Automatic cell counting and classification in sugar beet tissue using a microscope image clustering method

Nelia Nause, Facundo R. Ispizua Yamati, Christa M. Hoffmann

Institute of Sugar Beet Research, Göttingen, Germany

Background

- Significant differences in tissue strength between sugar beet genotypes
- Relationship between cell size and mechanical tissue properties expected
- Heterogenous tissue with big differences in cell size between tissue types in roots
- Exemplary manual measuring of cell sizes of small samples

Methods

- 9 sugar beet genotypes and 1 fodder beet
- Tissue cuboids of 1 x 1 x 2 cm
- Sectioning into 10 µm thickness
- Mosaics of microscopic images
- Data preprocessing techniques with R
- Manual cell counting as ground truth

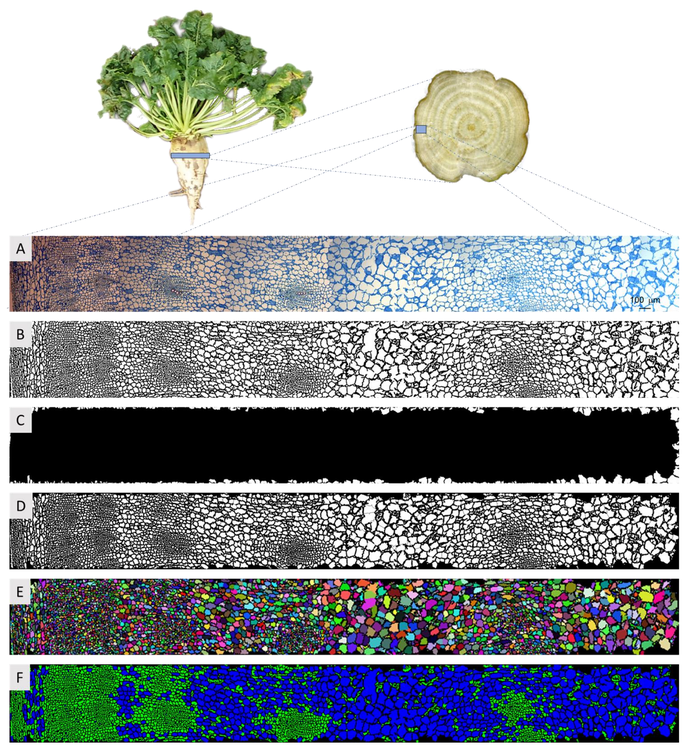

Fig. 1: Steps of digital image processing

Results

Main steps for cell detection:

- Conversion of the original image (A) to black and white (B)
- Identification of incomplete edge cells (C)
- Removal of egde cells (D)
- Inclusion of open cells by closing them (D)
- Individual cell identification by watershed segmentation and labelling (E)
- Individual cell characteriziation catalogue (eg. shape, position etc.)
- Identification of number and position of cambium rings
- Clustering by k-means algorythm (F)

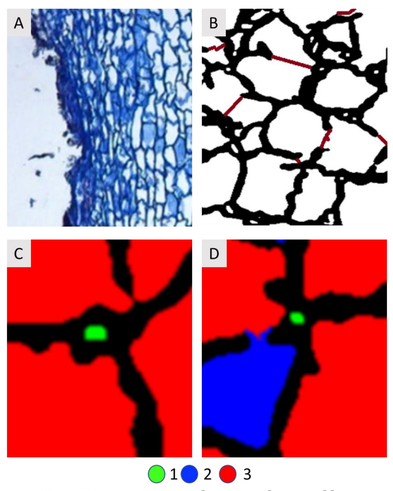

Fig. 2: Processing details

Challenges	Solutions
Elimination of the irregular edge of the peridermal area (A)	Applying “opening” - suppression of local disturbances of bright pixels and filtering out small structures
Cell walls damaged during tissue preparation process (B)	Closuring by permutation of the distance of each pixel from its neighbor, to generate a continuum of the cell wall
Intercellular spaces and artifacts (C &D)	Comparison of neighboring cells after cluster analysis using three clusters

Significant differences between *Beta* genotypes in:

- Number of cells
- Mean cell diameter (Fig. 3)
- Proportional distribution of clusters

No significant differences between genotypes:

- Number of cambium rings

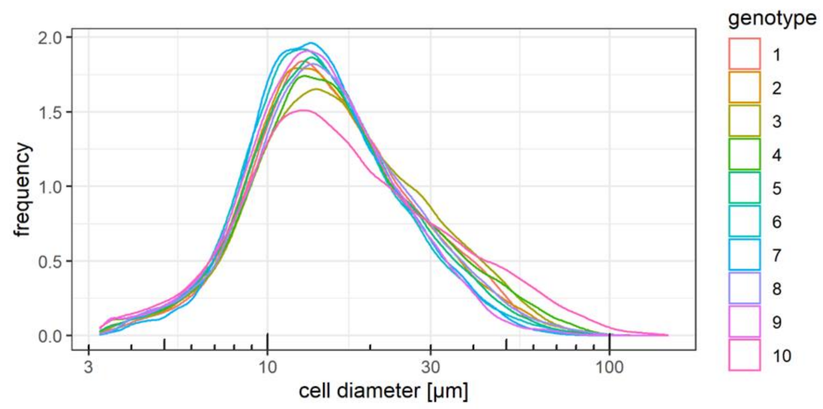

Fig. 3: Frequency of cell diameters in root tissue of different sugar beet genotypes

Conclusions

We developed a procedure of digital image processing which enables an automated evaluation of histological images of sugar beet root tissue.

- Cells can be reliably discriminated from intercellular spaces.
- For genotypes extreme in tissue strength, differences in the mean cell size have been identified.
- A classification of cells with similar morphological features by k-means clustering has been established, allowing a comparison of different tissue types between genotypes.

This work has been funded by SESVanderHave and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2070 – 390732324

IIRB Congress, 21.-23.06.2022, Mons, Belgium