

Quantification of histochemical detection of polyphenolic compounds in somatic embryos of Norway spruce



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INTRODUCTION

Somatic embryogenesis is the developmental process by which somatic cells, under suitable conditions, form somatic embryos (SE) that can germinate and new plants can develop. In the course of development, storage compounds are deposited, embryos acquire resistance to drought or develop defence system against other abiotic or biotic stresses. Under standard cultivation conditions, Norway spruce SEs accumulate polyphenolic compounds mostly in the root cap (Fig. 1 A,B,F). UV-B radiation is an abiotic stress triggering the cascade of defence reactions in plants. In response to UV-B, the accumulation of polyphenolics increases in protodermal cells and subprotodermal cortical cells and idioblasts in hypocotyl and cotyledons (Fig. 1 C,D,E) where they can attenuate UV-B stress.

AIM

To develop a method of quantification of polyphenolic compounds accumulated in somatic embryos of Norway spruce on the histological level

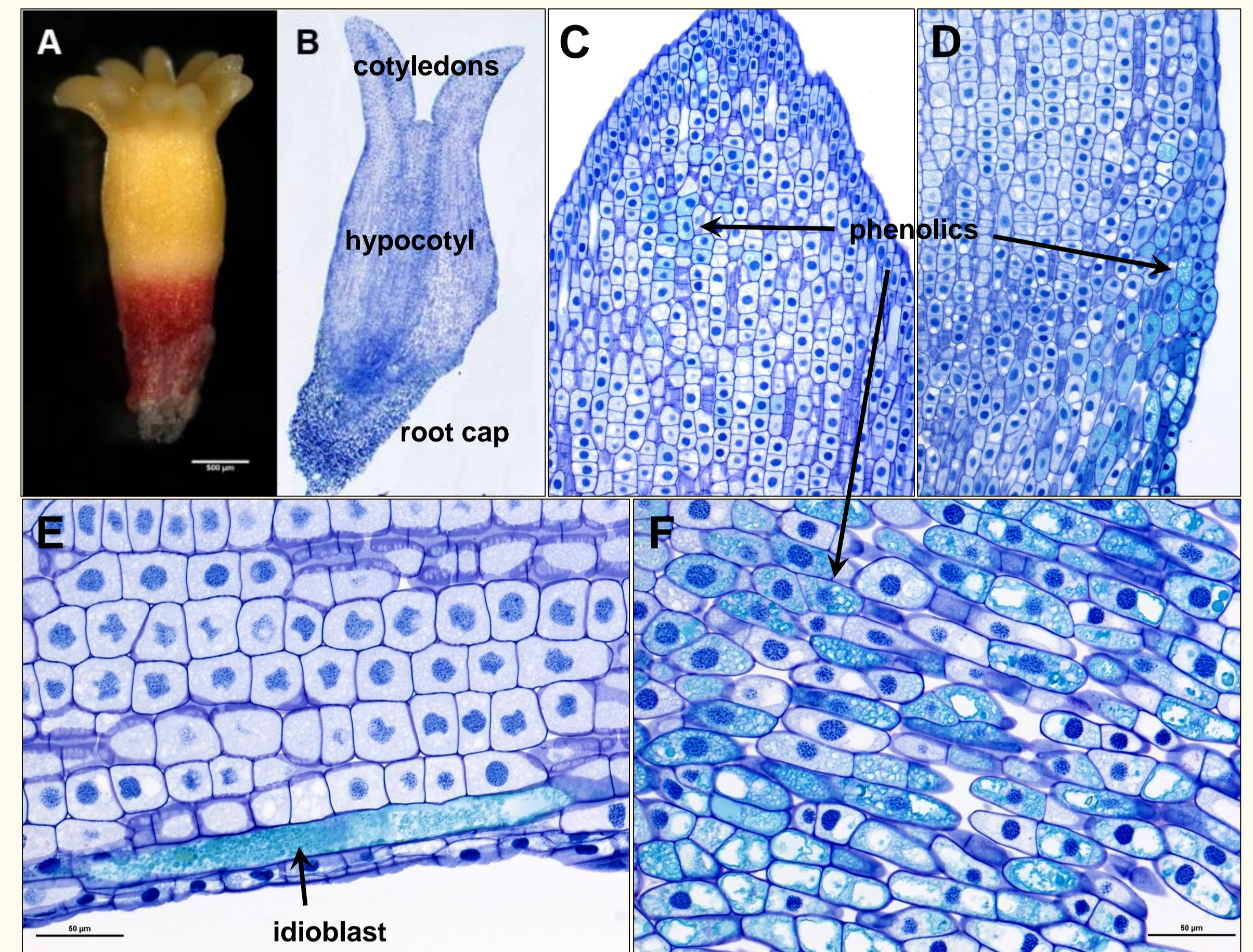
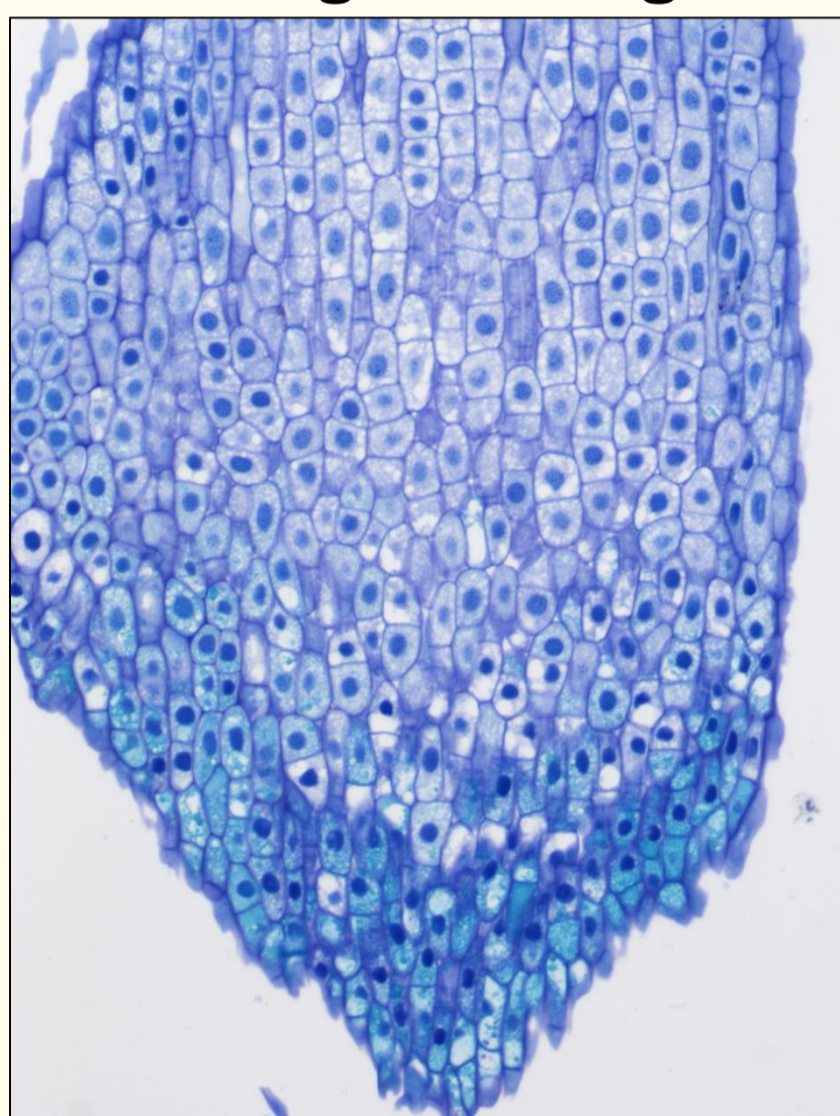
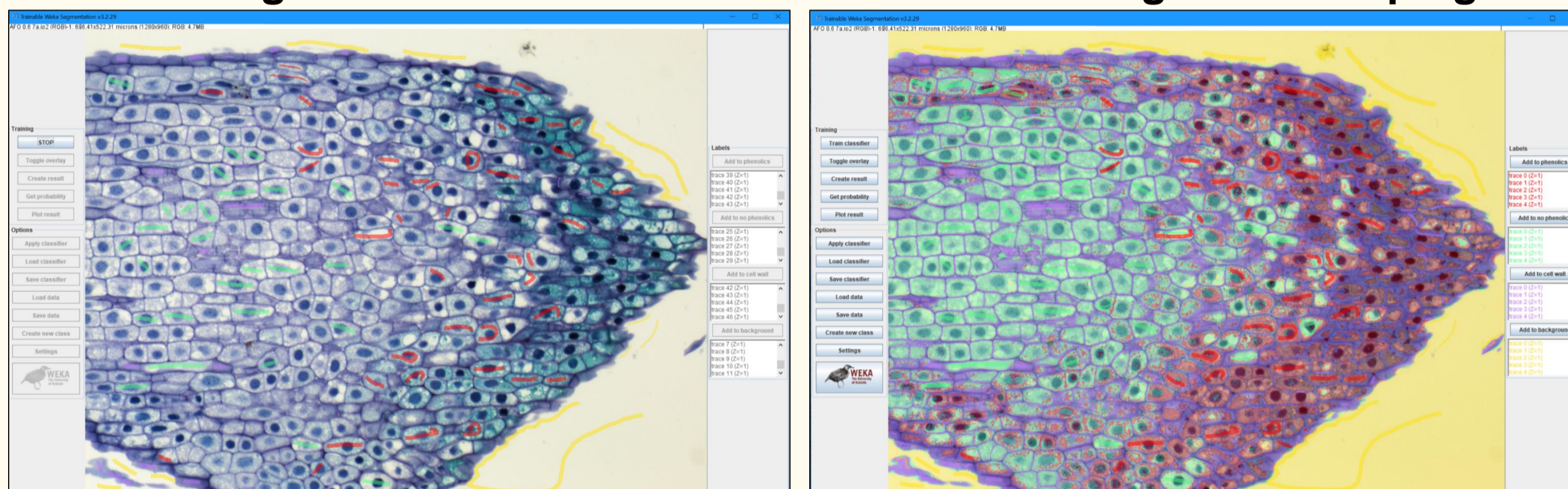


