LemnaTec High-Content Screening

ARABIDOPSIS

ASSESSMENT IN TIME AND SPACE

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Single point assessment of Arabidopsis plant size

When assessing Arabidopsis plants manually, the visual single point assessment is commonly used. This method enables for example classification and, if performed in a more comprehensive way, semi- quantitative estimation of plant size and colour at a specific time. Recognition of the biggest and smallest plant or rough size classifications are also possible.

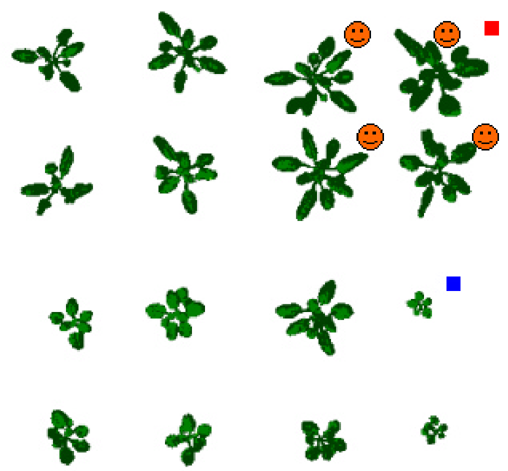

Image 1: Classification of an Arabidopsis tray highlighting the four biggest plants, the biggest plant and the smallest plant

If quantifications are to be made, they are restricted to parameters whose assessment remains labour-intensive, like rosette diameter or growth stages. So problems are still the high labour input and lack of reproducibility of qualitative classification as well as lack of continuous quantitative data.

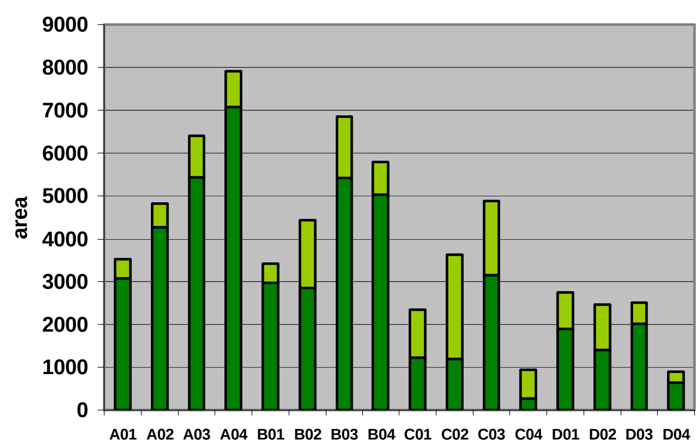

Fig. 1: Quantitative area measurement and colour classification, provided by the LemnaTec Scanalyzer

Image processing provides reliable quantitative data, making possible various kinds of statistics, a calculation of mean values in treatment groups and identification even of small but significant differences. But information about how the plants reached their size and colour up to that point remains very limited.

Multiple point assessment of Arabidopsis plant growth

The LemnaTec Scanalyzer 3-D and Plant Scanalyzer guarantee highly automated, fast and efficient imaging in time series. The primary data results produced automatically by the LemnaTec HTS Bonit Software after imaging are shown in image 2 and figure 2.

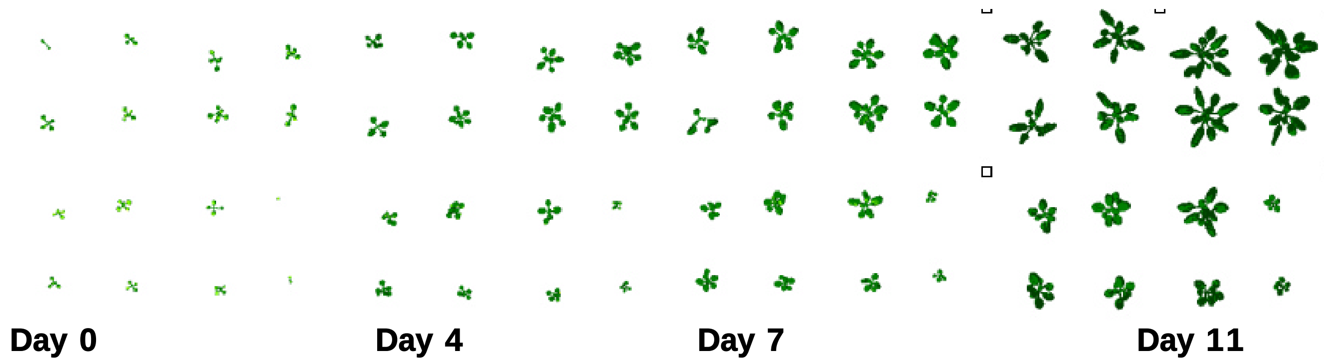

Image 2: Time series of an Arabidopsis tray showing the colour classified image produced by LemnaTec HTS Bonit software

