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# New Parameters for Seedling Vigor Developed via Phenomics

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**Abstract:** Early seedling establishment in rice (*Oryza sativa* L.), which is measured by primary/secondary tiller, shoot length, biomass, root-related traits, and leaf area index, is an important trait because it helps to compete for light, air, and water for better tolerating various abiotic stresses. Consequently, it can affect the yield. However, there are not many research studies on this subject. Furthermore, previous studies have only measured the target traits once. However, this does not reflect the variation of growth rate during the seedling stage. Thus, two data points, two weeks and four weeks after planting, were used in the current study. As a result, two QTL regions were detected for the growth differences via plant height and green area (reflecting tillering). We expect that these results can be utilized by breeders to evaluate and select vigorous seedlings for their breeding programs.

**Keywords:** plant phenomics; recombinant inbred line (RIL); rice; initial growth; QTL

## 1. Introduction

Early seedling establishment in rice (*Oryza sativa* L.) is a crucial trait to compete for light, air, and water for better tolerating various abiotic stresses. It also contributes to subsequent tillering quality [1]. Thus, it can highly affect the grain yield [2]. Kumar et al. [3] reported that early vigor has a very strong correlation with yield (0.84). Early seedling establishment is described in many research papers as seedling vigor and measured using several traits, such as primary/secondary tiller, shoot length, biomass, root-related traits, and leaf area index [1–13].

With the fact that the yield itself is one of the most poorly inherited traits in crop breeding [14], it would be very advantageous to invest in data collection during the early stage via examining seedling vigor. Among those traits for early seedling vigor, the majority of research has studied plant height and tillering because they are associated with yield. Plant height can help compete for light and indirectly affect biomass, which has a high impact on grain fill and grain weight. Tillering is highly related to the panicle number and eventually grain number. Other traits such as roots are also highly associated with the yield, while they are laborious and time-consuming to measure.

The labor-intensive and costly conventional phenotyping was the only method until the advent of high throughput phenotyping [15]. However, it has not been widely adapted in breeding programs.

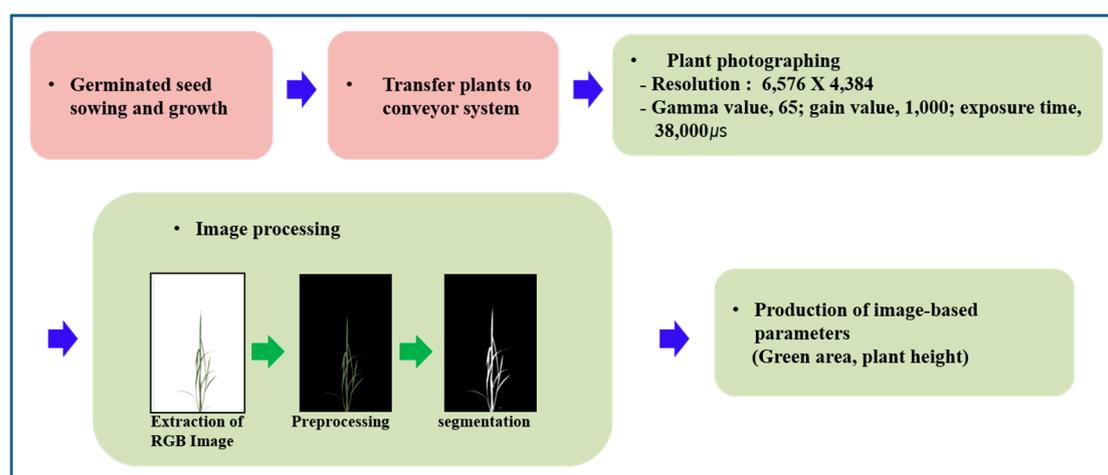
In rice, there are two research papers that have been published that collected data in the greenhouse [16] and field [17] using mature plants. However, the beauty of phenomics is the non-destructiveness, which means that the same objects can be repeatedly measured in different time intervals. Further, it can snap the data of many samples within a very short period of time very accurately and precisely. These are excellent features for collecting data in the early stage since the growth stage shift is fast in that stage. However, previous studies on seedling vigor only collected data once [7,12,13]. Although two data points were collected in one study, it did not examine the difference between the two data points [5]. As Hittalmani et al. [18] concluded that the quantitative trait loci (QTLs) on chromosome 1 for different traits, including projected plant height, panicle number, and panicle length, suggest the pleiotropism and or tight linkage of those traits, sharing several traits in the same region on chromosome 4 might be explained by pleiotropism as well.

There are several growth stage shifts, which means that many changes are involved in this time period. Thus, even the same trait, such as plant height and biomass, can be very different within this time span. Further, there is no research on the growth rate that has been conducted by measuring two different time points for growth comparison, which would enable researchers to understand what really occurs in the early stage. Hence, the objective of the current study is to measure the growth rate using plant height and green area which are highly associated with yield in second and fourth week in order to find out if these traits are related with any QTLs. The result would help us to understand how each phase of vegetative growth in the early stage moves. Furthermore, it would be the true feature of seedling vigor since it can measure the growth rate within a two to four-week time span.