Fig. 1: Somatic embryo of Norway spruce
A – fresh desiccated SE; B – section of the whole SE; C, D, E, F – details of different parts of SE with phenolics accumulated in cells

1. Original image



2. Training of the classifier of the Trainable Weka Segmentation plugin

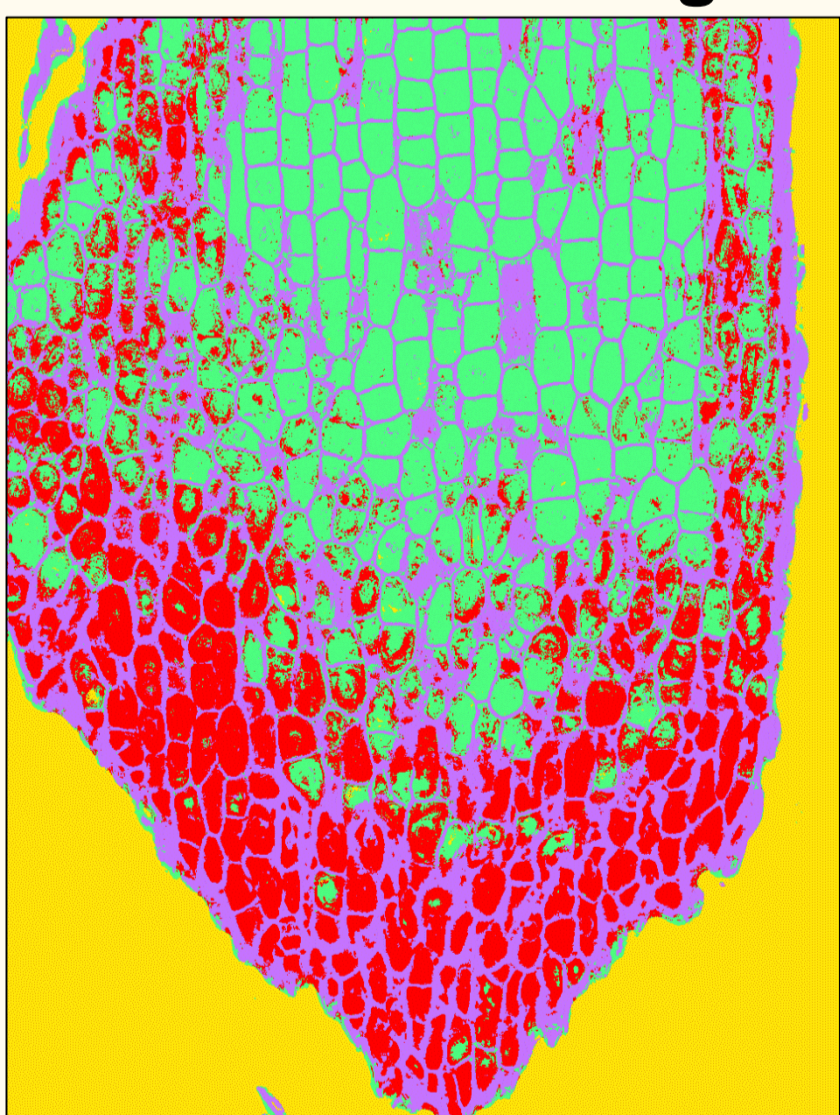


