LemnaTec High-Content Screening

ARABIDOPSIS

MORPHOLOGICAL PHENOTYPE ASSESSMENT

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Automated quantitative phenotyping of complete plants

LemnaTec's new Morphological Assessment Toolbox (MAT) provides an almost unlimited set of morphological parameters easily to be correlated with biological effects.

The great number of different, automatically quantifiable parameters allows comprehensive, high-content screening, statistics on morphological patterns and changes of these patterns over time.

The following examples are parameterised for Arabidopsis, but similar approaches are available for a wide range of other biological applications.

In all cases data are shown in an xy-graph to visualise the parametric values and show examples of group forming, depending on which parameter is used and which morphological traits are to be grouped. Analysing greater numbers of plants with advanced statistical grouping procedures makes multidimensional grouping possible, based on data provided by the LemnaTec Morphological Assessment Toolbox. Such datasets are ideal to use in QTL-studies, when unbiased, reproducible datasets on great numbers of samples are needed. While the parameters shown below are intended to be discernable by the human eye, further analysis and especially correlations between parameters may reveal highly significant correlation not so easily to be "seen", but presumably useful if a biological correlation can be found.

Moreover, if any additional visual phenotype is found during screening later on, one that has not yet been clearly represented in any parameter analysed and quantified, the storage of all images will allow a consistent reanalysis of ALL images of ALL plants ever grown within the imaged screening project. This conservation of raw data can dramatically reduce costs and time of screening programs, increasing the significance and worth of the library of screened plants, as costly repetitions of experiments are unnecessary. On the other hand, quantitative reproducible analysis allows for a high-level quality control, e. g. by monitoring growth conditions with control plants or testing biological reproducibility of phenotype development.

All following parameters are referring to complete plants. Phenotype traits related to growth patterns and single leaf morphology are presented in other application papers.

LemnaTec would like to thank Prof. Eevi Rintmäki from the University of Turku for the basic image with different Arabidopsis phenotypes shown in figures 1–8,10.

Compactness

Compactness is calculated based on the rotational momentum of the plant, irrespective of size . Figure 1 shows compactness versus area of the plant.

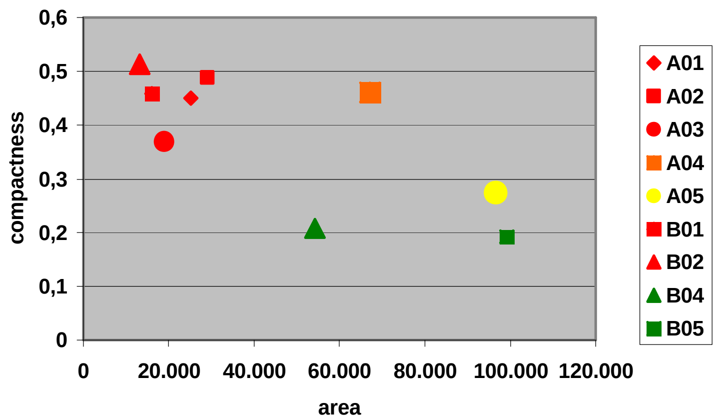
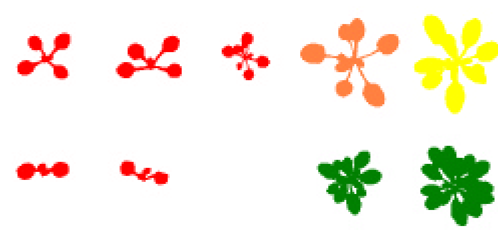


Fig. 1: Display of compactness versus plant leaf area (left). Colours in the diagram correlate with colours of the plants shown below



Compactness describes if the leaves are nearer around the centroid or farther away from it, e. g. by having longer stipes. The orange plant in this case is separated from the red plants by its size, which shows how grouping criteria can be defined for separating phenotype traits. As being size-independent, the compactness value itself is in the same range for both the red and the orange plant classes. While the green plants are obviously quite compact, the yellow plant is an intermediate, which is clearly reflected in the parameter for compactness.

Rotational Symmetry

Rotational symmetry of the complete plant is calculated based on the size-independent second moment principal axis ratio. This parameter integrates about the whole shape of the plant and is size-independent.

