LemnaTec High Content Screening

ARABIDOPSIS

MORPHOLOGICAL PHENOTYPE ASSESSMENT

LemnaTec GmbH
Matthias Eberius
Schumanstr. 18
52146 Würselen
02405 / 4126-0
matthias.eberius@lemnatec.de
www.lemnatec.de

Automated quantitative phenotyping of full plants

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

The high number of different parameters quantified automatically allows comprehensive high content screening and statistics on morphological pattern and changes of patterns in 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 how groups can form depending on which parameter is used and which traits are intended to be grouped. Analysing higher numbers of plants with advanced statistical grouping procedures allows multidimensional grouping based on the data provided by the LemnaTec Morphological Assessment Toolbox. Such datasets are ideal to use them in QTL studies as unbiased reproducible datasets over high numbers of samples are provided. While the parameters shown below are intended to be comprehensible to the human eye further analysis and especially correlations between parameters may reveal highly significant correlation not as easy to “see” but presumably helpful if a biological correlation can be found.

Additionally if any additional visual phenotype is found during screening later on which is not clearly represented in any parameter analysed and quantified up t this moment, the storage of all images will allow a consistent reanalysis of ALL images of ALL plants ever grown within the imaged screening program. This conservation of raw data can reduce costs and time of screening programs dramatically while boosting the significance and value of the plant library screened as costly later repetition of experiments are not necessary. On the other hand quantitative reproducible analysis allows high level quality control e. g. by monitoring growth conditions with control plants or testing biological reproducibility of phenotype development.

All following parameters are related to the full plant. Phenotype traits related to growth patterns and single leaf morphology are presented in different application papers.

LemnaTec thanks 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 size independent rotational momentum of the plant. Fig. 1 shows the compactness versus area of the plant.

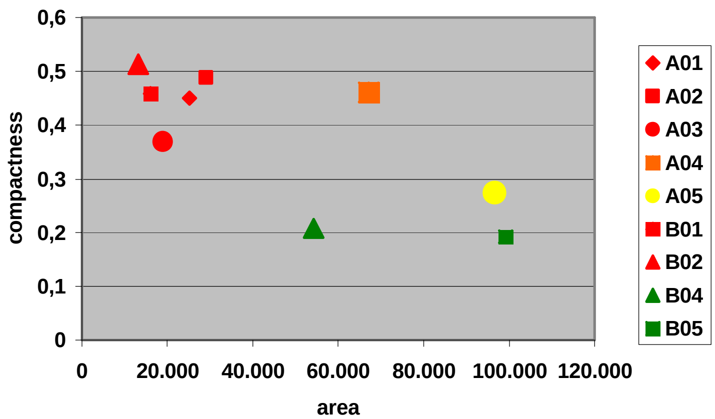
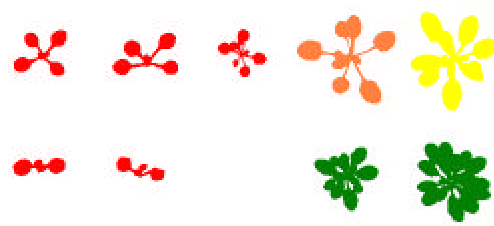


Fig. 1: Display of compactness versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



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

Rotational symmetry

Rotational symmetry of the whole plant is calculated based on the size independent 2nd moment principal axis ratio. This parameter integrates about the whole shape of the plants and is size independent.

