

1 **Developmental Normalization of Phenomics Data Generated by High Throughput Plant Phenotyping** 2 **Systems**

3
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16 **Abstract**

18 **Background**

19 Sowing time is commonly used as the temporal reference for *Arabidopsis thaliana* (*Arabidopsis*) experiments in
20 high throughput plant phenotyping (HTPP) systems. This relies on the assumption that germination and seedling
21 establishment are uniform across the population. However, individual seeds have different development
22 trajectories even under uniform environmental conditions. This leads to increased variance in quantitative
23 phenotyping approaches. We developed the Digital Adjustment of Plant Development (DAPD) normalization
24 method. It normalizes time-series HTPP measurements by reference to an early developmental stage and in an
25 automated manner. The timeline of each measurement series is shifted to a reference time. The normalization is
26 determined by cross-correlation at multiple time points of the time-series measurements, which may include
27 rosette area, leaf size, and number.

29 **Results**

30 The DAPD method improved the accuracy of phenotyping measurements by decreasing the statistical dispersion
31 of quantitative traits across a time-series. We applied DAPD to evaluate the relative growth rate in *A. thaliana*
32 plants and demonstrated that it improves uniformity in measurements, permitting a more informative comparison
33 between individuals. Application of DAPD decreased variance of phenotyping measurements by up to 2.5 times
34 compared to sowing-time normalization. The DAPD method also identified more outliers than any other central
35 tendency technique applied to the non-normalized dataset.

36

37 Keywords

38 High-throughput plant phenotyping, development, growth, computer vision, machine learning, phenomics

39

40 **Background**

41 Sowing-time is often taken as the initial time point for measuring plant phenotypic traits in HTPP systems [1].

42 Traits of a number of plants are typically measured between two defined timepoints, chosen relative to the time of
43 sowing. However, the germination of individual seeds in a genetically identical population is normally distributed,
44 even under uniform conditions [2]. Differences in the timing of germination and seed establishment increase the
45 dispersion of time-series measurements because individual plants will be at different developmental stages at
46 any given time point. This may create difficulty in drawing reliable conclusions from the data.

47

48 Data normalization is often applied to analyze datasets that have high dispersion, but many methods are not
49 suitable for time-series plant phenotyping data. Traditional methods such as the z-score, min-max, and decimal
50 scaling are not appropriate because of statistical parameters such as mean and standard deviation change over
51 time. Time-series normalization methods can numerically fit the time-series measurements to a single timeline
52 and reduce dispersion. However, these methods do not take into consideration developmental information nor
53 the effect of the allometric scaling of growth; individual seedlings can have similar trait values but maybe at
54 different developmental stages.

55

56 Plants progress through specific developmental stages that are consistent between genetically identical
57 individuals grown in uniform conditions. For example, the adjusted BASF, Bayer, Ciba-Geigy (BBCH) scale
58 describes *A. thaliana* developmental stages using seed germination, leaf development, rosette growth,
59 inflorescence emergence, flower production, silique ripening and senescence as significant markers [3].
60 Considering the availability of clear developmental stage scales, development normalization could be an
61 appropriate method to normalize HTPP data. Developmental normalization would arrange time-series plant
62 phenotype measurements based upon plants being at similar developmental stages. However, developmental
63 normalization has not previously been implemented in an automated manner suitable for image-based HTPP
64 datasets, likely due to the technical difficulty [4].

65

66 Image-based plant phenotyping systems are now widely used [5]. However, image processing remains one of the
67 most considerable difficulties for these systems, especially image segmentation of shoots and leaves.

68 Performance depends heavily on the complexity of images, which frequently include interference, light reflection,
69 leaf overlap, and foreign objects that must be removed (for example, pots, and soil) [6]. Furthermore, the

70 identification of multiple leaves at the same time (multi-instance segmentation) is difficult due to their similarity in
71 shape and appearance [4]. Without algorithms that extract accurate measurements, it is more complicated to
72 scale the principal environmental variables influencing the phenotype and underlying physiological processes [7].

