Utilizing Artificial Intelligence in Red Clover Breeding

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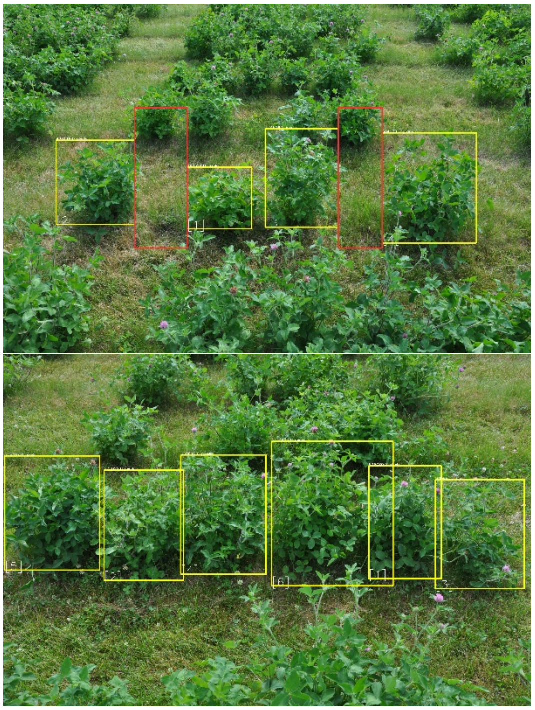

Increasingly plant breeders are exploring and implementing automated phenotyping in breeding programs (Shakoor et al., 2017). Is this technology just another fad or will it have staying power? We delve into this questions by exploring automated phenotyping in red clover (*Trifolium pratense* L.) breeding. As with DNA marker assisted breeding (Riday, 2013) our goal is to explore inexpensive and robust phenotyping systems requiring minimal capital costs and technological infrastructure. Currently our red clover breeding program uses visual plant vigor scores of individual space-plants as a biomass proxy (Riday, 2009). This system has been effective at generating biomass yield and persistence selection gains in red clover. To enhance our phenotyping system we acquired digital image sets of six space plant-slot red clover plots (Figures). Our goal was to create software and use it in conjunction with available software to identify individual space plants within images for further analysis. Rather than identify fixed areas in images where plants should be; we took the approach of having the software teach itself via machine learning to identify space plants within images. The program after viewing training images successfully learned to identify individual plants and box them (Figures). Coding was accomplished in the python language and incorporated freely available modules. Code was added to also determine missing plants among the six expected plant-slots. Boxed images of each plant can now be extracted for further image analysis allowing possible determination of: leaf-size, plant height, foliage density and color, plant maturity etc. This further image analysis will require further coding; although it may already be possible using height, width, and area of plant boxes to generate crude plant size scores. Further images will be taken in more difficult situation (e.g., taller companion grass). Field validation to determine efficacy of traits phenotyped using image analysis will also be conducted. We are impressed with how feasible it is to implement these technologies; and if fully realized offers a multitude of possibilities. For example multiple traits can be phenotyped by an unskilled operator who takes sets of six plant-slot images. Following image analysis phenotypic data is automatically electronically recorded for breeding analysis.

Riday, H. 2009. Correlations between visual biomass scores and forage yield in space-planted red clover (*Trifolium pratense*) breeding nurseries. Euphytica 170:339-345.

Riday, H. 2013. Marker assisted selection made cheap and easy. *In*: S. Barth and D. Milbourne (ed.) Breeding Strategies for Sustainable Forage and Turf Grass Improvement. Springer: Dordrecht. p. 21-27.

Shakoor, N., S. Lee, and T.C. Mockler. 2017. High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field. Curr. Opin. Plant Biol. 38:184-192.