

Overview

Grain yield is a key determinant of cereal crop productivity, with spike morphology playing a crucial role in its assessment. Traditional spike characterization methods are often labor-intensive and time-consuming, limiting their scalability for large-scale breeding programs. To address this challenge, we present an advanced image-based tool leveraging deep learning methodologies for non-invasive, high-throughput spike phenotyping. A U-Net model achieved robust spike segmentation, with adaptive thresholding and Segment Anything Model (SAM) reaching Dice scores of 0.95 and 0.98. The post-processing of segmented grains extracts key morphological traits, including spike length, grain number, grain width, grain length, and grain area, facilitating precise yield analysis. This innovation enhances the efficiency of crop breeding research by providing an accurate, non-destructive, and scalable solution for spike trait analysis.

Methodology

Dataset preparation

We trained the U-Net model on a dataset of 200 annotated spike imaged in an X-ray tube, employing data augmentation techniques like rotation and scaling (Fig. 1).

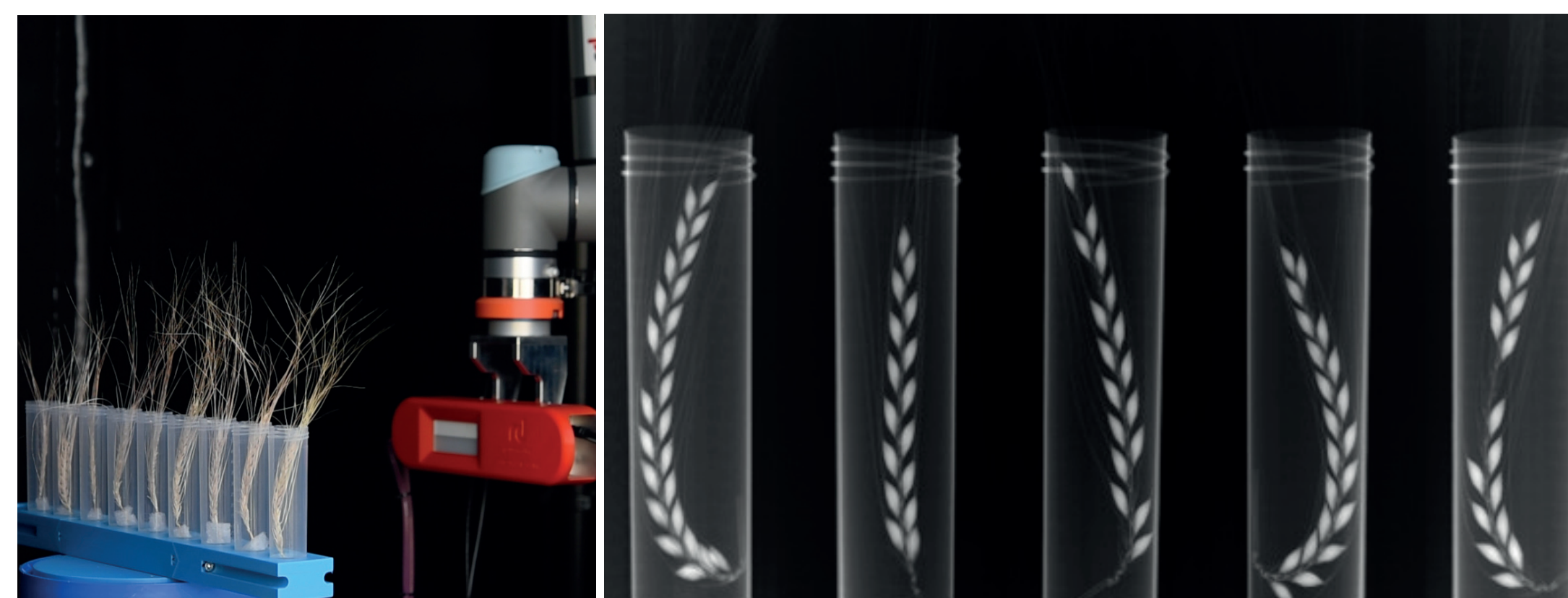


Fig.1 Grayscale X-ray image of a wheat spike inside the sample tube acquired with the PSI X-ray system.