73
74 Several approaches have been developed to address the challenge of image segmentation. They can be
75 categorized into four groups; shape analysis, watershed-based, machine learning, and graph-based. Shape
76 analysis algorithms rely on assumptions regarding plant geometrical features and structure, but they may fail
77 when encountering new data, which limits their applicability [8], [9]. Watershed-based methods consider a grey
78 image as a topographic surface produced by its intensity gradients, where light pixels are represented as high-
79 intensity values and dark pixels as low-intensity values [4]. However, the performance of watershed algorithms is
80 compromised due to over-segmentation when leaves overlap. Machine learning segmentation approaches can
81 be unsupervised or supervised. Unsupervised learning algorithms are mainly used for pixel clustering. They
82 identify individual leaves by grouping pixels which share a similar feature pattern such as color, texture, and
83 others. Supervised learning algorithms analyze and compare the input plant images with annotated images or
84 labels [10]. Graph-based methods segment individual leaves by applying graph-based noise removal and region
85 growing techniques [8].

86
87 We developed DAPD, which combines time-series trait measurement with normalization by developmental stage
88 in an automated manner. DAPD synchronizes the start-point of timelines for all plants in the population by their
89 number of leaves. To achieve this, we also developed a new leaf segmentation algorithm that utilizes both shape
90 analysis and supervised machine learning algorithms. This combined approach overcomes the limitations of
91 individual segmentation methods. We applied DAPD to evaluate the rosette area in *A. thaliana* and demonstrated
92 that it improved uniformity in measurements, enabling a more informative comparison between individuals. The
93 algorithm allows users to select and define the starting leaf number relevant to their experiment. Our code is
94 available for reuse at https://github.com/diloc/DAPD_Normalization.git.

95

96 **Results**

97

98 **DAPD time normalization of plant phenomics data**

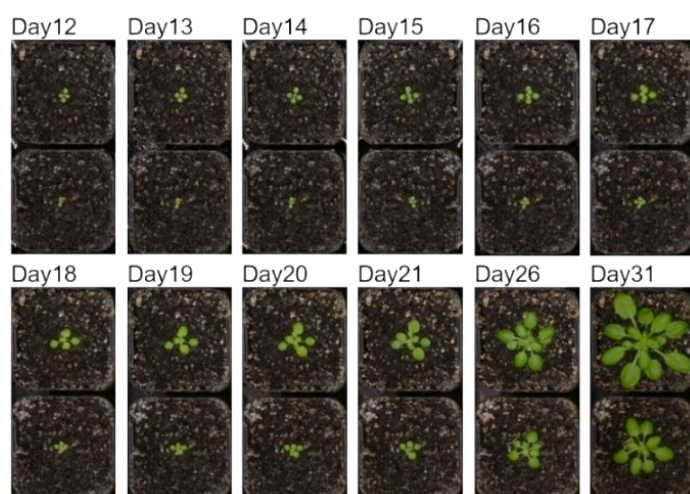
99 Our major aim was to produce an automated method for the developmental stage synchronization of HTPP data.
100 To achieve this, we developed DAPD, which synchronizes shoot phenotypic measurements of multiple
101 Arabidopsis plants by normalizing time-series measurements to a reference time point (i.e., a day). DAPD does
102 so by identifying the highest cross-correlation score between leaf number and day-after-sowing (DAS) of
103 seedlings of the same genotype. First, seedlings were grouped by genotype, then their leaf number was

104 assessed by applying the DAPD-Leaf Counting algorithm. Next, a daily leaf number was associated with a
105 specific day in the DAS timeline, and the degree of similarity between them was calculated by cross-correlation.
106 The highest score indicates the day where the leaf number of time-series are best aligned. We intended that the
107 application of DAPD would decrease the dispersion of time-series phenotype measurements.