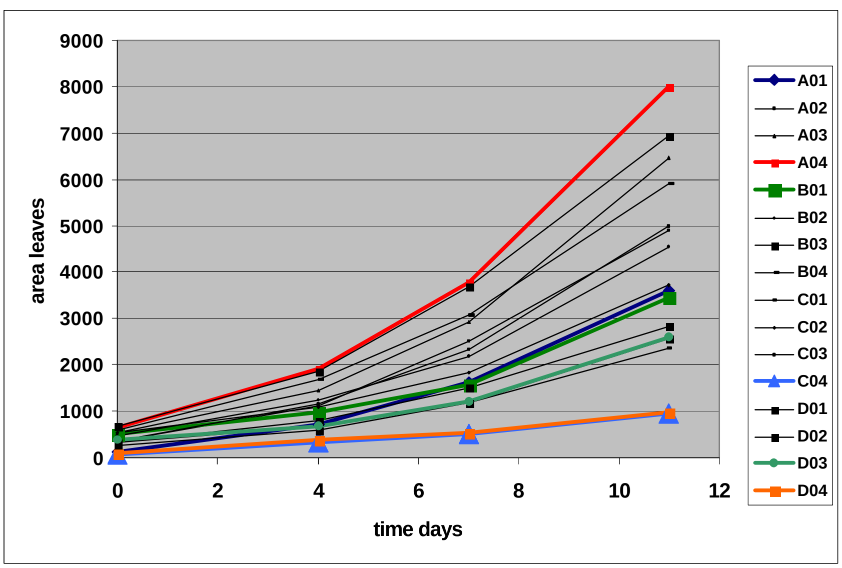

Figure 2: Growth curves of the Arabidopsis plants shown above. Highlighted are the biggest plant (A04), the two smallest plants (C04, D04) and some others (A01, B01, D03) mentioned later

It is trivial but important that plants being big at the first observation time quite often become the biggest ones at the end of the test, and the same is true for other sizes.

Differences in size at the first measurement point may be attributed to different germination times.
But area-based growth curves only show a minimal part of the information available from these raw data.
To evaluate if there are more differences between plants than just their different germination times, the most appropriate way is to look for growth rates, as plants of the growth stage analysed here show growth patterns much closer to exponential than to linear .
To visualise growth rates, one approach is to display growth curves with a logarithmic y-axis. In this case the slope of the curve represents the growth rate, while straight lines display constant exponential growth. The data of fig. 2 are represented accordingly in fig. 3.

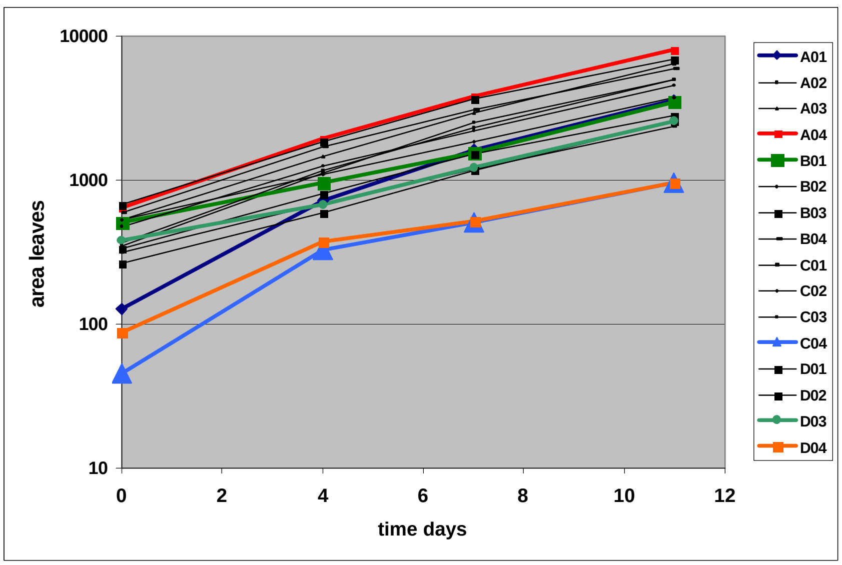

Fig. 3: Logarithmic growth curves of 16 Arabidopsis plants. Highlighted are the biggest plant (A04), the two smallest plants (C04, D04) one plant with an initially deviating growth pattern (A01) and some others, mentioned later (B01, D03)

While growth of the plants at first looked extremely heterogeneous, when looking at the plant images and at the growth curves in fig. 2 growth (described as growth rate, i. e. slope of the curves) is quite constant for most plants. The smallest plants (C04, D04) did not only start to grow later, but their growth rate remained constantly lower during the entire observation period. In contrast to this plant A01 started later, but from day 4 on reached growth rates quite similar to the others.
By just displaying the growth rates, as done in figure 4 below, it becomes even more obvious which plants show the general growth pattern and which deviate from it.

