

Advancements in sunflower multiparental population phenotyping for *Verticillium* Wilt using UAV-based multispectral imagery

Matías Domínguez¹, Denis Colombo², Alexandra Dillcheneider², Javier Lavandera¹, Andrés Corro Molas³, Carolina Troglia⁴ and Norma Paniego^{5*}

¹ INTA, EEA Pergamino, Argentina; ² INTA, EEA Anguil, Argentina; ³ INTA, AER General Pico, Argentina; ⁴ INTA, EEA Balcarce, Argentina; ⁵ INTA, Instituto de Agrobiotecnología y Biología Molecular, Argentina.

*paniego.norma@inta.gob.ar

Abstract: Here we present progress in phenotyping a sunflower Multiparent Advanced Generation Inter-Crosses (MAGIC) population for *Verticillium* wilt (VW), one of the most important sunflower diseases in Argentina. In addition, the implementation of high-throughput phenotyping (HTP) using unmanned aerial vehicles (UAV) is being explored to complement manual phenotyping and integrate it into breeding pipelines. A subset of 349 F2-MAGIC families was studied during the 2020/21 summer season in a VW-infested field in the EEA INTA Balcarce (37°50' 0" S, 58°15' 33" W, Argentina). Eighty F5-MAGIC contrast families for VW were selected from the 2020/21 phenotyping trial and phenotyped in another VW-infested field in the EEA INTA Anguil (36° 32'17" S, 63° 59' 20" W) in the 2023/24 summer season. VW incidence, severity and disease severity index (DSI) were recorded for each plot (one row of 5 m length). In the 2020/21 season, the trial was flown once during the flowering period (R5) using a Parrot Disco-Pro Ag drone with a Parrot Sequoia camera with 4 spectral bands, including green (G) (550nm ± 40nm), red (R) (660nm ± 40nm), red edge (RE) (735nm ± 10nm) and near infrared (NIR) (790nm ± 40nm). The flight altitude was 50 m. In the 2023/24 season, we used a Phantom 4 drone with a multispectral camera with five bands, including the blue (B) (450nm ± 16nm), G (560nm ± 16nm), R (650nm ± 16nm), RE (730nm ± 16nm) and NIR (840nm ± 26nm). The flight altitude was 40 m and the trial was flown four times during the flowering and grain-filling period from R1 to R9. The image processing was done with Agisoft Metashape for building the orthomosaics and with QGIS for creating the grid plot, extracting the reflectance and the vegetation indices (VIs) values. The Normalized Difference Vegetation Index (NDVI), the Normalized Water Vegetation Index (NWVI), the Optimized Soil-Adjusted Vegetation Index (OSAVI), and the Leaf Chlorophyll Index (LCI) VIs were estimated for the 2020/21 season. For the 2023/24 season, the NDVI, the Green Normalized Difference Vegetation Index (GNDVI), the Enhanced Vegetation Index (EVI), the Normalized difference red edge index (NDRE), the Green Red Vegetation Index (GRVI), the Green Leaf Index (GLI), the Plant Senescence Reflectance Index (PSRI), the Differenced Vegetation Index (DVI), the Visible Atmospherically Resistant Index (VARI) and the Chlorophyll Index Red Edge (CIRE) were extracted from each flight. Using the information from the spectral bands and the VIs, different machine learning models (MLM) were applied to classify each plot as susceptible or resistant to VW using the CARET library in R. The results confirmed the phenotypic variability of the MAGIC population for VW. Thirty resistant MAGIC F5 families exhibiting a DSI below 5 % were identified as valuable candidates for future breeding purposes. The MLM achieved a prediction accuracy of about

65 % in both trials, with the XGBoost model showing better prediction performance. Overall, the results highlight the potential of HTP for sunflower disease phenotyping and its applicability in sunflower breeding programs.

Keywords: MAGIC, Machine learning, disease phenotyping, prediction

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