108

109 We tested DAPD on two independent experiments comprised of many *A. thaliana* ecotype Col-0 and Cvi
110 replicates. In the first experiment, we used 355 individuals of Col-0 only and, in the second experiment, 140
111 individuals of each ecotype. Replicate plants of the same ecotype within experiments were at different
112 developmental stages, as assessed by leaf number, despite being sown at the same time and having been
113 grown from seeds of plants cultivated under conditions that would not result in seed dormancy (Figure 1).

114



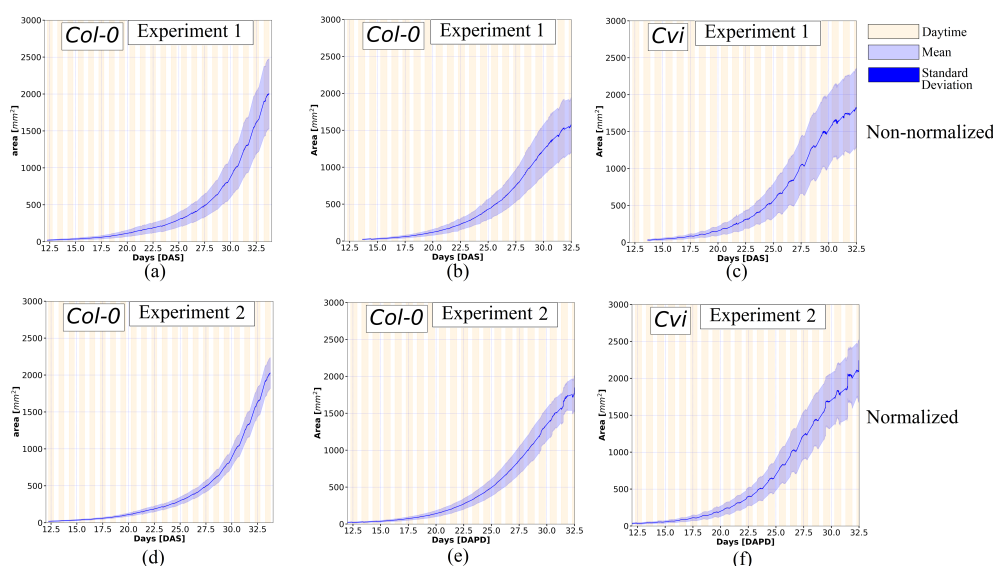
115

116 *Figure 1 Developmental differences in two neighboring Arabidopsis seedlings (ecotype Col-0) grown under*
117 *uniform environmental conditions.*

118

119 The rosette area measurements of all individual Col-0 and Cvi plants were obtained at different time points during
120 the daytime from 12 to 32 days. The mean and standard deviation of these non-normalized measurements were
121 calculated (Figure 2). The overall dispersion of the non-normalized datasets consistently grew from day 12 to day
122 32, as assessed from the standard deviation of the rosette area measurements (Figure 2 a-c).

123



124

125 *Figure 2 Mean and standard deviation of the non-normalized and normalized projected rosette area datasets.*

126 *The top row shows the non-normalized datasets: (a) Col-0 plants in experiment 1, (b) Col-0 plants in experiment*

127 *2, and (c) Cvi plants in experiment 2. The bottom row shows the normalized datasets: (d) Col-0 plants in*

128 *experiment 1, (e) Col-0 plants in experiment 2, and (f) Cvi plants in experiment 2. The light purple strip indicates*

129 *the standard deviation and the solid blue curve in the mean area.*

130 DAPD was applied to normalize the Col-0 and Cvi rosette area datasets. The standard deviation and mean

131 values were calculated at multiple time points to assess the dispersion of the data (Figure 2d-f). The normalized

132 data preserved the exponential growth pattern observed in the non-normalized data, but the overall statistical

133 dispersion of the normalized data was considerably smaller. Daily oscillations in leaf area were also observed,

134 most notably in the Cvi dataset. These occur due to the diurnal change in elevation angle of Arabidopsis leaves,

135 which increases and decreases the angle of the leaves relative to the cameras. Notably, DAPD preserves these

136 signals post-normalization because it shifts the time-series in whole day increments.

