LemnaTec High-Content Screening

ARABIDOPSIS

MORPHOLOGICAL PHENOTYPE GROUPING

Feasibility study

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Grouping of Plants Based on Morphological Parameters

The current version of the Lemnatec SAW Bonit software is already containing a set of powerful tools to characterise and classify plants based on size, growth rate and morphological parameters.

The updated version further increases the amount of available phenotyping parameters.

LemnaTec's aim has always been to demonstrate the biological relevance of parameters, and a feasibility study was made to demonstrate the possibilities of correlating phenotyping parameters and plant characteristics.

The results of this study are shown below. No biological and/or genetic data is available for this set of plants. Such correlations are to be established by the users, who should produce their own datasets with plants of different growth patterns. Of course, LemnaTec offers assistance with extracting a maximum amount of phenotypic parameters.

All results are based on a set of four images, taken from one tray at different measurement points. The classification was based on visual assessment of polar graphs grouped for similarities.

The subsequent mathematical clustering is based on the high-quality results shown here, suggesting a great deal of "visual" correlation between images and morphological parameters.

For a final validation with specific datasets, it is recommended to identify some of the users' own measurement series, showing the top 4 or more development stages of those plants where the genetical background is known. Again Lemnatec will assist users with the most efficient extraction of phenotypic features. And conversely, any information about the importance of specific traits will help LemnaTec to prepare, improve and develop additional useful parameters in the future.

The following analysis employs the parameters displayed in figure 1, as they seem to be most significant in this context. Values were normalised to the average of the whole tray.

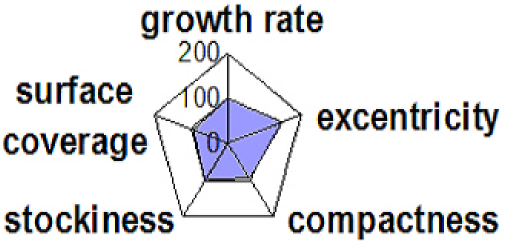

Fig. 1: Polar diagram of growth rate, excentricity, compactness, stockiness and surface coverage. The parameters used are explained in a separate paper ("*Arabidopsis* Morphological Phenotype Assessment").

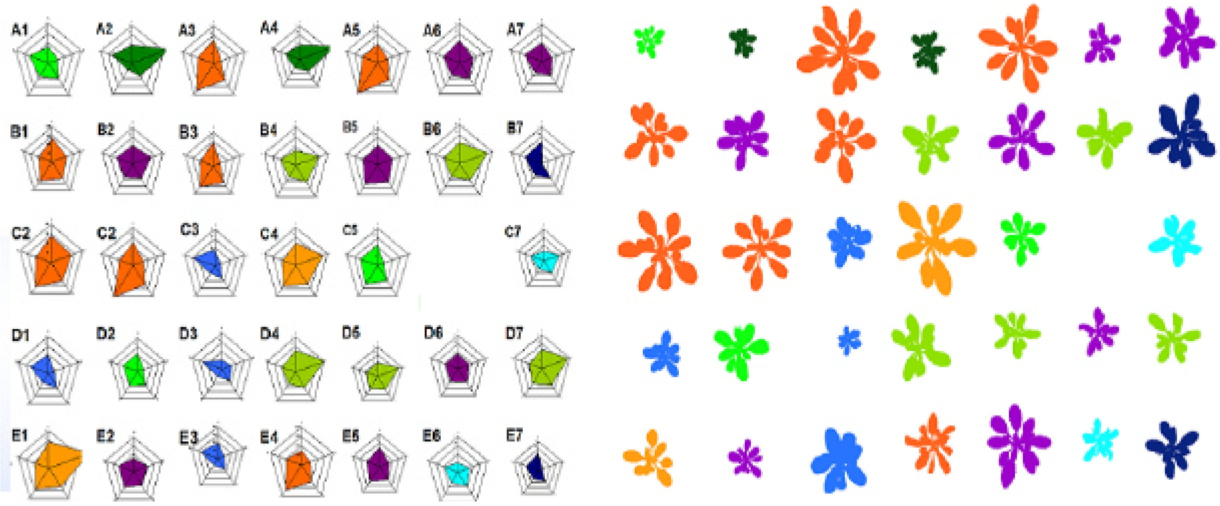

Fig. 2: Static (one point of measurement and overall growth rate) phenotype grouping of plants, based on 5 automatically measured traits (see figure 1, growth rate, excentricity, compactness, stockiness and surface coverage). Classification in colour classes was performed visually, based on the shapes in the polar diagram. Colours of polar diagrams correspond to plants on the right side. The polar diagram fingerprints look well correlated with the visual assessment of an *Arabidopsis* tray (day 13 of the test). Such data may well be used for QTL-studies.

Applying the concept of dynamic phenotyping pattern changes, the following overlay of 4 measurement points of the same tray was developed:

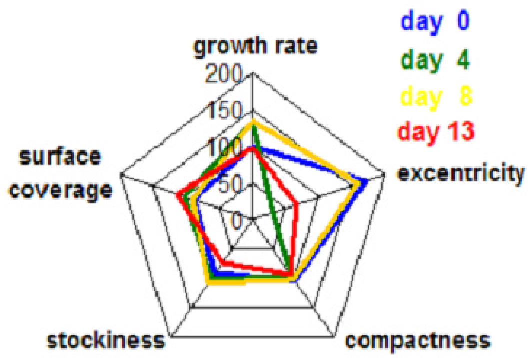

Fig. 3: In addition to the phenotypes, features are displayed for all four measurement points. Growth rate at day 0 is set to be 100 % as no specific number is available for all plants.

This kind of analysis may even be correlated with biological data showing the up- and downregulation of genes during specific phases of a plant's life-cycle as quantified on the images.

Again the classification was made visually, based on various specific traits mentioned below. Mathematical analysis of the results and correlation to genes may yield still more information.

Fig. 4: The full power of image-based phenotyping becomes obvious when classification includes the time-dependent development of all phenotypic parameters (growing stockiness, diminishing stockiness, all except excentricity nearly constant, growth rate slowing down). This provides important insights into the dynamic relation between gene, proteome and growth dynamics.

Conclusion

The LemnaTec analysis based on dynamic phenotyping will open up completely new prospects for automated high-throughput phenotyping.

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