Using the Dice coefficient as the loss function and the Adam optimizer, the model was trained for 60 epochs with early stopping to prevent overfitting (Fig. 2). Continuous validation ensured the model's accuracy and robustness for precise spike grain image segmentation. We also used a conventional adaptive thresholding method to segment the spikes.

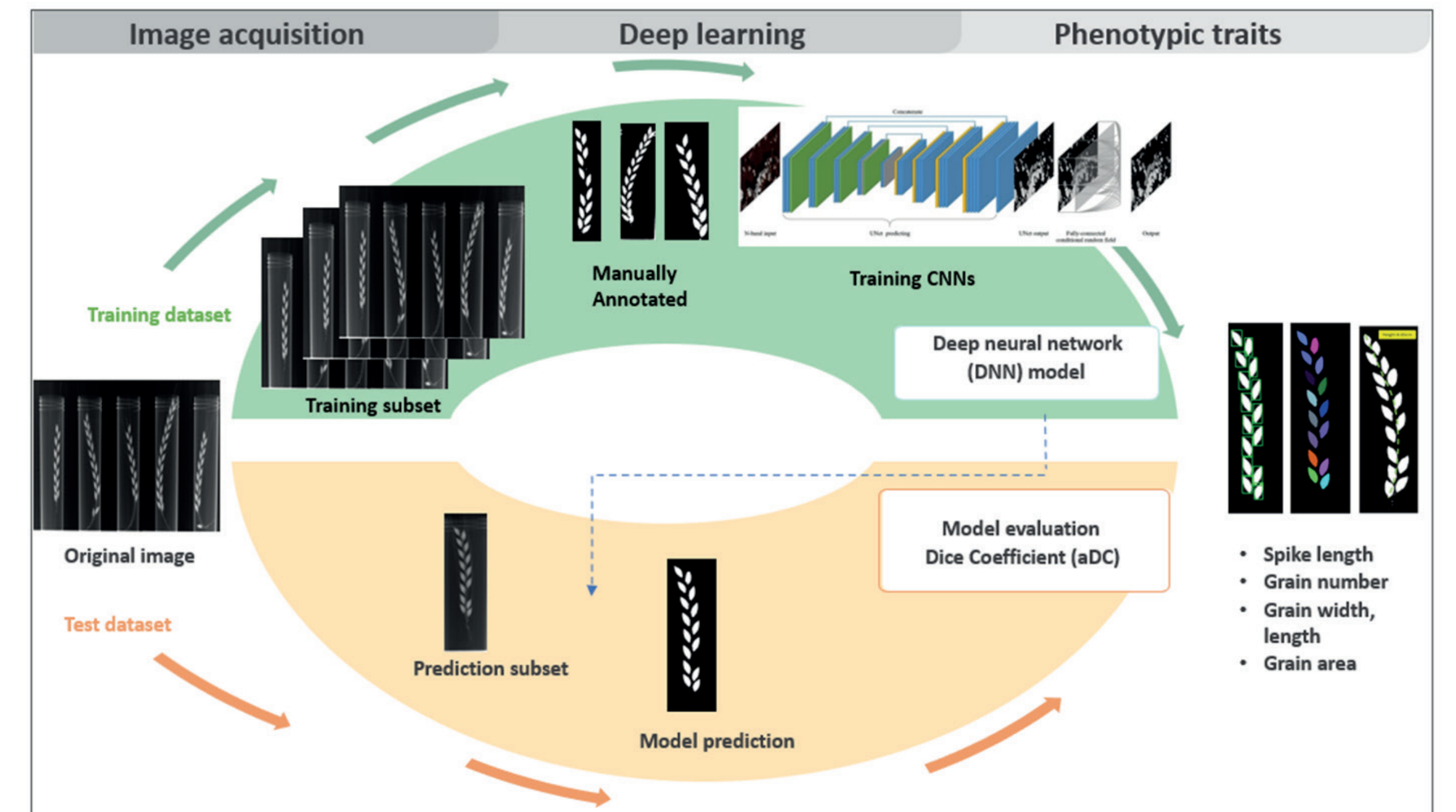


Fig.2 Workflow for supervised individual spike phenotyping: X-ray spike images are manually annotated and split into training and test sets; a convolutional neural network (CNN) is trained to segment grains, and its performance is assessed by the Dice coefficient (aDC), and the resulting masks yield spike length, grain number, size, and area.