137

138 Examining the standard deviation over the time-series confirmed the observation that DAPD time normalization

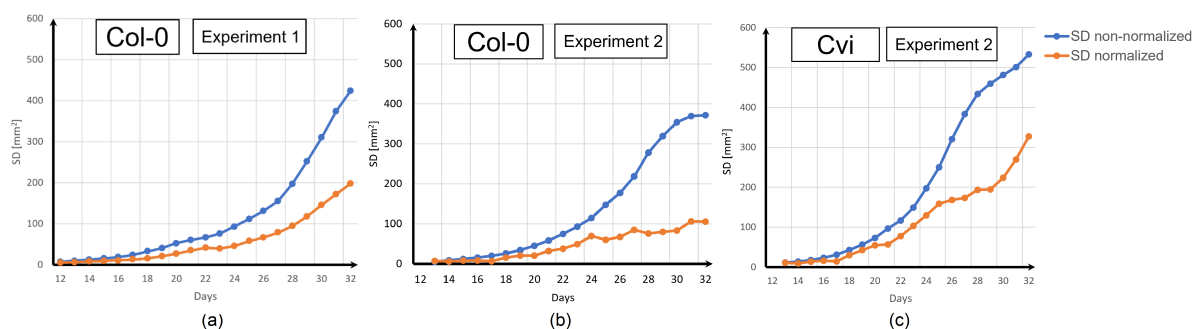
139 reduces the dispersion of rosette area measurements (Figure 3). During the period from day 13 to day 32, the

140 standard deviation of the non-normalized dataset exponentially increased in the three datasets whilst the

141 standard deviation of the normalized dataset linearly increased. DAPD normalization reduced the dispersion of

142 the measurements at day 32 by between 1.5 and 3.5 times.

143



144

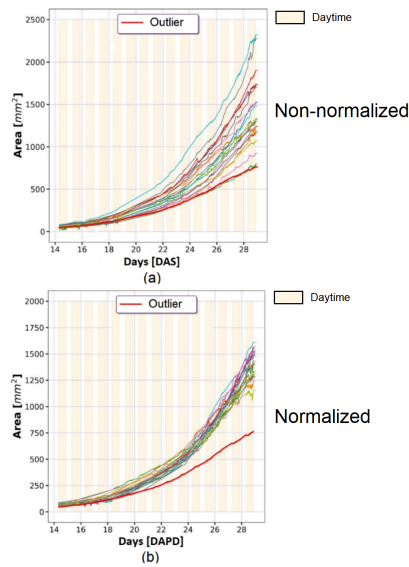
145 *Figure 3 Standard deviation (SD) comparison between the non-normalized and normalized datasets. (a) Col-0*
146 *plants in experiment 1, (b) Col-0 plants in experiment 2, and (c) Cvi plants experiment 2.*

147

148 **DAPD detects outliers with different growth traits amongst a population**

149 A powerful application of DAPD is detection of outliers in HTPP datasets. It is common in large-scale phenomic
150 experiments to observe a small number of individual plants that develop abnormally, despite being of the same
151 genotype as all other members of the population and being grown in uniform conditions. These individuals may
152 have been affected by unintended stresses, and, in some situations, it is reasonable to remove them from
153 datasets. We assessed the ability of DAPD to detect outliers on 24 wild-type Col-0 individuals grown two different
154 trays (Figure 4). It was difficult to confidently identify outliers amongst the non-normalized growth curves by visual
155 inspection or application of a central tendency metric (mean, median, mode of rosette area). However, after
156 applying DAPD normalization, a putative outlier was identified clearly. This plant did not follow the same growth
157 trajectory as the rest of the population, having a smaller rosette area from day 20 onwards. Visual inspection
158 determined that this plant was infected by a pathogen (Figure 5). These results demonstrate DAPD can be
159 applied to detect outliers in HTPP datasets systematically.