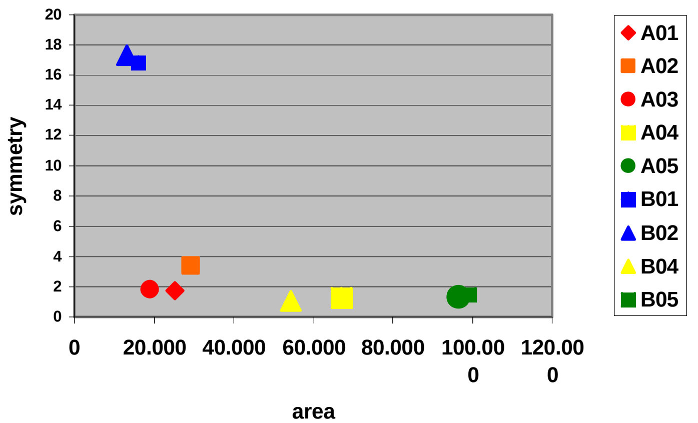
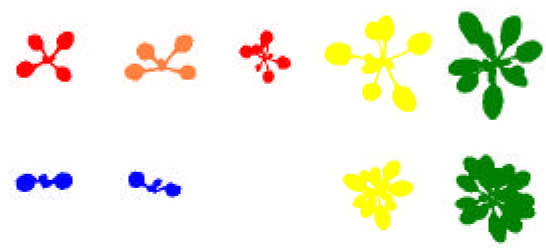


Fig. 2: Display of symmetry versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



Rotational symmetry describes in how far the leaves altogether show a symmetric plant. As seen easily this leads to completely other grouping than before-mentioned compactness. Now the two blue marked plants are strongly separated from all other ones due to its predominant two 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 value-differences have a distinct biological basis or are just real biological variability is an interesting scientific question to be answered by analysing larger numbers of plants from the same strain under similar conditions. As continuous reproducible data are provided by the LemnaTec Scanalyzer HTS software any small difference can be identified and used as classifier as long as statistics identify it as significant. This may open quite powerful windows e. g. for QTL analysis on the one side and on the other side help experienced phenotype assessors transform their experience and intuition into hard quantitative numbers.

Excentricity

While calculated with a different algorithm excentricity (Fig. 3) provides here quite similar grouping results as rotational symmetry (see Fig. 2 above).

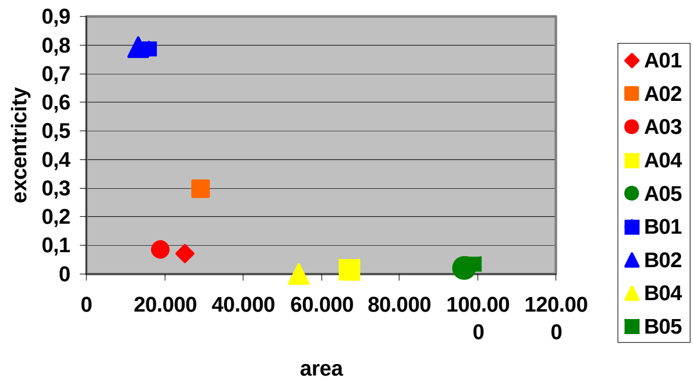
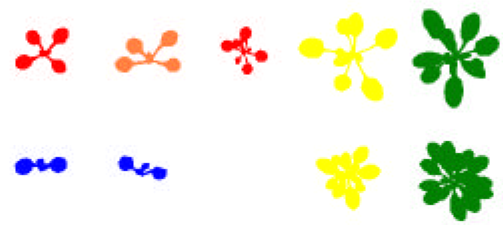


Fig. 3: Display of excentricity versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



Nevertheless the orange plant A02 shows more significant distance to A01 and A03 (red) than with rotational symmetry. This example shows that having a wide range of morphological algorithms available is a good choice to find the most appropriate classification parameter.

Surface coverage

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

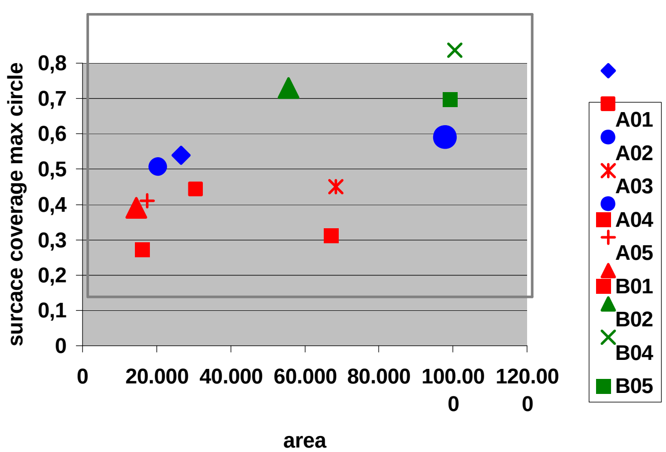
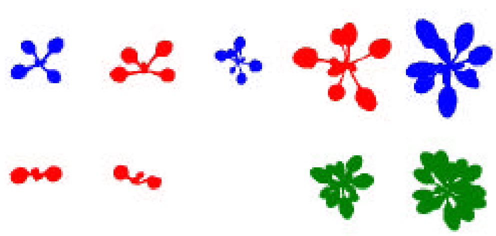