Results

By comparing our UNet model with the Segment Anything (SAM) model, which achieved an average dice coefficient (aDC) of 0.98. Minor adjustments to the model parameters are necessary to account for variations in the illumination conditions of the spike images.



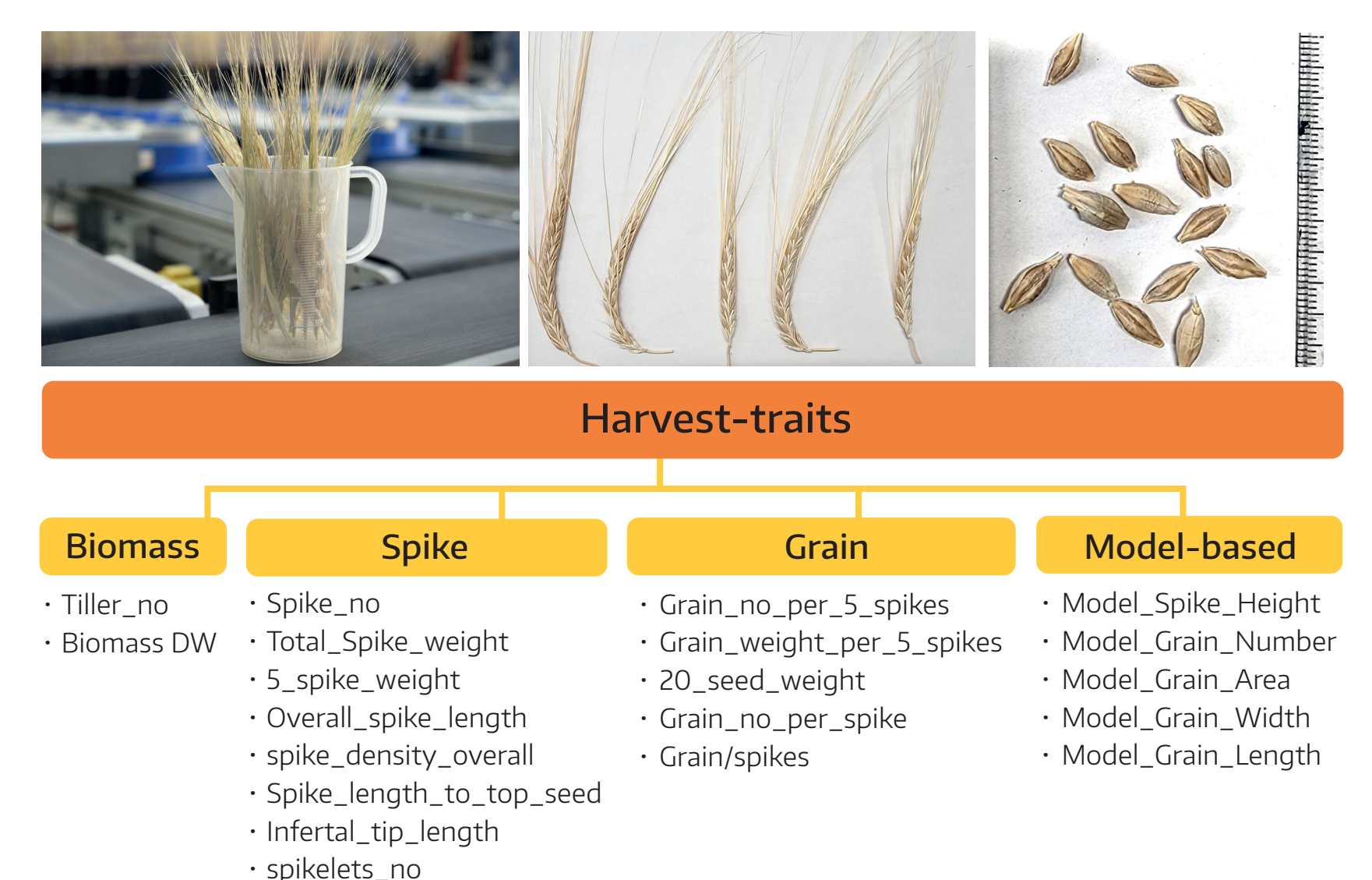
Fig.3 Instance segmentation of grain in spikes.

Training set	Test set
200	50
Segmentation model	Average dice coefficient
U-Net	0.90
Adaptive Thresholding (Otsu)	0.95
SAM	0.98

Table 1 Evaluation of the test set for different models with the average Dice coefficient.