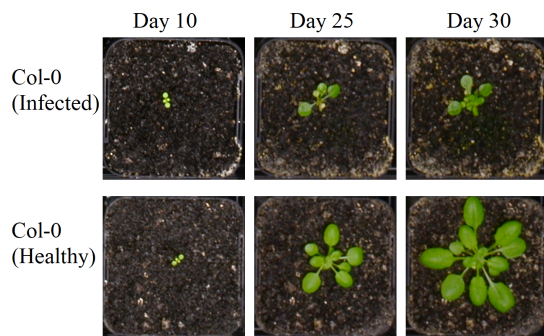
160



161

162 *Figure 4 Non-normalized and normalized rosette area curves of 24 Col-0 individuals in experiment 1, (a) non-*
163 *normalized Col-0 curves, and (b) normalized Col-0 curves. The red and thick curve represents the rosette area of*
164 *a pathogen-infected individual (outlier) before and after normalization.*

165



166

167 *Figure 5 A pathogen-infected plant with outlier growth traits detected automatically using DAPD. The pathogen*
168 *infected plant had a smaller number of leaves and rosette area than the healthy plant.*

169

170 **DAPD image segmentation out-performs other image segmentation methods**

171 DAPD depends upon a novel image segmentation method that we developed to improve accuracy and
172 applicability across new datasets compared with existing methods. The method depends on an algorithm that
173 combines shape analysis and supervised machine learning. We benchmarked the accuracy of the DAPD image
174 segmentation algorithm on public datasets (A1, A2, A3, A4) and our in-house generated dataset using dice,
175 precision, recall, and Jaccard metrics (Table 1) [11], [12]. These datasets contain ground-truth RGB and binary
176 images of *A. thaliana* and tobacco plants. DAPD performed consistently well in all metrics across all five
177 datasets. Notably, performance on the tobacco images (A3) comparable to performance on the *A. thaliana*
178 images, indicating DAPD is adaptable to plants with different structure.

179

180 *Table 1 Accuracy results of the DAPD image segmentation algorithm on four public datasets and our dataset.*

181 *The numerical values in each cell represent the mean and standard deviation (in parentheses). The last row*

182 *indicates the overall performance of our algorithm.*

183

Dataset	Accuracy (%)			
	Precision	Recall	Jaccard	Dice
A1	92.05 (3.65)	96.87 (4.81)	89.26 (4.43)	94.26 (2.59)
A2	90.90 (8.60)	97.45 (6.76)	88.83 (10.43)	93.71 (6.69)
A3	95.86 (5.14)	93.88 (14.48)	89.85 (13.85)	93.86 (10.97)
A4	94.89 (4.29)	98.37 (2.50)	92.56 (5.09)	96.06 (2.87)
Our dataset	96.05 (5.42)	98.57 (1.61)	93.29 (2.98)	96.58 (1.64)
Mean	93.95 (5.42)	97.03 (6.03)	90.76 (7.36)	94.90 (5.78)

184

185

186 We compared the performance of the DAPD image segmentation algorithm with three others (Table 2); the
187 Rosette Tracker algorithm [13], the probabilistic parametric active contours algorithm [14], and the Image-based
188 plant phenotyping with incremental learning and active contours [11]. The comparison was conducted by running
189 all five algorithms on the same datasets (A1, A2, A3, A4, and our dataset) and calculating the mean of each
190 performance metric. DAPD image segmentation strongly out-performed active contours and rosette tracker in
191 three of four metrics. Incremental learning performed well but was still not as strong as DAPD image
192 segmentation. These results demonstrated DAPD image segmentation performs well relative to other commonly
193 used approaches.