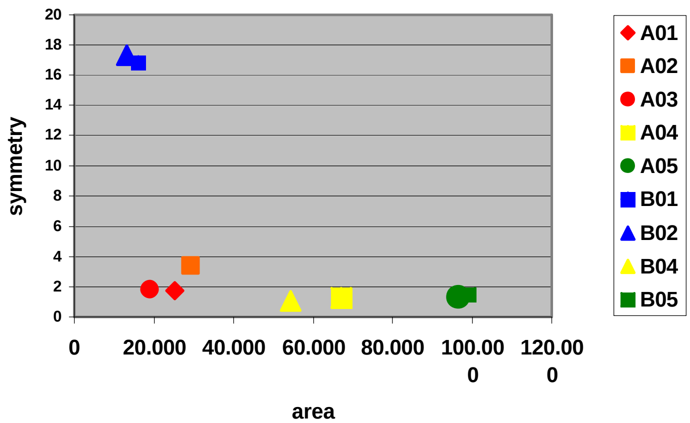
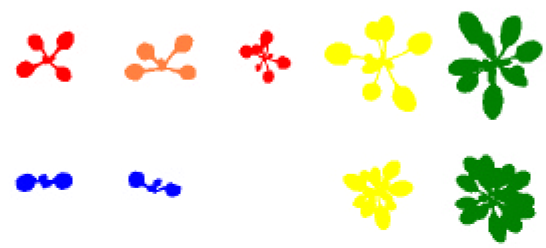


Fig. 2: Display of symmetry versus plant leaf area (left). Colours in the diagram correlate with colours of the plants shown below



Rotational symmetry describes in how far the leaves as a whole show a symmetric plant. It can be easily seen that this leads to a completely different grouping than the compactness mentioned before. Now the two blue-marked plants are distinctly separated from all others due to their two predominant leaves. But even smaller differences as between the red and orange plants are reflected in the data providing a higher value for the orange one. Up to which degree such differences in value have a distinct biological basis or are just normal biological variability is an interesting scientific question, to be answered by analysing greater higher numbers of plants from the same strain under similar conditions. As continuous reproducible data is provided by the LemnaTec Scanalyzer HTS software, any small difference can be identified and used as a classifier, as long as statistics identify it as significant. This may show up quite important new perspectives, e. g. for QTL-analysis, and can also help experienced phenotype assessors to turn their experience and intuition into definite quantitative numbers.

Excentricity

While calculated with a different algorithm, eccentricity (fig. 3) provides grouping results quite similar to those of rotational symmetry (see fig. 2 above).

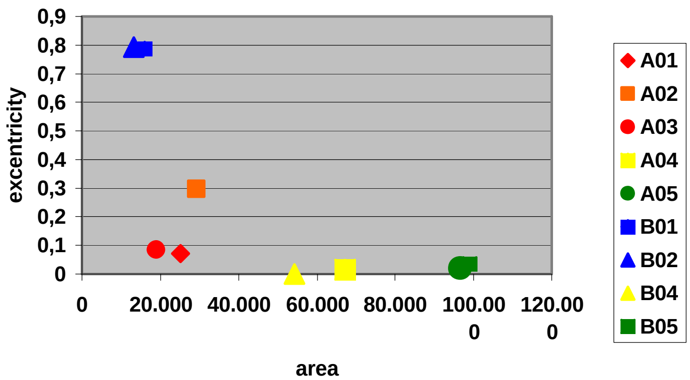
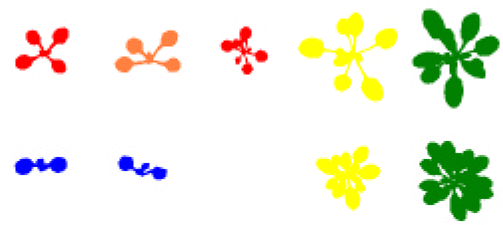


Fig. 3: Display of excentricity versus plant leaf area (left). Colours in the diagram correlate with colours of the plants shown below



Nevertheless the orange plant A02 shows more significant separation from A01 and A03 (red) than with rotational symmetry. This example demonstrates that having a wide range of morphological algorithms available is convenient for finding the most appropriate classification parameter.

Surface Coverage

Surface coverage compares the plant area to the area of a circle covering the plant.

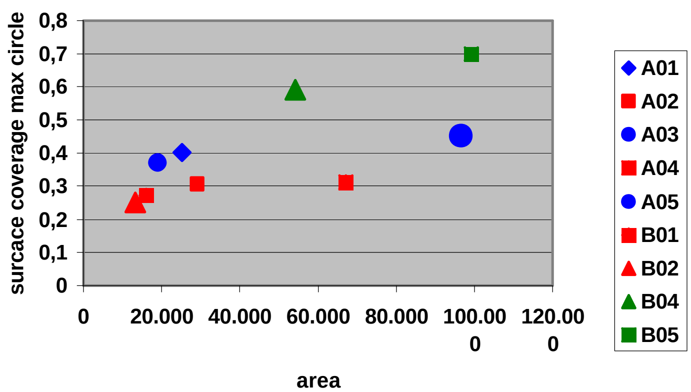
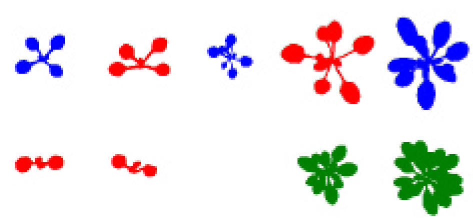


