Comparison of leaf morpho-anatomical characters in Amaranthus spp.: phenotyping as an investigative tool for environmental and agricultural

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Comparison of leaf morpho-anatomical characters in *Amaranthus spp.*: phenotyping as an investigative tool for environmental and agricultural sciences

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INTRODUCTION

Plant phenotyping is an investigative method that can provide insight into the interaction between plants and the environment, especially when paired with genotype studies [1]. The resulting knowledge can be useful in eco-physiological research, to understand how species adapt to their growing conditions and to biotic interactions. In recent years, phenotyping techniques for research in plant physiology and anatomy have been developed in the field of the image processing, ranging from microscope acquisitions up to high-scale imaging through remote sensing [2,3].

In this work, we focused on the detailed study of leaf morphometric traits through the processing of photographic images and reflection confocal microscopy acquisitions on nail polish replicas. Four phylogenetically-related species of *Amaranthus* (*A. hybridus* L., *A. palmeri* S. Wats., *A. retroflexus* L., *A. tuberculatus* (Moq.) J.D. Sauer) have been selected, due to their high impacts in agricultural crops. The study aims at developing an easily replicable survey method that would be suitable for both agronomical and environmental purposes.

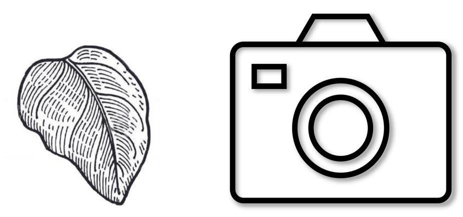
MATERIALS & METHODS

A. hybridus
A. palmeri
A. retroflexus
A. tuberculatus

Collection of 18 young leaves per species (13-16 stage of BBCH scale [4])

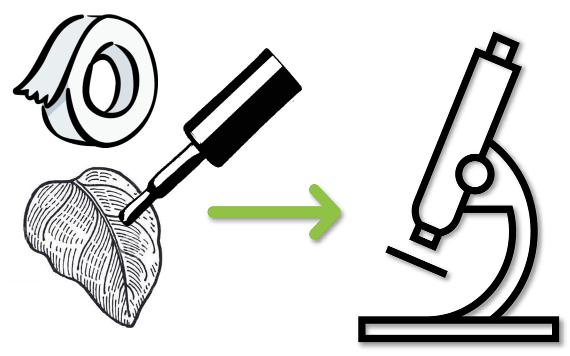
1. Photographic acquisition

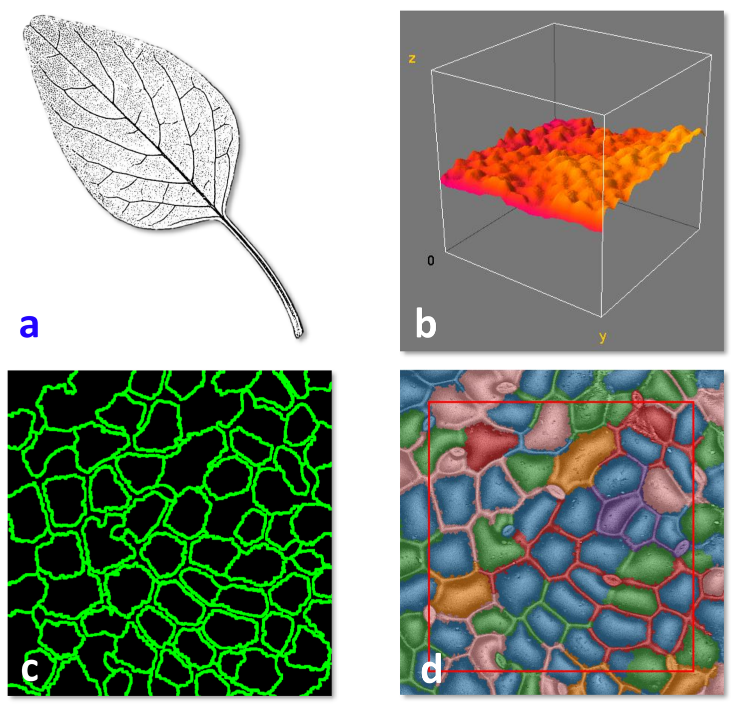
of the adaxial leaf page (backlit leaf)



2. *Stripping* technique

- Application of two coats of transparent nail polish to obtain a mold
- Mold removal with tape and tweezers
- Slide mounting and **confocal microscopy** analysis in **reflection mode** (488 nm)