Fig. 4: Display of surface coverage versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



This parameter is intended to provide a value 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 ones, it is interesting to see that the coverage of the highly asymmetric plants is not so much lower that of plants A02 and A04 (all red), despite of the high 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 full plant area by image processing as shown in Fig. 4.

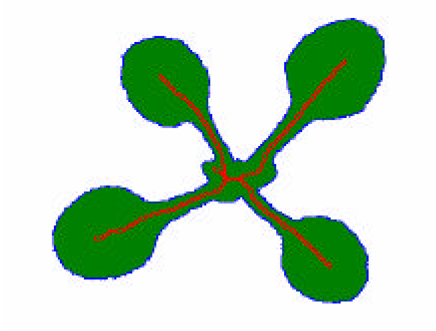


Fig. 5: Plant area and plant skeleton derived by image processing

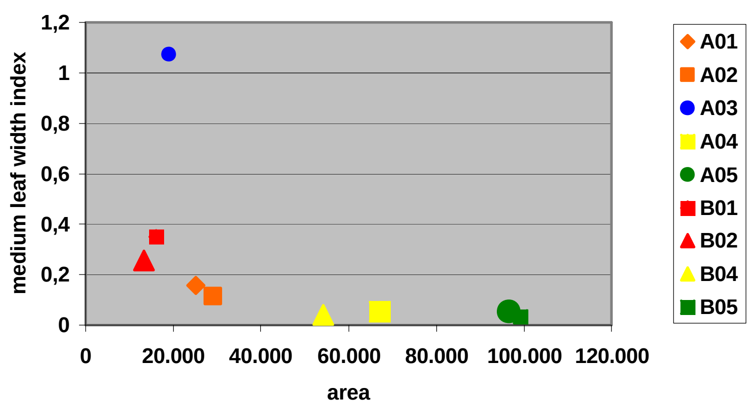
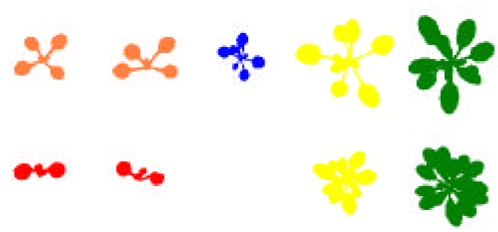


Fig. 6: Display of medium leaf width index versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



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 produces very high values, the other plants show smaller differences in the index especially the yellow and green ones separated by size. This corresponds well to the human 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 additional classification of morphological traits.

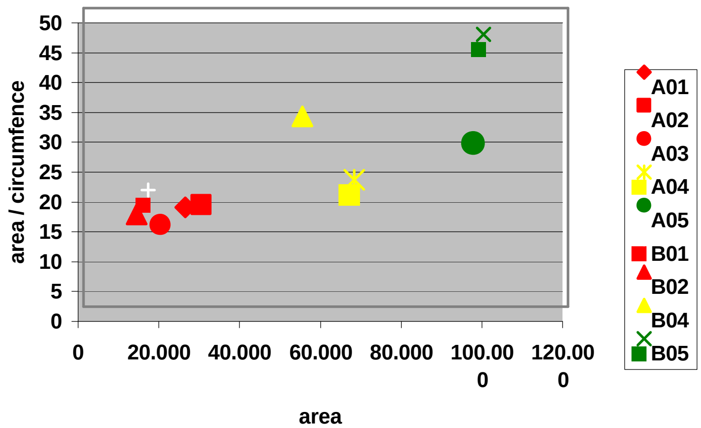
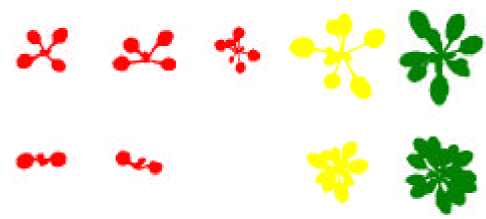