## 2. Materials and Methods

### 2.1. Plant Materials and Growth Condition

As plant materials, 162 recombinant inbred lines with eight sub-samples were used, which were made by the combination of Milyang 23 and Gihobyeo. These groups were developed more than F25 generation by the SSD method, and a physical map was made by Indel, STS, RTM marker [19]. Sowing and growing were carried out in a 50-well tray in a complete randomized block design and grown for two and four weeks in a 14-h light/10-h dark condition. At the time of imaging, the outermost growing plants were removed to exclude the edge effect (Figure 1).



**Figure 1.** The process of image acquisition.

### 2.2. Image Acquisition

The image of the rice was photographed using a 3D-scanner (LemnaTec, Germany) and analyzed using a Matlab (2013b, The MathWorks Inc., Natick, MA, USA) program. RGB (red, green, and blue)

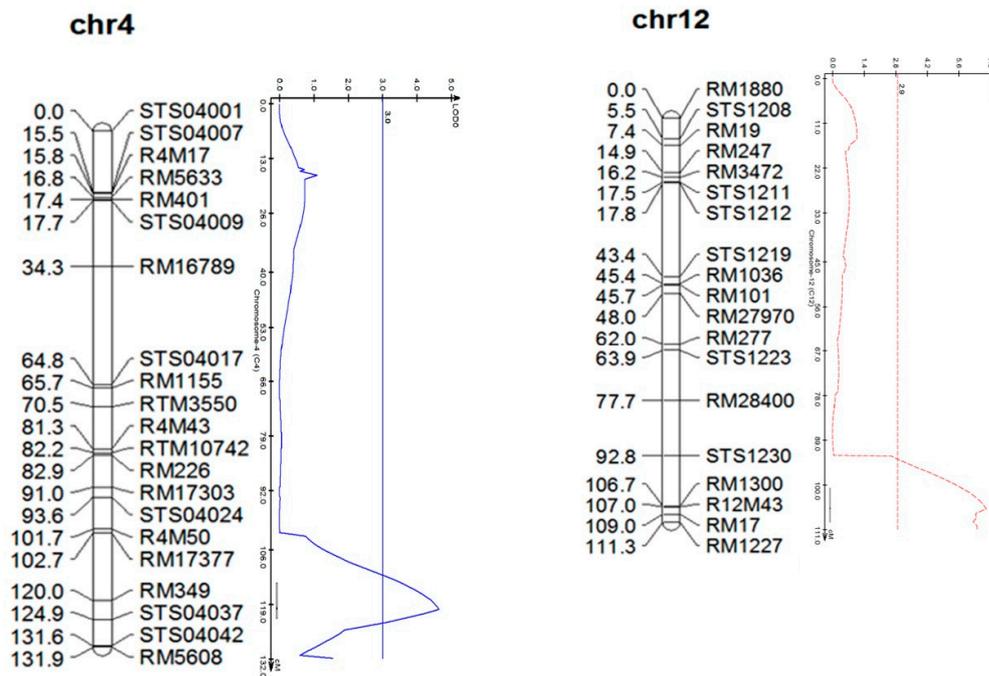
images of plants were obtained with the resolution of  $6576 \times 4384$ . Light condition was constantly maintained by the gamma value of 65, gain value of 1000, and exposure time of  $38,000 \mu\text{s}$  (Figure 1). At this time, the number of pixels was calculated by separating the green area of the leaf and stem. The plant height using image information was also measured using the Matlab program. Afterwards, each trait was summarized in Excel format and used for QTL analysis as follows: After changing the RGB image extracted from the region of interest (ROI) into the HSI color space, a green region containing 73 to 180 degrees of the HUE channel was obtained. The plant height was obtained by using the 'regionprops' function in Matlab to create the minimum rectangle surrounding the ROI, followed by its longitudinal axis length. The obtained data of the green area and plant height were converted into Excel format using the 'xlswrite' function.

### 2.3. Algorithm Application in Image Analysis

Effective image analysis was performed using the intermediate value filter, fill area filter, and multi-step morphology by the Matlab program. The intermediate value filter was used as a median filter to remove small speckle noise using the 'medfilt2' function [20]. The untreated areas of images generated in the noise removal process were processed by using the fill area filter function 'imfill' [21]. Through the 'imdilate' and 'imerode' functions in the multi-step morphology process, morphological dilation and erosion eliminated problems of the reduction of the plant area or the background addition [22]. In order to improve the data processing speed, the obtained image was cut out of the plant area only by removing the background as much as possible. In the case of RGB, it is difficult to investigate the color value effectively because it uses the additive color mixture of the three primary colors. Color classification was performed by replacing RGB images with HSI and Lab channels [23]. HSI means Hue, Saturation, and Intensity, and Lab means L for lightness and a and b for the color opponents green–red and blue–yellow. The color classification process was used to obtain the maximum color information that appears mainly throughout the growth of rice [24–28]. The HSI-H, Lab-a, and Lab-b channels excluded the red and blue hue regions and mainly used the green and yellow hue regions (Figures 2 and 3).