3. Data processing

- Image processing with ***ImageJ Fiji*** [A] *and* ***LeafNet*** [B] software: measure of leaf **morphometric traits** (a) and surface ***roughness*** **parameters** (b), as well as **cell segmentation** (c) and **cell morphology analysis** (d).
- Statistical analysis: **MANOVA** (Multivariate Analysis of Variance) and **CDA** (Canonical Discriminant Analysis)

RESULTS

Fig. 1 - CDA one dimension plot of the all leaf-trait considered. A CDA was conducted on the MANOVA model results ($P < 0.001$), applied to all non-autocorrelated variables ($r<0.75$). The Canonical Variable 1 explains the highest percentage of variance. *A. hybridus*, *A. palmeri* and *A. retroflexus* showed a positive score (panel A) linked to a high number of variables (panel B), while *A. tuberculatus* showed negative score. The variables represented by the longer vectors (*Leaf circularity* and *Hairiness Aspect Ratio*) are those showing a greater effect in species differentiation.

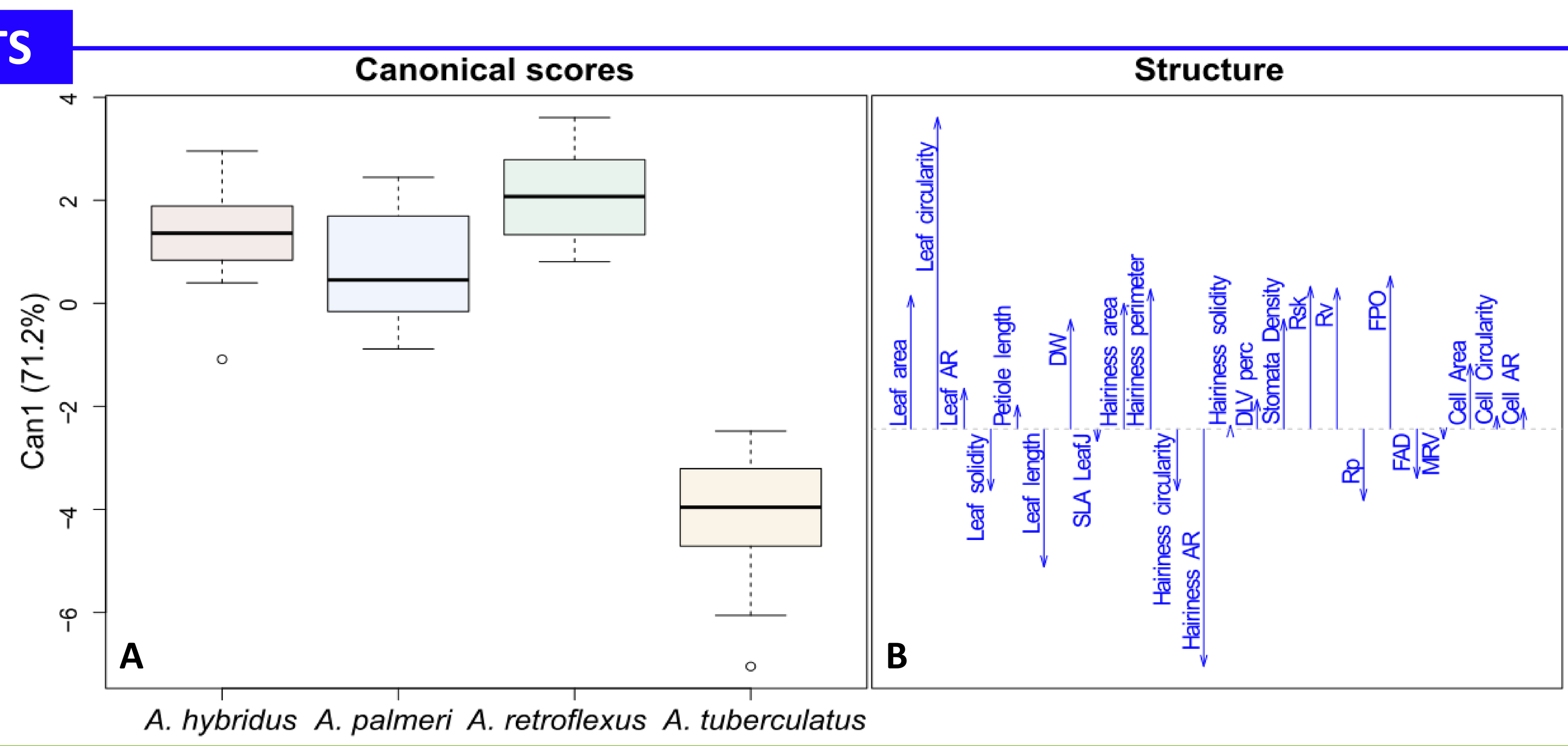
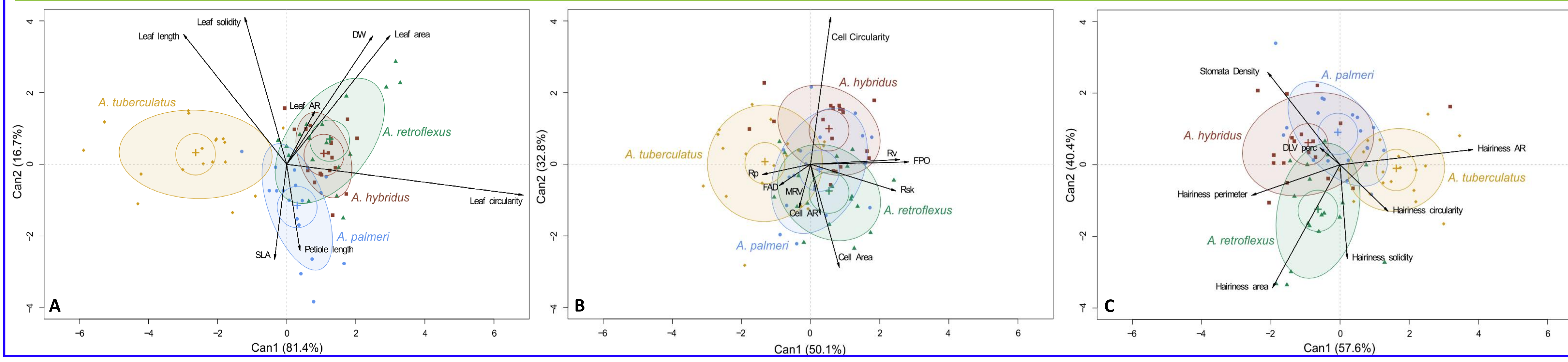


