Plant Photosynthetic Heterogeneity Reveals New ...

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Abstract

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Author Summary

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Introduction

Using sunlight, water and CO_2, plants produce sugars and release O_2 with photosynthesis [1]. The process involves the formation of high energy intermediates capable of generating reactive oxygen species. The photosynthetic apparatus, chloroplast and surrounding leaf tissue is inherently susceptible to oxidative damage, especially under stress conditions when the supply of light energy exceeds the capacity to utilize it [2,3]. Plants have evolved a number of mechanisms, such as photosynthetic apparatus damage and repair [4], to dissipate excess light energy to minimize the potential for damage at the expense of photosynthetic efficiency [5,6]. However, these mechanisms are sensitive to leaf development and thus may change from one leave tissue to another, resulting in heterogenous photosynthetic patterns (see Figure **??**). These patterns also vary with the position, size and growth rate of leaves, since leaves at the same node is unique in age. By integrating plant morphological and physiological features, measuring plant photosynthetic heterogeneity aids interpretation of the sophisticated photosynthesis mechanism, particularly important for plant primary productivity estimation and modeling [7].

Heterogeneity is a concept relating to the uniformity in a substance. The granularity of photosynthetic heterogeneity ranges from cells to tissues, leaves, and even the whole plant. While in-leaf variability in photosynthetic activity has been well-studied for the understanding of the effects of stomatal conductance [8, 9], recent works show that photosynthetic capacity may decline with vertical gradient and leaf age [10, 11], indicating that leaf-based photosynthetic heterogeneity is a key towards the understanding of plant photosynthesis.

The leaf heterogeneity in photosynthesis was firstly been studied with a simulation model [10]. Due to the lack of high-throughput phenotyping technologies, the authors determined the effects of biochemical variability via the Farquhar model incorporating defined degrees of spatial variability of its parameters. The Farquhar model is a mechanistic, biochemical model widely used to describe steady-state CO_2 assimilation in leaves [12,13]. Spatial heterogeneity in photosynthesis was found to have an effect on the ability of the Farquhar model to accurately characterize photosynthesis at the leaf level [10].

Recently, with the advent of advanced technologies of biomedical imaging, directly measuring heterogeneity has recently assumed new importance [14–17]. The rapid development of lighting and imaging techniques enables real-time non-invasive monitoring of photosynthesis [14, 18], resulting in vast amount of images of plant photosynthesis [19]. These images can be used to quantify photosynthetic behavior

in genetically diverse populations, enabling to measure variability of photosynthetic parameters at high resolution across leaves, leading to better understanding of the underlying mechanisms that control the photosynthetic properties [20, 21].

In order to measure leaf-based photosynthetic variability in a large-scale phenotyping experiment, in which hundreds of plants are screened simultaneously, it is required to automatically segment each leaf from top-view images, and then measure the variations of photosynthesis parameters across leaves. Photosynthesis images are false-color images, where the light intensity of every pixel is proportional to photosynthetic efficiency [22] (see Figure **??**). Differences between individual leaves with similar photosynthetic efficiency can be subtle, making the boundaries between them difficult to define and creating a significant challenge for subsequent shape analysis. The difficulty even arises when individual leaves overlap and occlude one another in these false-color images. ...

In this paper, we present a new computational framework called leafPH and use the tool to minitor the leaf-level photosynthesis heterogeneity of more than 100 Arabidopsis chloroplast mutant strains, each with at least four replicates. The mutant screen analysis reveals that ...

Results

In plants, photosynthetic heterogeneity refers to a plant comprising multiple regions, many of which have different photosynthesis properties, potentially because of vastly different leaf developmental stage and tolerance level to environmental changes. By developing an efficient plant photosynthetic heterogeneity measure leafPH and applying it on more than 100 Arabidopsis chloroplast mutant strains, we show that ...

