UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Plant Health

Assessment of cultivated and wild species in response to water stress conditions

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Introduction

- Significant impact on agriculture.
- Drought: limiting factor for plant growth.
- Implementation of defense mechanisms.
- Physiological, morphological, and biochemical characteristics.

Objectives

- Evaluate the effects of the water stress treatments on the growth, development and photosynthetic activity of nine different plant species.

Learning objectives

- Learn and practice data management, execute statistical analyses in R, generate several type of graphs, and develop correct interpretation of results.

Hypotheses

- **H0.** There will be no significant effects of the water stress treatments on the growth, development and photosynthetic activity of the evaluated plant species.
- **H1.** There will be significant effects of the water stress treatments on the growth, development and photosynthetic activity of the evaluated plant species.

Materials and Methods

Plant Material.
9 species. *Amaranthus retroflexus*, *Beta vulgaris*, *Hordeum vulgare*, *Lolium perenne*, *Portulacea oleracea*, *Raphanus sativus*, *Solanum lycopersicum*, *Sonchus oleraceus*, *Spinacia oleracea*.

Substrate and pot preparation.
210 one-liter pots, washed, absorbent paper in base. Substrate was peat moss and perlite (3:1), mixed and hydrated, pots filled to the top, located in 15 trays, 14 pots per tray.

Transplant.
1-week old seedlings transplanted individually in pots. Labels with species, treatment, repetition. Transplant date: 28/9/2022.

Water stress treatments

Table 1. Amount of water per tray (L) provided for each treatment.

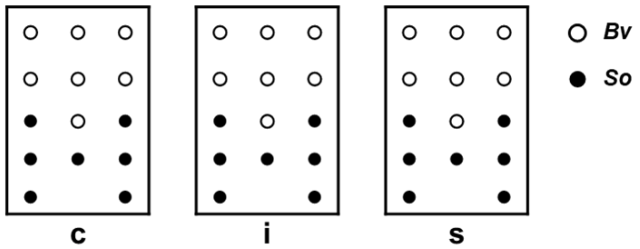
		Amount of water per tray (L)		
Week	Date	Control	Intermediate	Water Stress
W0	28/09/2022	1.5	1.5	1.5
W1	05/10/2022	2.0	1.0	0
W2	13/10/2022	2.0	1.0	0
W3	19/10/2022	2.0	1.0	0
W4	26/10/2022	2.0	1.0	1.0
W5	02/11/2022	2.0	1.0	0.5
W6	09/11/2022	2.0	1.0	0.5

Sampling.

Final measurements, careful extraction of plants, shaking substrate off, washing of roots, plants laid out, photographs, estimation of LA and RL with Digimizer. Plants divided into shoots and roots, weighed (FW), stored in Al foil for dehydration in oven (DW).

Experimental design

Study Factor A was Water Stress, with three levels or treatments: Control (c), Intermediate (i), Water Stress (s). Study Factor B was Plant Use, with Cultivated (crop) and Wild (weed). 7 repetitions per treatment and species, 14 plants per tray, 3 trays per group, 42 experimental units per group, each one constituted by a potted plant. 210 in total.



15 plant parameters measured and registered for analyses: Soil Humidity (SH), Electrical Conductivity (EC), Plant Height (PH), Number of Leaves (NL), Leaf Length (LL), Leaf Width (LW), Chlorophyll Content (CC), Leaf Area (LA), Aerial Fresh Weight (AFW), Aerial Dry Weight (ADW), Aerial Water Content (AWC), Root Length (RL), Root Fresh Weight (RFW), Root Dry Weight (RDW), Root Water Content (RWC).

Data analysis

1 Data Overview: All species & all variables

- Excluded: *Hordeum vulgare* and *Lolium perenne*
- ANOVA
  - ➢ Heatmap of p-values
- Principal component analysis (PCA)
  - ➢ **What are the most important variables that determine the behavior of plant species?**

2 Data Filtering: Focus on the important

1. ANOVA: Avoiding biases in results
   - ➢ W1 should not show any significant differences among treatments
   - ➢ *Solanum lycopersicum* was filtered out.
2. ANOVA: cluster species
   - ➢ Gather species with more significant variables together

3

Final Data Analysis: Clean data and group comparisons

I. **PCA for each cluster**
   A. Goal: Determine the most important variables explaining the behavior of each pair of species