Fig. 4 Destructive harvest for cross-validation with model-based traits.



Case Study Validation

In this study, nine spring barley (*Hordeum vulgare* L) lines with different genetic backgrounds were selected to investigate the effect of drought stress on yield-related traits. During the senescence phase, 5 spikes were harvested from 3 replicates per treatment. The spikes were placed in tubes and scanned with X-rays. Using the automated X-ray-based spike analysis to extract yield-related traits of many spikes, the instance segmentation result of the grain spike from SAM was generated, see Fig. 3.

Destructive analysis for ground truth cross-validation was performed after the scanning where 16 harvest-related traits including biomass, spike and grain were extracted (Fig. 4). The correlation analyses for (i) spike length predictions (Fig. 5) and (ii) grain number (Fig. 6) showed strong correlation ($R^2 = 0.801$ and 0.86 , respectively) with ground truth measurements.

By investigating the effect of drought, the results showed a similar trend in spike length and grain number from both model-based and ground-truth estimates. The effect of drought stress was pronounced in the selected genetic line L1, showing a reduction under drought compared to the control. However, on grain-related traits, the drought effect was reflected in grain development in L2 with a significant reduction under drought.

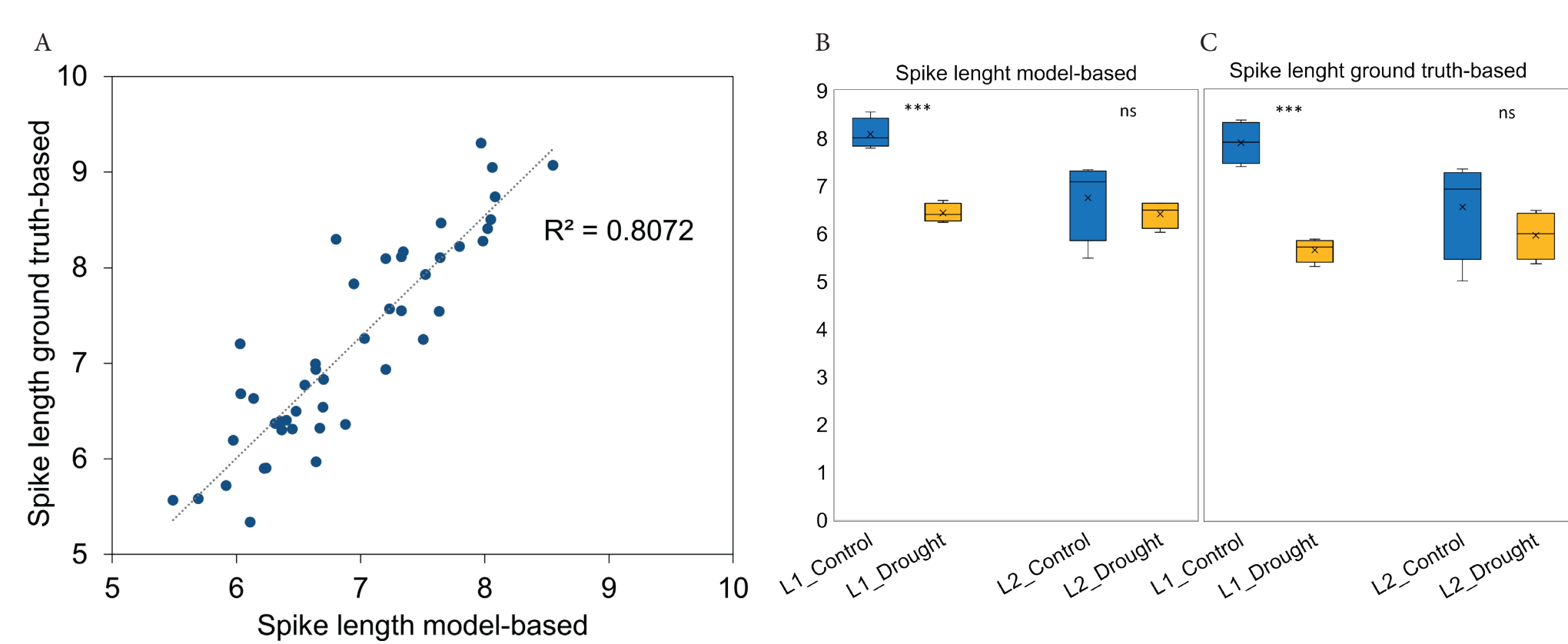


Fig.5 Spike length assessment. A) Comparison between model-based spike length estimations and ground truth measurements. B) Boxplots showing the model-based and C) ground-truth based on two selected barley lines under control and drought stress conditions. Significance levels are indicated: ns ($p \geq 0.05$) and *** ($p < 0.001$).