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201 *Table 2 Accuracy comparison between active contours, rosette tracker, incremental learning, graph*
202 *segmentation, and DADP algorithm. Each cell shows the mean and standard deviation of the four metrics*
203 *(precision, recall, Jaccard, and dice) across the five datasets (A1, A2, A3, A4, and our dataset).*

Method / Algorithm	Accuracy (%) (A1, A2, A3, A4 and our dataset)			
	Precision	Recall	Jaccard	Dice
Active contours	42.25 (26.05)	99.66 (26.18)	42.19 (25.66)	59.34 (26.20)
Rosette Tracker	54.29 (18.11)	99.97 (17.79)	54.29 (19.22)	70.37 (15.00)
Incremental learning	89.87 (13.68)	91.94 (2.96)	83.90 (14.44)	89.72 (12.36)
DAPD	93.95 (5.42)	97.03 (6.03)	90.76 (7.36)	94.90 (5.78)

204

205

206 **Discussion**

207 Traditional growth analysis in HTPP systems relies on sowing-time as the temporal start point. This assumption
208 could lead researchers to believe, for instance, that application of an experimental treatment elicits changes in
209 growth at a given time point. However, these analyses may be confounded by differences in germination time or
210 true leaf emergence between genotypes.

211

212 We propose the DAPD method to control for temporal differences in development within plant phenotyping
213 datasets. This method uses plant developmental stages to normalize the timeline of phenotyping measurements.
214 We demonstrate the utility of DAPD to normalize rosette area measurements, but it can be similarly applied to
215 normalize any phenotypic measurements. Furthermore, the analysis we present is on Arabidopsis plants, but
216 DAPD could be used to normalize HTPP data from any species, with two contingencies: First, that a defined and
217 relevant developmental scale can be provided to normalize to, and; second, that an algorithm is available to
218 measure the phenotypic feature of interest in high throughput.

219

220 DAPD normalization improved the detection of outliers. Typically a small proportion of plants develop abnormally
221 in large-scale experiments. There are many causes, such as seed quality, low-frequency pathogen infection or
222 unintended stress. The DAPD method enabled clear, systematic detection of anomalous individuals (outliers)
223 within datasets, which is not possible by inspecting central tendency metrics of the non-normalized datasets.

224 DAPD includes a new algorithm to extract the rosette area and count the number of leaves from top-view images.

225 This method outperformed other image segmentation methods when accuracy metrics were assessed on five
226 different ground-truth datasets.

227

228 Comparisons between traditional analysis, where sowing time is taken as the reference point, with DAPD
229 normalization illustrated that DAPD decreased dispersion of measurements. The difference in data dispersion
230 between the two approaches gradually increased from the start of experiments to the end. This occurred because
231 dispersion increased more rapidly over the plant lifecycle in the traditional (non-normalized) analysis than the
232 DAPD normalized data. In principle DAPD normalization will enable more sensitive detection of significant
233 differences in trait values between plant lines or ecotypes, due to this decreased variance in measurements.
234 DAPD normalization might also identify differences in germination phenotypes that might otherwise go unnoticed.
235 For example, a mutant with an apparently greater rosette area than wild type during an early growth stage might
236 either have a higher growth rate or germinate earlier. Assuming development was otherwise unchanged, DAPD
237 normalization would eliminate the former possibility, allowing researchers to focus on subsequent experiments.
238 Our code is available for reuse at https://github.com/diloc/DAPD_Normalization.git.

239

240 **Methods**

241 **Plant material and growth conditions**

242 Seeds were sterilized in a desiccator using chlorine gas for 150 minutes then stratified for three days in 0.1%
243 agarose in the dark at 4°C. Afterwards the seeds were sown in vermiculite, perlite, and soil mixture (1:1:3), with
244 20 pots per tray. After germination, only a single seedling was retained per pot. The seedlings were grown in a
245 controlled environment room at 20°C with 50% humidity and were watered with 500 ml water every four days
246 after sowing. Illumination was with an LED light source; it used seven light wavelengths including near-infrared
247 (850 nm), far-red (740 nm), deep red (660 nm), red (618-630 nm), green (530 nm), blue (450-460 nm) and cold
248 white, supplied by PSI Instruments. The average irradiance output on the chamber was set at 150 μ mol m⁻² s⁻¹ in
249 the photosynthetically active radiation spectrum.