Fig. 4: Display of surface coverage versus plant leaf area (left). Colours of the diagram and of the plants correlate



This parameter is intended to provide a value that shows how dense the plant covers the soil in its immediate growth area. While it is extremely obvious that the green plants are the most dense, it is interesting to see that the coverage of the highly asymmetrical plants is not so much lower than that of plants A02 and A04 (all red), despite of the great asymmetry.

Medium Leaf Width Index

The medium leaf width index is calculated as the square of the length of the plant skeleton divided by the plant area. For this purpose, the plant skeleton is derived from the entire plant area by image processing, as shown in fig. 4.

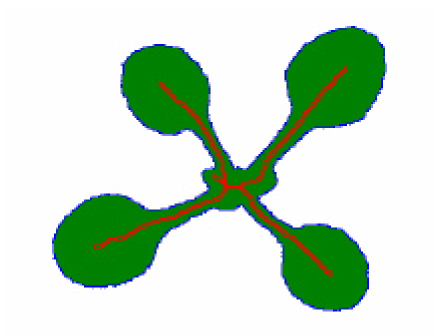
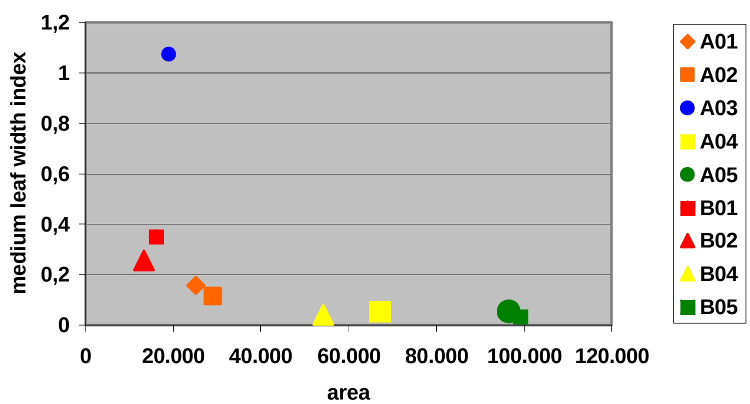


Fig. 5: Plant area and plant skeleton, derived by image processing



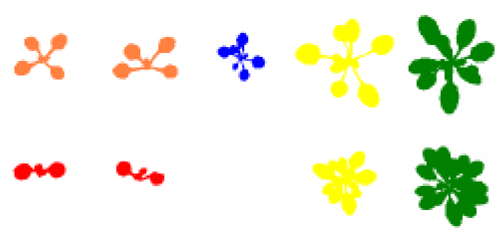


Fig. 6: Display of medium leaf width index versus plant leaf area (left). Colours in the diagram correlate with colours of the plants shown below

The medium leaf width index describes size-independent differences of „leaf width“, integrating stipes, leaves and overlapping effects. While the blue plant has the smallest leaves, but comparatively long stipes, it produces very high values, whereas the other plants show smaller differences in the index, especially the yellow and green ones separated by size. This corresponds well to the visual impression that leaf width integrating the stipes is relatively similar for these plants. The red and orange plants show higher values as leaves are smaller and stipes shorter and relatively thicker.

Area/Circumference

While having some size-dependency left, the ratio of leaf area divided by plant circumference may allow for additional classification of morphological traits.

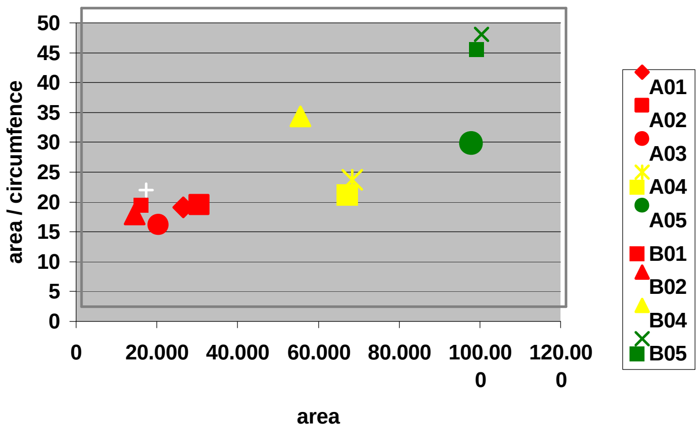
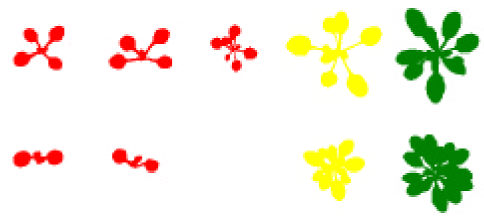


