominant insensitive to ethylene, may work in the constitutive activation of ETR1 protein activity. Double loss-of-function mutants (*ers1-2*, *etr1-6*; *ers1-2*, *etr1-7*) show a severe constitutive ethylene response phenotype consistent with the negative regulator model for receptor function (Wang et al., 2003). Second, if ethylene binding exerts a negative control on ETR1 activity, other factors may have similar functions. ETR1 protein has a GAF domain involved in energy sensing (Aravind and Ponting, 1997) and related to the PAS domain, another ubiquitous signaling and sensory transducer (Ho et al., 2000). Oxygen binding to the FIXL protein in *Sinorrhizobium meliloti* results in negative regulation of kinase activity (Silva Sousa et al., 2007). Finally, it has been demonstrated that ETR1 activity is regulated by hydrogen peroxide in stomatal cells (Desikan et al., 2005). Thus, ETR1 is a global sensor whose activity may be regulated by the redox or energy status of the cell and which affects global developmental aspects such as seed shape.

The morphological description of plant structures is a requisite for understanding the relationships between structure and function in evolution, and may contribute to defining developmental situations associated with genomic composition and activity. Changes in shape may be either the result of developmental programs in a "regular" environment or the response to changes (stress) in environmental conditions (Cervantes and Tocino, 2009). Modeling the *Arabidopsis* seed shape by an elongated cardioid may help us to understand and quantify morphological variation in seeds, changes in the course of imbibition and alterations in mutants as well as differences between related genotypes.

Ethylene insensitive genotypes have lower curvature values in their root apex (Cervantes and Tocino, 2005; Noriega et al., 2008), and alterations in polarity in the course of imbibition were detected in seeds of these and other ethylene mutant genotypes (Robert et al., 2008).

In this work, a new method for the description of seed shape was optimized. Modeling of the *Arabidopsis* seed shape by an elongated cardioid may contribute to increased understanding and quantification of morphological variation in seeds, changes in the course of imbibition and alterations in mutants, as well as differences between related genotypes. Its application to analysis of shape in ethylene mutants revealed general shape differences in the ethylene insensitive mutant *etr1-1*. It may be of interest to determine whether changes in shape associated with this or other genotypes are related to particular environmental factors. This is an important research objective for future work in model systems, an in particular, in *Arabidopsis*.

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References

Abeles FB, Morgan PW, Saltveit ME. In: Ethylene In Plant Biology. Oxford: Elsevier Books; 1992.
Aravind L, Ponting CP. Trends Biochem. Sci. 1997;22:458–9.
Baluska F, Brailsford RW, Hauskrecht M, Jackson MB, Barlow PW. Bot. Acta 1993;106:394–403.
Bleecker A, Schaller G. Plant Physiol. 1996;111:653–60.
Cervantes E. Oxygen sensing in plants. In: Lopez-Barneo J, Weir K, editors. Oxygen regulation of ion channels and gene expression. New York: Futura Publishing Co. Inc.; 1998. p. 57–66 Chapter 5.
Cervantes E, Tocino A. J. Plant Physiol. 2005;162:1038–45.
Cervantes E, Tocino A. Plant Signaling Behav. 2009;4(5):1–5.
Chae HS, Faure F, Kieber JJ. Plant Cell 2003;15:545–64.
Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. Science 1993;262:539–41.
Desikan R, Hancock JT, Bright J, Harrison J, Weir I, Hooley R, et al. Plant Physiol. 2005;137:831–4.
Gielis J, Gerats T. Proceedings of the international conference on computing, communications and control technologies, vol. VI, 2004. p. 265–72.
Guzmán P, Ecker JR. Plant Cell 1990;2:513–23.
Hall AE, Bleecker AB. Plant Cell 2003;15:2032–41.
Ho Y-SJ, Burden LM, Hurley JH. EMBO J. 2000;19(20):5288–99.
Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. Cell 1993;72(3):427–41.
Noriega A, Cervantes E, Tocino A. J. Plant Physiol. 2008;165:960–6.
Robert C, Noriega A, Tocino A, Cervantes E. J. Plant Physiol. 2008;165:911–9.
Silva Sousa EH, Tuckerman JR, Gonzalez G, Gilles-Gonzalez M-A. Biochemistry 2007;46:6249–57.
Wang W, Hall AE, O'Malley R, Bleecker AB. PNAS 2003;100(1):352–7.
Woeste KE, Ye C, Kieber JJ. Plant Physiol. 1999;119:521–8.