Fig. 2 - CDA bi-plots showing the effect of variables divided into groups on *Amaranthus* species. A: Leaf morpho-anatomical traits; B: Leaf surface-related variables (cells and *roughness*); C: Conductivity-related variables (hairiness, stomata, veins). The Canonical Variable 1 explains the highest percentage of variance, most markedly in the case of leaf morpho-anatomical traits. These variables in fact mainly influence the distinction of *A. tuberculatus* from the other species, also evidenced by the greater overlap of the four ellipsoids in panel B (less evident in Panel C). Also in this case, the variables represented by the longer vectors differentiate species to a greater extent.



CONCLUSIONS

The present work considered the leaf analysis of well-known invasive *Amaranthus* spp. in order to carry out a phenotypic characterization. It has been shown that the determination of leaf traits at different investigation levels can highlight species-specific traits even at a juvenile stage, a crucial phenological stage in the plant development. The methodology proved to be a promising approach for the functional characterization of phylogenetically-related species due to its replicability and low-impact sampling method and managed to give a statistically measurable value to characters involved in taxonomy and biodiversity studies. With a view to its improvement with further advanced detection techniques, such as remote sensing, it could become a useful tool for quantitative analysis of adaptative responses-related traits and to define strategies for the sustainable management of the agro-ecosystem.

IPSP Istituto per la Protezione Sostenibile delle Piante Consiglio Nazionale delle Ricerche

References:

[1] **Li *et al.*, 2020** - https://doi.org/10.1016/j.compag.2020.105672

[2] **Araus *et al.*, 2022** - https://doi.org/10.1007/978-3-030-90673-3_27

[3] **Li *et al.*, 2014** - https://doi.org/10.3390/s141120078

[4] **Hess *et al.*, 1997** - https:// doi.org/10.1046/j.1365-3180.1997.d01-70.x

Software References:

[A] **Schindelin *et al.*, 2012** - doi:10.1038/nmeth.2019

Plugins used: LeafJ (a,c,d), Vessel Analysis (a), Extended Depth of Field (b), SurfCharJ 1q (b).

[B] **Copyright © Zhou Lab 2020 – 2021**: https://leafnet.whu.edu.cn/. Used for (c) and (d) analysis.

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