250

251 In experiment 1, three hundred and fifty-five individual plants of *A. thaliana* wild-type (Col-0) were grown under 12
252 h light / 12h dark cycle. In experiment 2, one hundred and forty individuals each of *A. thaliana* ecotypes Cvi and
253 Col-0 were grown under long-day conditions (14 h of light/10 h of dark).

254

255 **Imaging**

256 Images were acquired every 15 minutes during the daytime using an HTPP system with 30 RGB cameras that
257 formed a stereo vision system. After the acquisition, images were pre-processed to reduce the noise and correct
258 the color and lens distortion. Subsequently, image segmentation algorithms extracted the rosette area and shoot
259 phenotypic measurements such as the rosette area, growth rate, and leaf number were calculated. The image
260 processing steps and details are described in the following.

261

262 ***Image pre-processing***

263 Image pre-processing algorithms corrected the lens and color distortion. The lens correction algorithm calculated
264 the optimal rotation and translation camera parameters using a chessboard pattern and the pin-hole camera
265 model. Color distortions were removed using a mapping function between the light intensity and color checker
266 cards, which had been included in the imaged area.

267

268 ***Rosette segmentation***

269 The projected rosette area of individual plants was extracted from pre-processed images by using a combination
270 of multiple image segmentation algorithms. First, individual pot regions were dynamically cropped from the RGB
271 images using an adaptive window. The resultant image was converted to HSV color space and the value (V)
272 channel extracted from it. The V channel was used to enhance the contrast of the image by applying the Contrast
273 Limited Adaptive Histogram Equalization (CLAHE) technique [15]. Subsequently, the green color component of
274 the image was obtained by the independent contribution of the RGB and HSV color space. The green component
275 image was binarized using Otsu's method for global automatic thresholding [16]. The salt-and-pepper noise of
276 the Otsu result was removed by applying a median filter, followed by using a Gaussian filter, which reduced high-
277 frequency spatial noise. The binary image was further processed by an area-fill operation to remove small
278 unwanted background regions or holes.

279

280 The resultant binary image was used to segment the plant shoot from the original HSV image. Undesired
281 background objects were identified and removed from it by grouping into coherent classes using K-means
282 clustering [17]. Then, a histogram of the Hue channel was extracted and smoothed using the Savitzky-Golay filter
283 [18]. A complementary spatial correlation test was used to determine the rosette objects. The test checked the
284 correlation between image clusters. These clusters were formed by selecting all colors in a distribution except by
285 color values beyond the curve's intersection point. Spatially correlated clusters belonged to the same object and
286 were retained. Non-correlated clusters were removed. The whole procedure was repeated multiple times, and at
287 each time, the gamma value was randomly modified. This iterative approach occasionally over-segmented some
288 rosette areas, but it was corrected by using Kriging [19]. Kriging is also known as Gaussian process regression,
289 which produced predictions of unobserved values from observations at nearby locations [20]. Finally, the
290 projected rosette area was obtained from the segmented rosette image. Unexpected power disruptions caused
291 some image data not to be acquired during the time-series. Considering the complete time series as being
292 images acquired every 15 minutes during the day, the data missing from experiment 1 was 14.83% of the total
293 data and in experiment 2 was 5.6%. To enable consistent downstream analyses we applied data imputation

294 techniques to estimate the missing data, including up-sampling to a higher frequency, spline interpolation and
295 curve fitting.

296

297 **Individual leaf Segmentation / Counting (DAPD segmentation)**

298 Our leaf segmentation approach combined two algorithms; edge contour extraction and marker-controlled
299 watershed [21]. The edge contour extraction process was applied to the rosette binary image to obtain the rosette
300 contour using topological analysis [22]. The center of mass of the rosette was calculated using the Hough
301 transformation. This information was used to calculate leaf morphology features such as tip, base, center, and
302 petiole. The watershed transformation was applied to the segmented rosette image, and centers of leaves were
303 used as initial markers. This identified overlapping leaves and separated convex and smooth rosette features that
304 touched. After applying the watershed transformation, the resulted number of markers represented the total
305 number of leaves in the rosette.