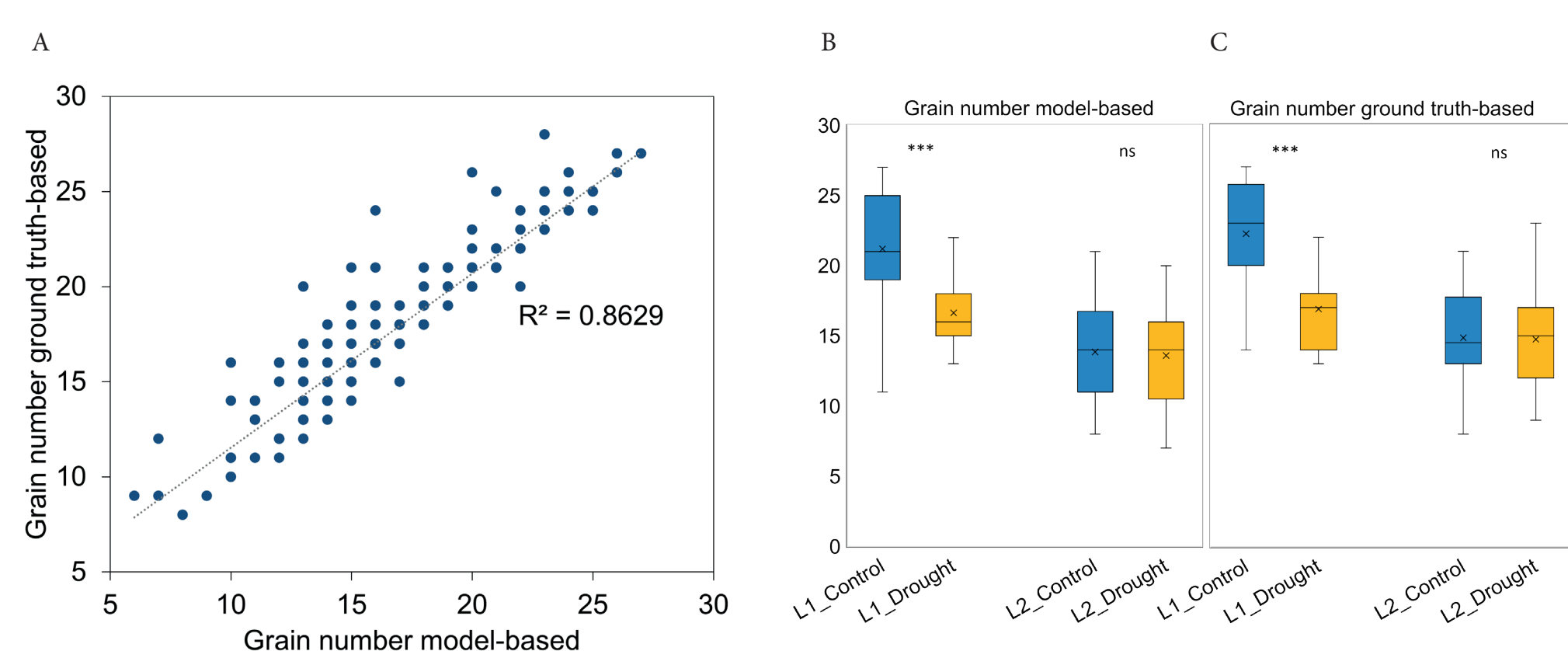


Fig.6 Grain number assessment. A) Comparison between model-based grain number estimates and ground truth with a strong linear relationship ($R^2 = 0.8629$). Each point represents an individual sample. B) Boxplots showing the model-based and C) ground-truth based on two selected barley lines under control and drought stress conditions. Significance levels are indicated: ns ($p \geq 0.05$) and *** ($p < 0.001$).