Fig. 7: Display of area divided by circumference versus plant leaf area (left). Colours in the diagram correlate with colours of the plants shown below



This grouping is a good example of plant grouping according to more than two morphological parameters, which can be displayed and used in a xy–graph. The best way to do this is by producing a reasonable ratio of two parameters. This can be achieved quite easily with the LemnaTec HTS Bonit software, displaying a secondary-derived morphological parameter like the ratio. Identifying such parameters and ratios provides a wide range of related parameters to be tested in QTL-studies for correlation to molecular biological data.

In some cases it is of special interest to keep a phenotypic parameter related to size, especially if the parameter on the x-axis is not size, but for example age of the plant or the cumulative water use in drought or water efficiency studies. These examples show the power of readily available quantitative data, and these are easily produced by the automated LemnaTec image processing.

Stockiness

Stockiness is in a mathematical sense the description of roundness. But the term roundness is much better applicable to, for example, egg-shaped objects in comparison to perfect circles

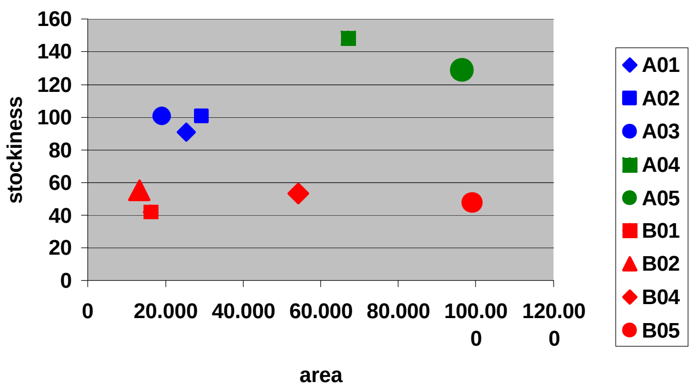
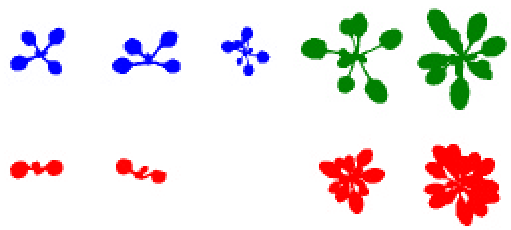


Fig. 8: Display of area divided by circumference versus plant leaf area (left). Colours in the diagram correlate with colours of the plants shown below



When applying standard morphological algorithms to biological objects, biological interpretation of the parameters is often needed. Applied to Arabidopsis images stockiness separates plants with invisible or relatively short, broad stipes from plants with long, smaller stipes.

While the green plants have long stipes and long leaves, the blue plants have rounder leaves and comparatively shorter stipes. With the red plants stipes are shorter and wider, if to be discerned at all.

Application to Separate Two Groups

The aim of the following example is just to separate two groups from each other. This method reveals that using stockiness and compactness as a separating factor for the two groups is working very well.