Framework of leafPH

We first introduce the framework of leafPH which consists two major modules: leaf alignment and tracking and heterogeneity measurement. The architecture of the application is shown in Figure ??. First of all, we have developed the leaf alignment algorithm [23]. This paper focus on the measure of

Comparing with the existing heterogeneity approaches, our method is novel in the following ways:

1. leafPH relies on a novel leaf alignment and tracking method...
2. leafPH models heterogeneity of photosynthesis with size, position and growth rate...
3. leafPH refers to the differences between individual leaf photosynthesis and the pooled photosynthesis across all the leaves, with the weights being those used in the pooling method. With leafPH, transient regional variation events that do not affect the whole plant photosynthesis, can be easily discovered.

Basic heterogeneity measurement

Let $T = \{T_1, T_1, \dots T_n\}$ be the set of effect estimates of a plant p with n leaves. In each study T_i, let u_i and u_p be the averaged photosynthesis values of leaf l_i and the averaged photosynthesis values of whole plant p, and $std(l_i)$ and $std(p)$ be the population standard deviations, respectively. Under the assumption of normal distribution and homoscedasticity, the effect estimate T_i is the standardized mean difference [24], which can be estimated by

$$T_i = \frac{c(l_i)\,(\mu(l_i) - \mu(p))}{S(l_i, p)} \tag{1}$$

where $mean(.)$ is the averaged photosynthesis value, $c(l_i)$ is a correction factor for the positive bias suffered by the standardized mean difference with small sample sizes, which can be estimated by $c(l_i) = 1 - 3/(4(|l_i| - 1) - 1)$ [25]. Such adjustment will reduce the effect estimate of small leaves that only have a few pixels, and thus increase the robustness of the heterogeneity model. The pooled estimate of the within-group standard deviation $S(l_i, p)$ can be computed with [24]:

$$S(l_i, p) = \sqrt{\frac{(|l_i| - 1)std^2(l_i) + (|p| - 1)std^2(p)}{|l_i| + |p| - 2}} \tag{2}$$

where $std^2(.)$ is the standard variance and $|.|$ is the total number of pixels.

Second, we apply Cochran's Q-test statistic for determining whether there is true leaf-based photosynthetic heterogeneity among the leaves:

$$Q = \sum_{i=1}^{n} w_i \left(T_i - mean(T)\right)^2 \tag{3}$$

where n is the number of leaves of plant p, and $w_i = 1/(\tau^2 + \delta_i^2)$. Note that we ignored the computation of the sampling variance of the effect estimate δ_i^2, since we count the photosynthesis value of every pixel in the test. The between-study variance τ^2 is the parameter in the statistical model that mainly represents the true heterogeneity among the true effects of the studies. Therefore, a good procedure for determining whether there is true heterogeneity among all leaves of a plant should be positively correlated with τ^2. At the same time, it should not be affected by the number of leaves, and should be scale-free in order to be comparable among different plants.

We estimate τ^2 using an estimator based on the method of moments [26]:

$$\tau^2 = \begin{cases} \frac{\widetilde{Q} - (n-1)}{\sum \widetilde{w_i} - \frac{\sum \widetilde{w_i}^2}{\sum \widetilde{w_i}}} & \widetilde{Q} > |T| + 1 \\ 0 & else \end{cases} \tag{4}$$

where $\widetilde{Q}$ and $\widetilde{w_{ij}}$ are the results of Q test of fixed effect estimate and the corresponding weight.

$$\widetilde{w_{ij}} = [Pos(L_i) \neq Pos(L_j)] \times \frac{min(|L_i|, |L_j|)}{(ST_{ij}^2 + c) \sum L} \tag{5}$$

One problem with the Q test is that its statistical power depends

Heterogeneity measurement of position, size and growth of leaves

leafPH on Arabidopsis data

The L'Abbe plot can be used to visually explore the inconsistency of leaf-based photosynthesis.

leafPH on synthetic data

Materials and Methods

Data Acquisition and Preprocessing

In the photosynthesis phenotyping experiment, hundreds of Arabidopsis thaliana plants (wild type, genetic variations with gene knockout or over-expression, ecotypes, etc) were grown side-by-side under three different light conditions (constant, sinusoid, fluctuate), for in total three days. Top-view fluorescence images were collected every 15 minutes in order to observe the photosynthesis activity of all of the plants simultaneously. Each fluorescence image is a grey-scale image with a resolution of 1M pixels at 12-bit intensity.