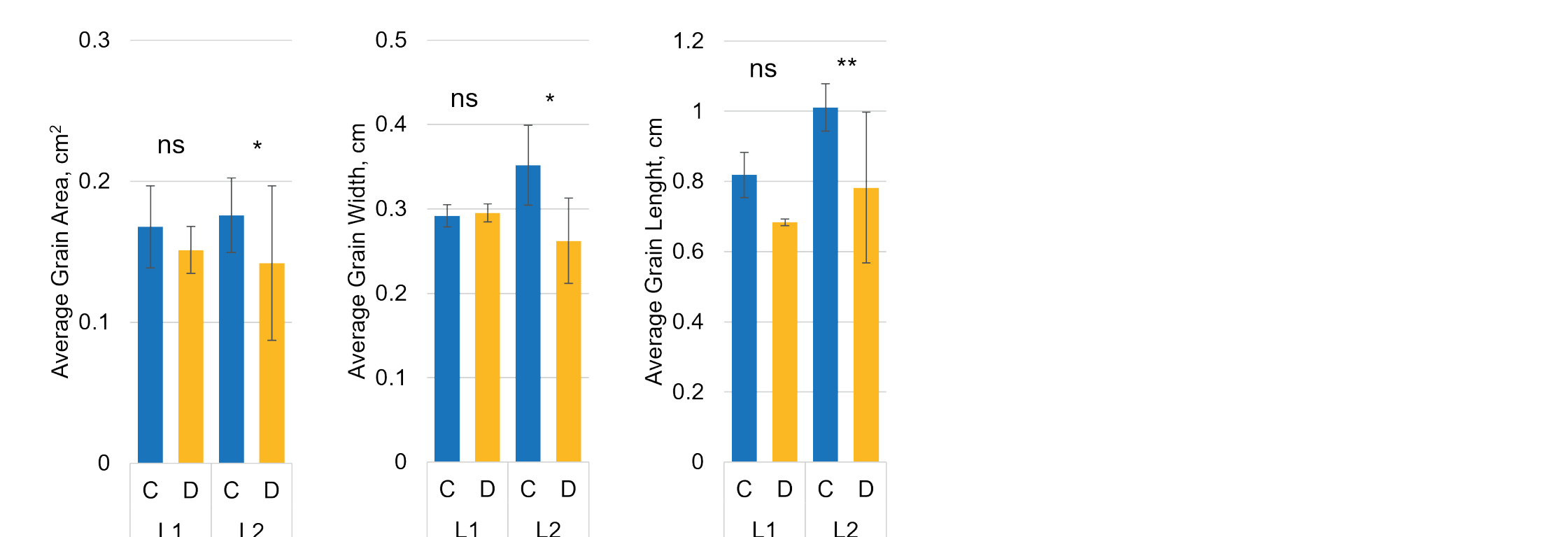


Fig. 7 Grain parametrization including area, width and length on two selected barley lines. Significance levels are indicated: ns ($p \geq 0.05$), * ($p < 0.05$), ** ($p < 0.01$)

For integrative visualization, a Pearson correlation matrix was performed on traits extracted by the model and other 16 harvest-related traits (Fig. 8). Key relationships for phenotypic selection, such as spike length and grain number model-based, emerged as useful traits as they correlated positively with most of the harvest-related components in this case study.