Fig. 7: Display of area divided by circumference versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



This grouping here is a good example how more than 2 morphological parameter can be displayed and used in a XY–diagram to group plants. The easiest way is just producing a reasonable ratio of two parameters. This can be done easily within 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 e. g. age of the plant or the cumulative water us in drought or water efficiency studies. These examples just show the power of data if once quantitative data are available and easily to be produced by automated LemnaTec image processing.

Stockiness

Stockiness is mathematically the description of roundness. But the term roundness is much better applicable to e. g. egg shaped objects in comparison to perfect circles than to highly structured objects.

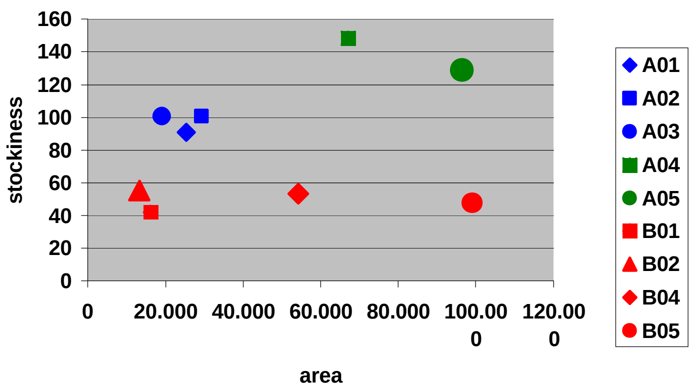
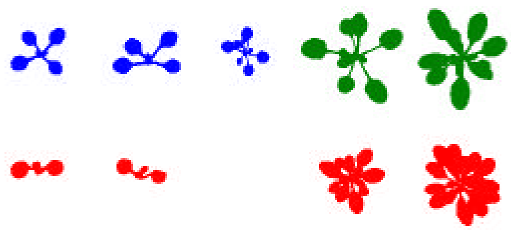


Fig. 8: Display of area divided by circumference versus plant leaf area (left). Colours in the diagram correlates to the colour of the plants shown below.



Applying standard morphological algorithms to biological objects often need biological interpretation of the parameters.. 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. For the red plants stipes are shorter and wider if to be seen at all.

Application to separate two groups

In the following example it is just the aim to separate two groups from each other. It reveals that using stockiness and compactness allowed to separate the two groups very well from each other.