3. Adding traces to classes by freehand drawing

3. Result of the 3rd run of the classifier training

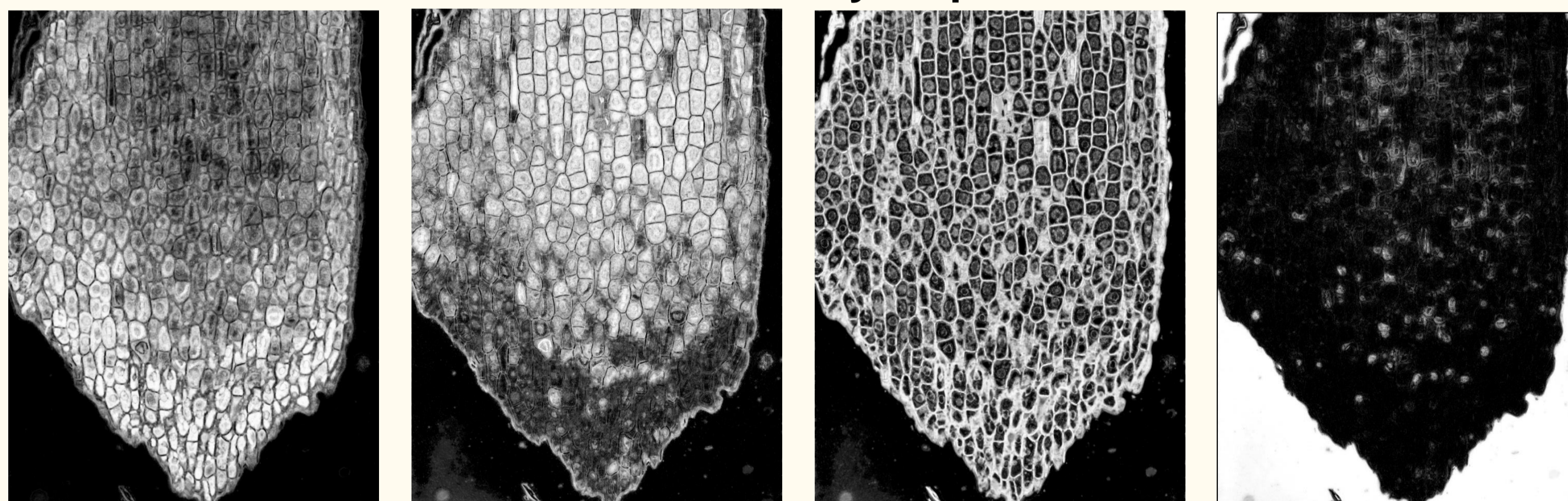
Results of segmentation

4. Classified image



Red – region of the cells with phenolic compounds
Green – region of the cells without phenolic compounds
Violet – cell walls
Yellow – background of the section