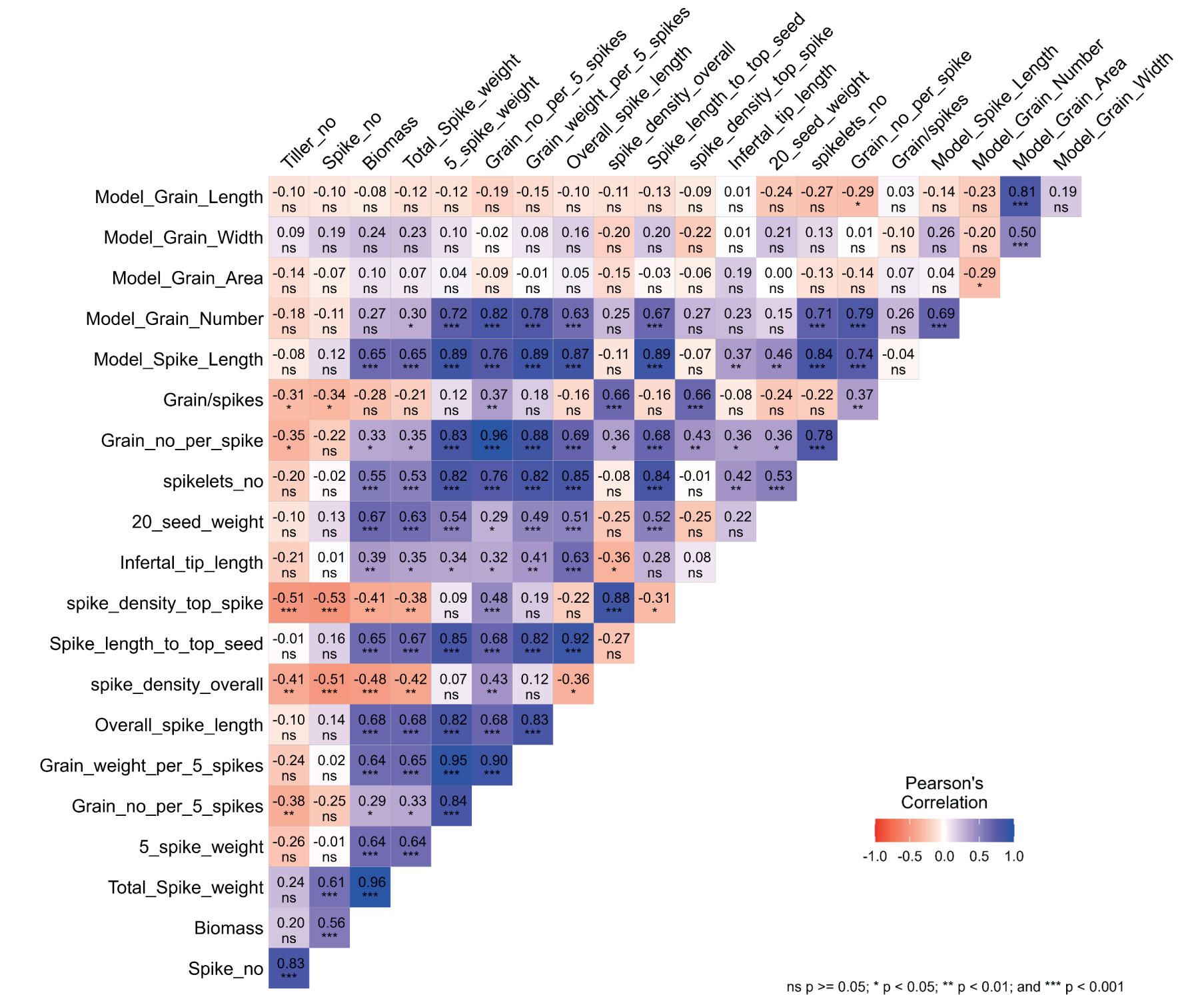
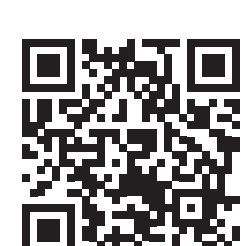


Fig. 8 Pearson's correlation matrix among biomass, spike, grain-related parameters. Correlation coefficients are color-coded from negative (red) to positive (blue) relationships. Significance levels are indicated: ns ($p \geq 0.05$), * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$)

Conclusion

- Fast, non-destructive X-ray imaging enables precise characterization of wheat and barley spike- and grain-related traits.
- Seamless high-throughput workflow speeds analysis from image to data in minutes
- Actionable insights for assessing yield potential in cereal crops for plant breeding



Acknowledgment

This work was supported from European Regional Development Fund-Project SINGING PLANT (No. CZ.02.1.01/0.0/0.0/16_026/0008446). Plant Sciences Core Facility of CEITEC Masaryk University is gratefully acknowledged for providing technical support.

Reference

Ullah, S., Panzarová, K., Trtílek, M., Lexa, M., Máčala, V., Neumann, K., Altmann, T., Hejátko, J., Pernisová, M., & Gladilin, E. (2024). High-Throughput Spike Detection in Greenhouse Cultivated Grain Crops with Attention Mechanisms-Based Deep Learning Models. *Plant Phenomics*, 6. <https://doi.org/10.34133/plantphenomics.0155>