**Figure 2.** Phenotype of two and four week-old plants.



**Figure 3.** Genetic map showing the growth rate-related QTLs between two and four week-old seedlings of the MGRILs. *qGA-4* in the blue peak indicates the green area; *qPH-12* in the red peak indicates the plant height.

#### 2.4. QTL Analysis for Calculating the Growth Rate

Data for QTL analysis were analyzed using the MapDisto program [28] for the genetic mapping of recombinant subspecies between Milyang 23 and Gihobyeo. Genetic mapping and physical mapping of each marker were performed using the MapChart program [19,26,29]. QTL analysis was performed using Windows QTL Cartographer V2.5 [30]. A significant logarithm of the odds (LOD) value was adopted for 1000 traits at a 95% significance level for each trait. The QTL results for the growth rate were analyzed through the program after calculating the differences in green area and plant height between two and four weeks. Green area QTL (*qGA*) was the total pixel number of the plant image, and plant height QTL (*qPH*) was calculated by the object extent Y of the Y-axis length of the least vertical oriented rectangle covering the object.

### 3. Results

The data for plant height and green area measured by image analysis followed a normal distribution and most of the variance in the growth phenotypes was explained by the genotype (data is not shown). QTL for plant height difference between two and four week-old seedlings was detected on chromosome 12 (Figure 3). The peak on chromosome 4 is located at 120 cM with LOD 4.7 (between R4M50 and STS04042) and that on chromosome 12 was at 105.0 cM with LOD 6.8 (between STS1230 and RM1227). QTL for the plant green area difference between two and four week-old seedlings was detected on chromosome 4, which is known to be associated with the plant height of mature rice, as shown in several studies such as [31]. Those regions on chromosome 12 and 4 are reported to be associated with plant height in mature rice [10,31].

### 4. Discussion

The seedling vigor is the early stage of the vegetative growth phase. Vegetative growth stages are characterized by a gradual increase in plant height, leaf emergence at regular intervals, and active tillering [32]. A recent study for plant height [16,17] in matured rice using the high throughput phenotyping method revealed a strong peak on chromosome 1, which is a known area where the

*semi-dwarf1* (*sd1*) gene is located. However, several studies using seedlings reported that the same peak of QTL on chromosome 12 found in the current study is associated with plant height and that it is associated with the tolerance of anaerobic conditions using three week-old seedlings [33], as well as using mature rice [34–38]. However, the seedlings in the current study were not submerged. Thus, something else might be involved in the elongation of leaves or (inter)nodes.

Tillering is an important agronomic trait for grain production. The plant height consists of leaf length and culm length, which are composed of nodes and internodes. Thus, in order to grow vertically, both the leaf and culm should be extended. However, the growth rate of leaf length and culm length can be different, as found in sorghum [36]. Both plant height by means of leaf elongation and tiller can contribute to a greater area of blade leaf. This is crucial for energy production for the yield because a blade leaf contains more chlorophyll [39]. Rice tiller is a specialized grain-bearing branch that is formed on the unelongated basal internode and grows independently of culm [40]. Yang et al. [16] reported that the green area of images taken for the whole plants was associated with the peak on chromosome 4, which is associated with plant volume that can be increased by tillers. This region shares the same peak with the current study for the green area difference between two- and four-week-old seedlings. The difference here comes from the tiller initiation in this early stage. The sheath and blades are each longer and wider than their counterparts on the previously emerged leaf, and this trend continues until the ninth complete leaf while the plant is in the vegetative stage [40]. Notably, tillering occurs before the ninth leaf of the main shoot is completed. This transit speed required to initiate the tillering stage could be very important for seedling vigor as not only does the crown develop noticeably at the base of the main shoot during tillering [39], but also the number of productive tillers has a direct positive effect on the number of filled grains per panicle and the grain yield [41]. The QTL peak on chromosome 4 in the current study is reported to be associated with several important agronomic traits in mature rice, including root thickness, root dry weight per tiller [42], crown growth [43], basal root thickness, root fresh weight, and root volume [43], which are observed in the tiller initiation stage. This region is also related to the number of spikelets, number of panicles, panicle length, and number of spikelets per panicle [18,44], which are directly related to the yield since grain yield is the combined result of those factors.

## 5. Conclusions

Early seedling embellishment is advantageous for plants, enabling them to be ready for stable growth during the growing season for a high yield. Among many other traits used to measure seedling vigor, the current study measured two important components for seedling vigor, plant height data and green area data collected at two different time points, two and four weeks after planting, in a very accurate and high throughput manner, which was not possible before; the result in the current study introduces new parameters for seedling vigor. Furthermore, these two components are highly associated with rice yield, as described above. Thus, the result with the new parameters for seedling vigor using high throughput phenotyping could be useful to breeders not only for fast seedling establishment, but also for high yield potential.

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