4. Probability maps



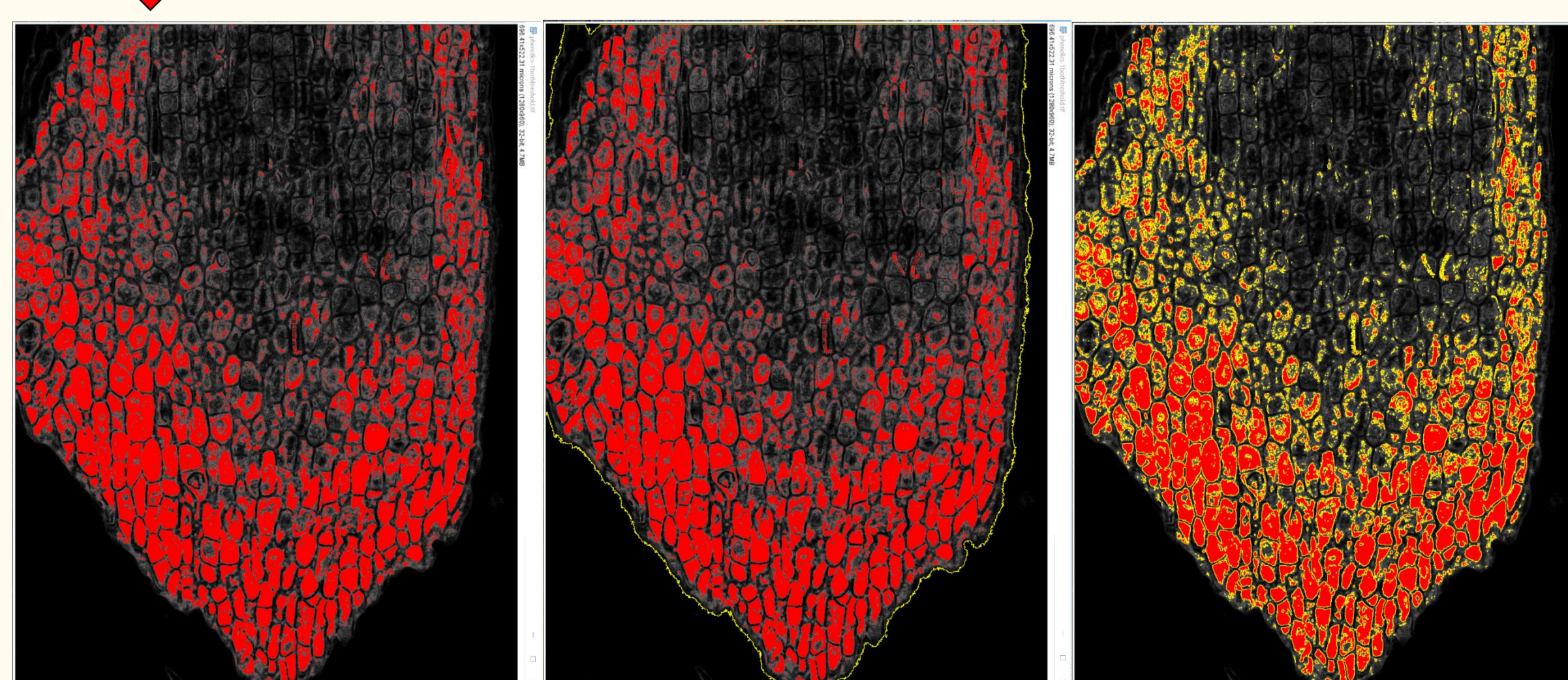
Phenolics in cells

Cells without phenolics

Cell walls

Background

5. Thresholding and Creation of Regions of Interest



Threshold of the area with phenolics

ROI 1 – whole section

ROI 2 – area with phenolics

Image analysis

1. Mature SEs were embedded in glycol methacrylate (Technovit 7100), sections (5µm) were stained with Toluidine Blue resulting in blue nuclei and cellulose cell walls and blue-green polyphenolic content of vacuoles. Microscopic images were acquired.
2. For evaluation of selected sections we used image analysis software ImageJ (Schindelin et al., 2012) and integrated plugin Trainable Weka Segmentation (Arganda-Carreras et al., 2017) that provides image segmentation based on the training of a classifier.
3. Four classes have been defined (cells with phenolics in the vacuole, cells without phenolics, cell walls and background around the section image) by trace freehand drawing.
4. Classifier training resulted in the classified image overlaid with the corresponding class colours and probability map for each class.
5. Thresholding of the phenolics probability map was applied and two Regions of Interest (ROIs) were created – ROI 1 for whole section and ROI 2 for the area where phenolics occurred in the cells (thresholded area with phenolics was marked using wand tool, than *Edit – Selection – Create Selection*).
6. The areas of ROI 1 and ROI 2 can be measured using ImageJ tools (*Analyze – Set Measurements – select Area and Display labels*; in ROI Manager: *Deselect – Measure*); results of area measurements are then displayed in the Result Table.
7. The proportion of the area of cells accumulating phenolic compounds to the whole area of the evaluated sections will be estimated.

References

Arganda-Carreras, I., Kaynig, V., Rueden, C., Eliceiri, K.W., Schindelin, J., Cardona, A., et al. (2017). Trainable Weka Segmentation: a machine learning tool for microscopy pixel classification. *Bioinformatics* 33(15), 2424-2426. doi: 10.1093/bioinformatics/btx180.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods* 9, 676. doi: 10.1038/nmeth.2019

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Summary

The method of image segmentation using trainable classifier and subsequent measurements of created ROIs is a convenient tool for quantification of polyphenolic compounds on the histological sections of SEs. To obtain unbiased estimation of the proportion of the „area with phenolics“ to the area of embryo the systematic uniform random sampling is necessary.