To accurately capture the photosynthesis activities of plants from fluorescence images, a image segment method is applied to remove the background, identify every piece of leaf [23], measure the intensity of pixels on leaves, and finally convert the intensity values to the measure of four kinds of photosynthesis

parameters. The extracted measurements of photosynthesis parameters are presented in the form of multi-dimensional time-series, one dimension for every photosynthesis parameter. Figure 1 shows that the measurements of a photosynthesis parameter Φ_2 at one time point are quite different for the leaves of the same plant (i.e. plant no. 3-7 and 5-5).

Leaf alignment and tracking

For completeness, we introduce the leaf alignment and tracking approach...

Heterogeneity Test

Cochran's Q-test is the classical measure of heterogeneity [27]. In leaf-based photosynthetic heterogeneity, Q is calculated as the weighted sum of squared differences between the photosynthesis value of a individual leaf and the pooled photosynthesis value across all leaves with the weights being those used in the pooling method. The distribution of Q is a chi-square statistic with $k-1$ degrees of freedom, where k is the number of leaves. Cochran's Q-test has been widely used in biomedical studies. For example, heterogeneity in the aggressiveness of tumor cell populations has been adopted as an essential feature in predicting treatment success [28]. However, due to the nature of plant, leaf-based photosynthetic heterogeneity often includes a small number of leaves, and thus the power of the Q-test in such circumstances is low [29, 30].

The I^2 statistic describes the percentage of variation across studies that is due to heterogeneity rather than chance [29, 31]. I^2 can be calculated as $I^2 = (Q - df)/Q$, where Q is Cochran's Q-test heterogeneity statistic and df is the degrees of freedom. A negative value indicates no observed heterogeneity, and larger values show increasing heterogeneity. I^2 is an intuitive and simple expression of the inconsistency. I^2 of leaf-based photosynthetic heterogeneity does not inherently depend upon the number of leaves, so that I^2 values of different plants become comparable. A confidence interval for I^2 is constructed using either the iterative non-central chi-squared distribution method [32] or the test-based method [31].

Based on Cochran's Q-test and the I^2 statistic, we develop a new approach that quantifies the effect of heterogeneity of photosynthesis across leaves of the same plant, and compare the degree of inconsistency among mutant strains in varying environmental conditions (see Section). The challenges of this work include the leaf alignment and the new heterogeneity measure that takes leaf position, size and growth into consideration.

Discussion

A consensus view of the data is that the photosynthesis ability of a plant is not uniform across the whole area (Charles 2008, Meng 2007). The photosynthetic properties of plants can vary dramatically across cells, tissues, and organs [], reflecting differences in development, stress responses, regulation of processes such as stomatal conductance [], photodamage [], and storage of photosynthate [] and contribute substantially to productivity [].

For example, we observed that the acclimation of photosynthesis in response to cold temperatures appears to be more rapid and robust in younger or emerging than older leaves, and ecotypes isolated from different latitudes show distinct heterogeneity patterns, implying that these responses are important for adaptation of photosynthesis to fluctuating temperatures. In other cases, exposure of plants to fluctuating light resulted in loss of photosynthetic capacity or increased photoinhibition in specific sets of leaves or leaf sectors. In many cases, older leaves are preferentially affected, suggesting that resources for maintenance or acclimation responses are preferentially directed to younger leaves. However, we have also identified mutant lines where younger leaves are preferentially affected, which presumably affect the development of photosynthetic robustness.

In order to systematically study the leaf level photosynthesis phenotypes, especially in a high-throughput screen manner, we developed a novel computational tool to automatically conduct statistical analysis on leaf based photosynthesis.

Acknowledgments

References

Figure Legends

Tables

Supporting Information Legends

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