

Recognizing and overcoming context dependency in the application of a machine learning tool for counting stomata in *Setaria* versus maize

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Stomata, microscopic pores on leaf surfaces, regulate the uptake of carbon dioxide and the simultaneous loss of water vapor by leaves. New image acquisition and analysis methods are allowing high-throughput phenotyping of stomatal patterning, which in turn have been applied to better understand the genetic basis of variation in certain species. However, it takes considerable data and effort to train the models, and their ability to accurately detect epidermal structures is constrained to morphologies found within the training data. This issue of context dependency, the inability to perform effectively in novel contexts, is the main hurdle preventing widespread adoption of machine learning in high-throughput phenotyping of intraspecific, interspecific, and environmental variation. Here we show the limited ability of a Mask-RCNN tool, which was previously trained and successfully applied to *Zea mays*, to analyze images from a closely related grass, *Setaria viridis*. We then demonstrate successful retraining of the tool to cope with the novel diversity presented by this new species. The stomatal complexes in optical tomography images of mature *Setaria* leaves were accurately identified by comparison to expert raters ($R^2 = 0.84$). This study highlights the challenge of context dependency for widespread application of machine learning tools for phenotyping plant traits, even in closely related species. At the same time, it also provides a new tool that can be applied to leverage *Setaria* as a model C₄ species, while also providing a roadmap for translation of a machine learning to analyze stomatal patterning in new plant species.