It is remarkable that in all cases the same set of raw data was used, provided by non-destructive image-based leaf area analysis, to which then an appropriate growth model (here: exponential growth) needs to be applied.

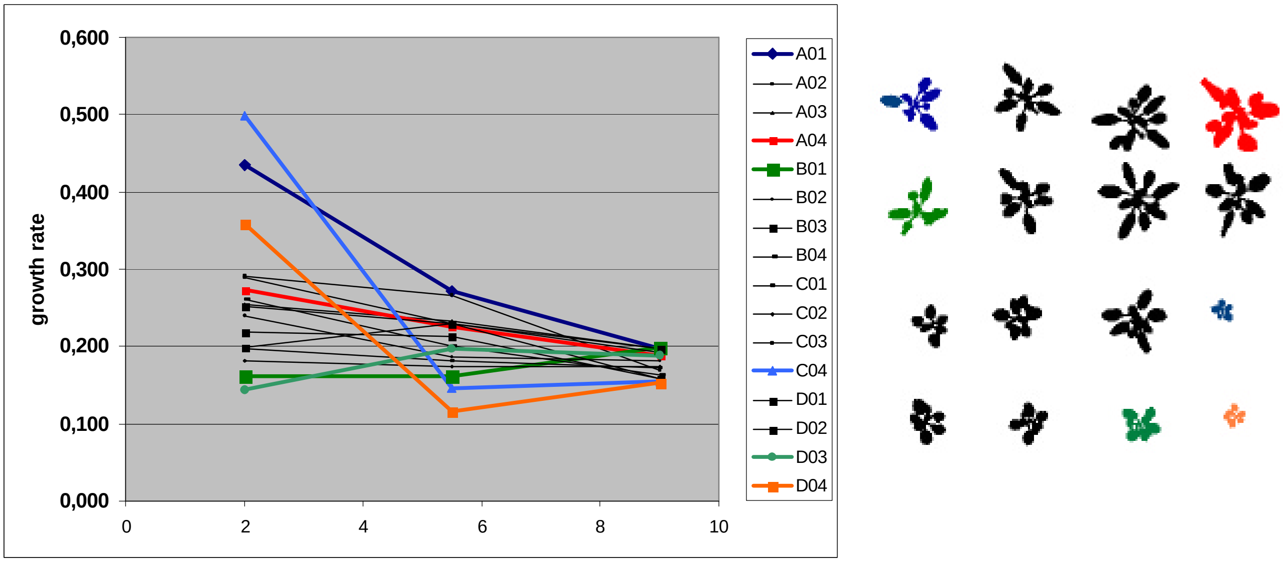

Fig. 3: Growth rate curves of 16 Arabidopsis plants. Highlighted are the biggest plant (A04), the two smallest plants (C04, D04) the plant with the highest average growth rate (A01) and two plants with the lowest average growth rate (B01, D03)

The growth rate graph shows more clearly that the biggest (A04) plants are not always the fastest growing (A01) during observation time. Some plants have interesting growth patterns (C04, D04) and slow growers (B01, D03) need not be the smallest ones (C04, D04) in the end. Plant C04 and D04 are also good examples for the ability of the LemnaTec system to quantify and display changes in growth rate provoked either by external stressors (drought, salinity, toxicity etc.) or genetics.

Reproducibility or area measurement

To assess reproducibility, a tray (side length about 40 cm) with 16 plants was turned around 10 times and each time the size of all plants was analysed.

Tray 1 Tray 2

Image 3: Trays with plants used for reproducibility testing

As a result the standard deviation for each plant was in a range of 3.5 (+/- 2.1) % for the smaller plants (plate 1) and 1.5 (+/- 0.7) % for the bigger ones (plate 2). The main reason for the absolute value of standard deviation and difference between images with big and small plants is the digitalisation effect which generally leads to higher values for small objects, due to higher ratio of rim to area.

This shows the very high reproducibility of leaf area measurement, even if complete trays are acquired on one image. If even smaller effects should be measured significantly , higher magnifications are easily possible, either by imaging just 1 or 4 plants in a Scanalyzer 3-D or by using full trays but multiple images per tray in a Scanalyzer HTS.

Thus even small effects far beyond the human eye's ability to discern them can be quantified and used to assess dynamic growth alterations, caused for example by external stressors.

Conclusion

1. **LemnaTec Scanalyzer 3-D, Scanalyzer HTS and Plant Scanalyzer provide all-embracing imaging technology needed for automated, fast and reproducible assessment of Arabidopsis plant growth.**
2. **Non-invasive measurement with the LemnaTec Scanalyzer units makes comprehensive data on growth dynamics readily available for research and screening.**
3. **Growth rate can eliminate normally unavoidable assessment artefacts, caused by common differences in plant size at the start of the test.**
4. **Using quantitative, reliable data and growth rate-based calculations reduces data "variability" and raises data quality for later assessments or statistics to higher levels.**
5. **Data provided can offer new insights into growth dynamics, especially under stress and recovery test designs.**

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