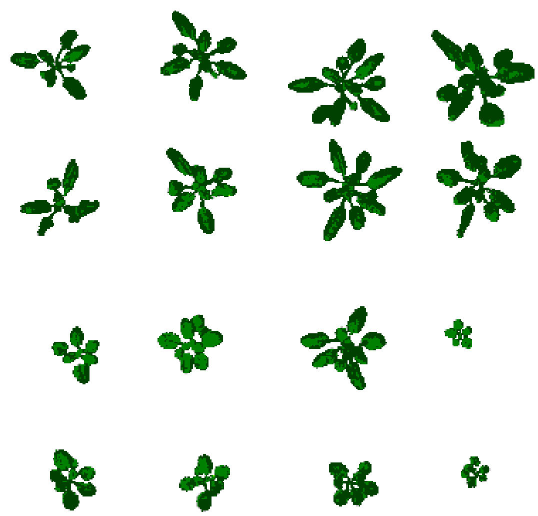
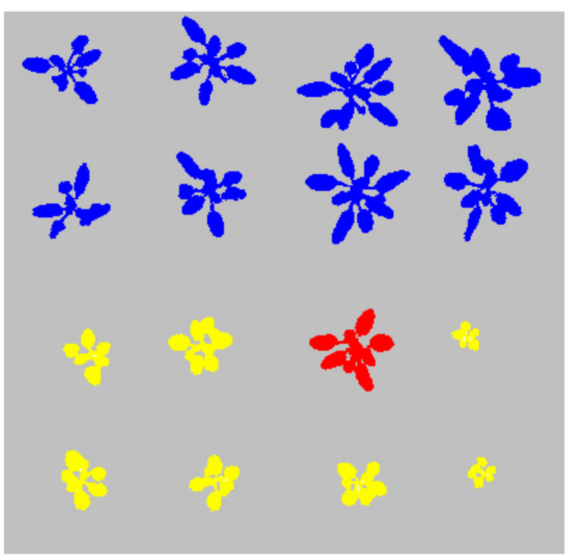
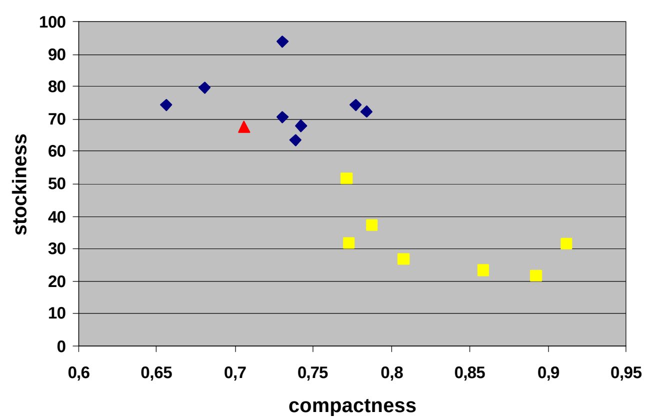


Fig. 9: The upper left image displays the leaf area image as produced by the LemnaTec HTS Bonit software. The image below shows a classification of two groups in blue and yellow, discriminating long leaf plants from round leaf plants. The plant highlighted in red seems to be an outlier from the grouping scheme in the tray, which is clearly represented in the numerical graph below plotting stockiness versus compactness





Conclusions

1. With the new Morphological Assessment Toolbox MAT LemnaTec provides a very powerful tool to assess a wide range of plant morphological parameters. Different morphological parameters allow for reproducible and different groupings of the plants easily to be visualised. This paper focuses on a selection of complete plant parameters like compactness, rotational symmetry, excentricity, surface coverage, medium leaf width index, area/circumference or stockiness. More parameters focussing on single leaves are discussed elsewhere.
2. Quantitative and reproducible assessment permits application of advanced grouping algorithms. Each quantitative parameter refers to a different morphological trait and may be used in high-content screening, e. g. for QTL-analysis.

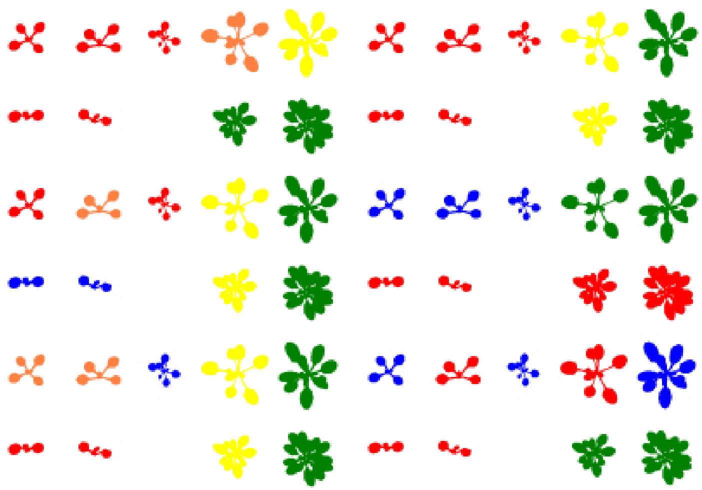


Fig. 10: Different morphological parameters classify plants in different phenotypic groups

3. As all images are stored and thus a tremendous amount of raw data is retained after a test, the LemnaTec image systems permit consistent reanalysis of ALL data over very extensive and long screenings, even if some of the parameters to be assessed are not yet known at the start of the screening. This library of screened plants is a tremendous treasure for each lab, because it shortens screening times and reduces costs, at the same time. No tests have to be repeated due to additional assessments that emerged while screening was already in progress.
4. Routine use of control samples allows for automatic and quantitative quality control of test conditions. This maximises quality and reproducibility of the test results.

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