306

307 **Developmental normalization (DAPD normalization)**

308 The normalization by development removed the time difference between plant measurements, which had similar
309 developmental stages. These stages were identified by the leaf number based on the adjusted BBCH scale [3].

310

311 The leaf number of each plant was measured by applying the DAPD segmentation algorithm to the rosette
312 images. The measured leaf number was a discrete function (1), which depended on the real leaf number, leaf
313 occlusion effect, and modeling error denoted $ME(t)$.

314

$$315 \text{Leaf}_{meas}(t) = \text{Leaf}_{number}(t) + \text{Leaf}_{occlusion}(t) + ME(t) \quad (1)$$

316 The variability of the measured leaf number function (1) was minimized by calculating the trend using curve
317 fitting. This trend assembled an exponential function in the early developmental stages (2). The coefficient was
318 the initial value of the function, and b was the growth rate.

319

$$320 \text{Leaf}_{trend}(t) = ae^{bt} \quad (2)$$

321 The leaf number trend may vary from plant to plant within the same line/mutant population at a time point. Then,
322 the average leaf number trend among all individuals was calculated to homogenize the trends (3).

323

$$324 \overline{\text{Leaf}_{trend}(t)} = \frac{\sum_{i=1}^N a_i e^{b_i t}}{N} \quad (3)$$

325 The time-difference of plants with similar leaf number was removed by finding the best timeline in (1) that fitted
326 (3). Multiple timelines were generated from the original timeline (t) by inserting a time delay (k). This time delay
327 could be a positive or negative integer number that shifted the original timeline in days (t – k). The mean squared
328 deviation (MSD) was calculated per each time delay (4). The time delay (s) that produced the lowest MSD was
329 selected to shift the leaf number (5) and rosette area (6) time series. However, this time delay (s) must be
330 adjusted because plants could have the same number of leaves, but the rosette area might be different due to
331 the maturity and expansion of leaves. Δt represented the time delay adjustment, as shown in equation (7).

332

$$333 \quad MSD = \frac{1}{n} \sum_{i=1}^n (Leaf_{meas}(t - k) - \overline{Leaf_{trend}(t)})^2 \quad (4)$$

$$334 \quad Leaf_{meas_shift}(t) = Leaf_{meas}(t - s) \quad (5)$$

$$335 \quad Area_{meas_shift}(t) = Area_{meas}(t - s) \quad (6)$$

$$336 \quad Area_{shift}(t) = Area_{meas}(t - s + \Delta t) \quad (7)$$

337

338 **Abbreviations**

339 BASF: German for Baden Aniline and Soda Factory; BBCH: BASF, Bayer, Ciba-Geigy; CLAHE: Contrast limited
340 adaptive histogram equalization; DAPD: digital adjustment of plant development; DAS: day-after-sowing; HSV:
341 hue, saturation, value; HTPP: high throughput plant phenotyping LED: light-emitting diode; RGB: red, green,
342 blue;

343

344 **Authors' contributions**

345 DL, OB, JW and ML designed the experiments. DL, XM and OB performed the experiments. DL acquired data,
346 processed images and analyzed data. DL, GD and EC developed algorithms. DL, OB, EC, GD and ML
347 interpreted the results and wrote the paper. All authors read and approved the final manuscript.

348

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364 Instrument for building the HTPP system.

365

366 **Competing interests**

367 The authors declare that they have no competing interests.

368

369 **Availability of data and materials**

370 Our code is available for reuse at https://github.com/diloc/DAPD_Normalization.git. Correspondence should be
371 addressed to m.lewsey@latrobe.edu.au.

372

373 **Consent for publication**

374 All authors reviewed and approved the final version of the manuscript for submission.

375

376 **Ethics approval and consent to participate**

377 Not applicable.

378

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