Quantifying the Spiral Leaf Trait of *Arabidopsis* from the 3D Shape Model Towards Computational Phenomics

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1 Introduction

Computational phenomics becomes more important in the recently remarkable advance of functional genomics. In the RIKEN Genomic Sciences Center, more than 50,000 mutant lines of the model plant *Arabidopsis thaliana* have been produced for the saturated mutagenesis of the genome [3]. For the research of functional genomics, to establish the high-throughput phenotypic screening system is a matter of vital importance [1]. Towards the high-throughput system, we have proposed a novel methodology of the phenotypic analysis which is based on 3-dimensional (3D) model reconstruction via surface measurement by the laser range finder (LRF) [5] and the computational screens of morphological traits on the 3D reconstructed model [2]. However, conventional description of morphological traits of *Arabidopsis* is mostly qualitative due to subjective human observations. Thus we need to define quantitatively individual morphological traits to be handled as objectively digital parameters. In this report, we focus on the spiral leaf trait [3, 4] as one of 3D-specific traits at rosette leaves, and propose a quantitative definition of the spiral leaf trait. Through an experiment to extract the quantitative spiral leaf trait at a wild-type and a mutant line, we exhibit the importance for quantifying traits precisely towards computational screens based on the 3D reconstructed model.

2 Method and Results

2.1 Quantitative Definition of the Spiral Leaf Trait

The spiral leaf trait is specified as a morphological trait of the leaf blade with helical growth for several mutants [3][4]. Two pictures in Fig. 1 (a) display a wild-type Col-0 in the left side, and a mutant Z035240 of Col-0 with a spiral leaf in the right side. The spiral of a leaf blade can be quantified by deflection of ridge (convex) or ravine (concave) of blade shape along the longitudinal axis of a blade. We approximate the deviation of ridge or ravine by a peak of a parametric curve based on the quadratic polynomial, $f(x_{tr}) = a_0 + a_1 x_{tr} + a_2 x_{tr}^2$, on a cross section along the transverse axis of a blade. Position of the peak is obtained by calculating $-2a_1/a_2$. However, a single sampling of a peak seems to be insufficient to quantify deflection from the longitudinal axis. Hence we increase the number of peak samples along the longitudinal axis in addition to the current peak point. Then we consider a line function approximating the peak points about the longitudinal axis, and quantify a slope of the peak line function as the blade spiral trait. When the peak line function is formalized as $g(x_{lo}) = b_0 + b_1 x_{lo}$, the slope of the peak line function is b_1. The slope is converted to a rotation angle θ from the longitudinal axis for convenience so that $\theta = tan^{-1} b_1$ as shown in Fig. 1 (b).

2.2 Experimental Condition

Arabidopsis seeds were treated at 4°C for 2 days, then transferred to continuous red light at 22°C for 2h. After red light treatment, seeds were sown in soil, and transferred to a plant growth room (16h

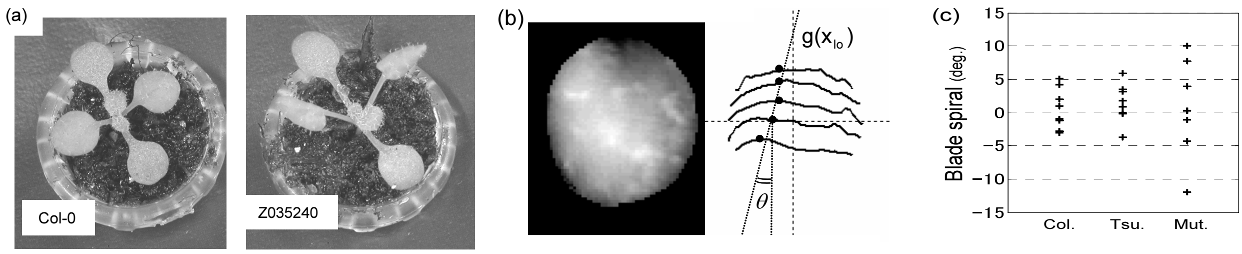

Figure 1: Spiral leaf trait: (a) Col-0 (left) and the mutant (right). (b) Range image of a leaf blade of Z035240 (left) and the angle of the spiral trait (right). (c) Extracted spiral leaf traits by each type.

light / 8h dark at 22°C). Average light intensity was 70 μ mol m^{-2} sec^{-1}. Plants were transplanted to 1.5ml micro tubes before laser surface scans. Due to the bounds in the range of measurement, *Arabidopsis* plants were measured at 13 days after sowing.

2.3 Result of Trait Extraction and Comparison Tests

Fig.1 (c) shows the spiral leaf traits of 8 rosette leaves by each type. From the left, Col-0, Tsu-0, and Z035240 blade spiral degree was represented. An ecotype Tsu-0 was added for reference, which has no spiral leaf blades as Col-0. It was indicated that the variance of spiral trait of the Z035240 mutant is larger than that of Col-0. The difference of variances between Z035240 and Col-0 is significant by the F-test (P <0.05). About the absolute values of the traits, the hypothesis testing for the difference of median is applied, however the difference of median is not significant. We observed that several mutant lines do not have larger rotation angles than those of Col-0. We choose the homozygous mutants for the extraction of the trait, the display of the trait should be equivalent to 100%. The reason of no phenotype in several mutants may be that they are too young to display the phenotype.

3 Discussion

In this report, we defined the quantitative spiral leaf trait as a part of quantifying morphological traits of *Arabidopsis* towards automatic mutant screening. Moreover, we investigated morphological differences between Col-0 and a mutant Z035240, and detected the significant difference of variances. At practical mutant screens, the heterozygous plants are used for phenotypic investigation. As respective percentages of dominant and recessive traits are ideally 75% and 25%, the detection of differences against the wild-type at the heterozygous plants is harder than that of the homozygous plants. We need to verify carefully whether any new definition of a trait is an effective index at homozygous plants before adopting at computational mutant screens.

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