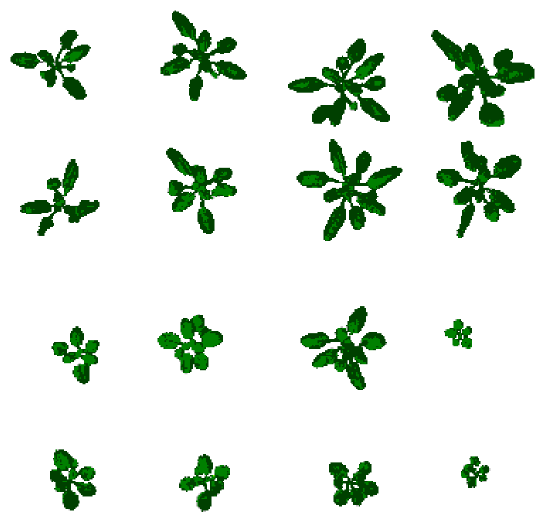
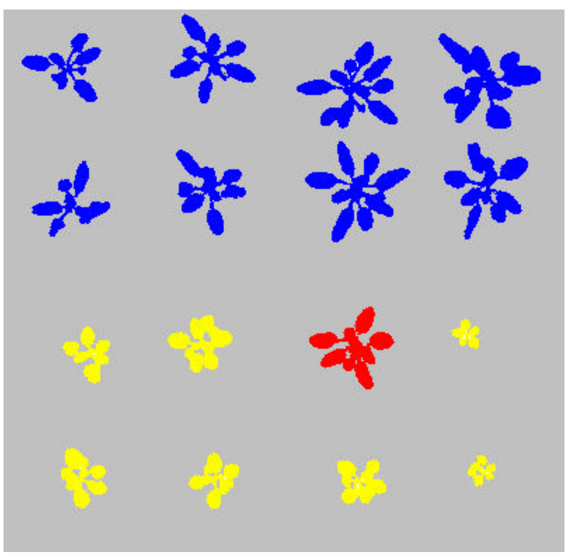
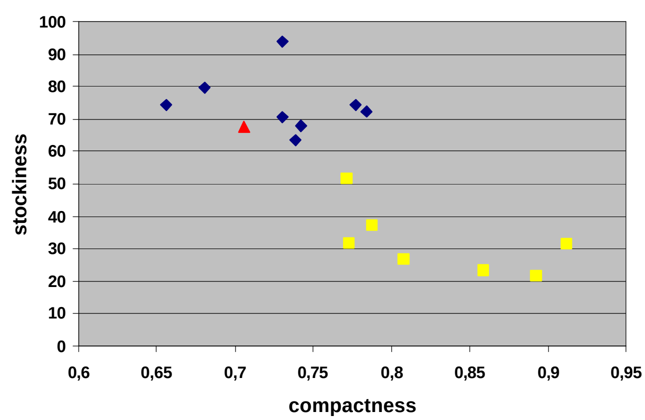


Fig. 9: The upper left image displays the leaf area image as produced by the LemnaTec HTS Bonit software. The image below shows a classification of two groups in blue and yellow discriminating long leaf plants from round leaf plants. The plant highlighted in red seems to be an outlier from the grouping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





Conclusions

1. With LemnaTec´s new Morphological Assessment Toolbox MAT LemnaTec can provide a very powerful tool to assess a wide range of morphological parameters for plants. Different morphological parameters allow reproducible different groupings of the plants easily to be visualised. The paper provided here focuses on a selection of whole plant parameters like compactness, rotational symmetry, excentricity, surface coverage, medium leaf width index, area/circumference, stockiness. More parameters focussing on single leaves are discussed separately.
2. Quantitative and reproducible assessment allows 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.

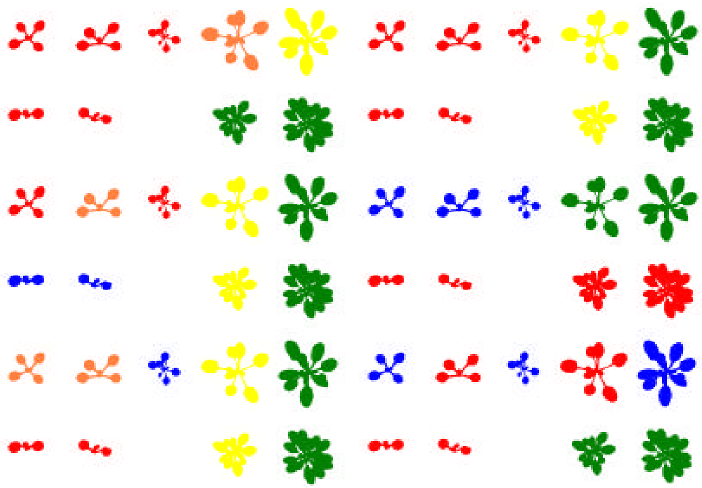


Fig. 10: Different morphological parameters classify plans to different phenotypic groups

3. As all images are stored and thus a tremendous amount of raw data conserved after a test, the LemnaTec image systems allow consistent reanalysis of ALL data over very big and long screenings even if some of the parameters to be assessed are not known at the start of the screening. This boosts the value of the screened plant library tremendously and shortens screening times while reducing costs as no tests have to be repeated due to additional assessments needed that just revealed while screening was already running.
4. Routine use of control plants allow automatic and quantitative quality control on test conditions to maximise quality and reproducibility of results.

For any further question please do not hesitate to contact:

Matthias Eberius, LemnaTec; matthias.eberius@lemnatec.de