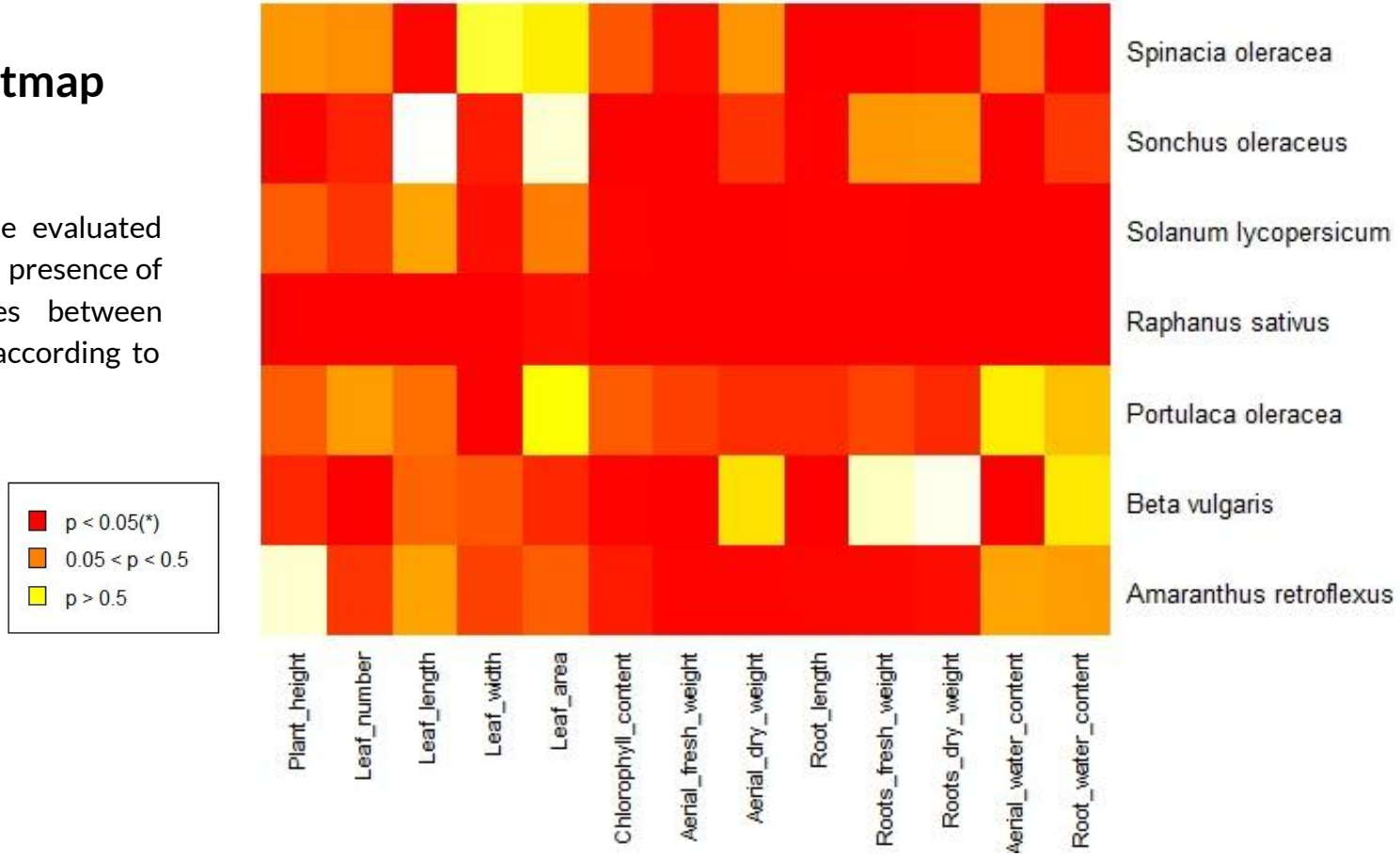
II. **Bonferroni test**
   A. Goal: Answer hypothesis
   B. Overall: were there any variable(s) that could describe plant responses under water stress treatment?

Results

1

ANOVA Heatmap

Heatmap showing the evaluated species, variables, and presence of significant differences between treatments ($p<0.05$) according to the ANOVAs.



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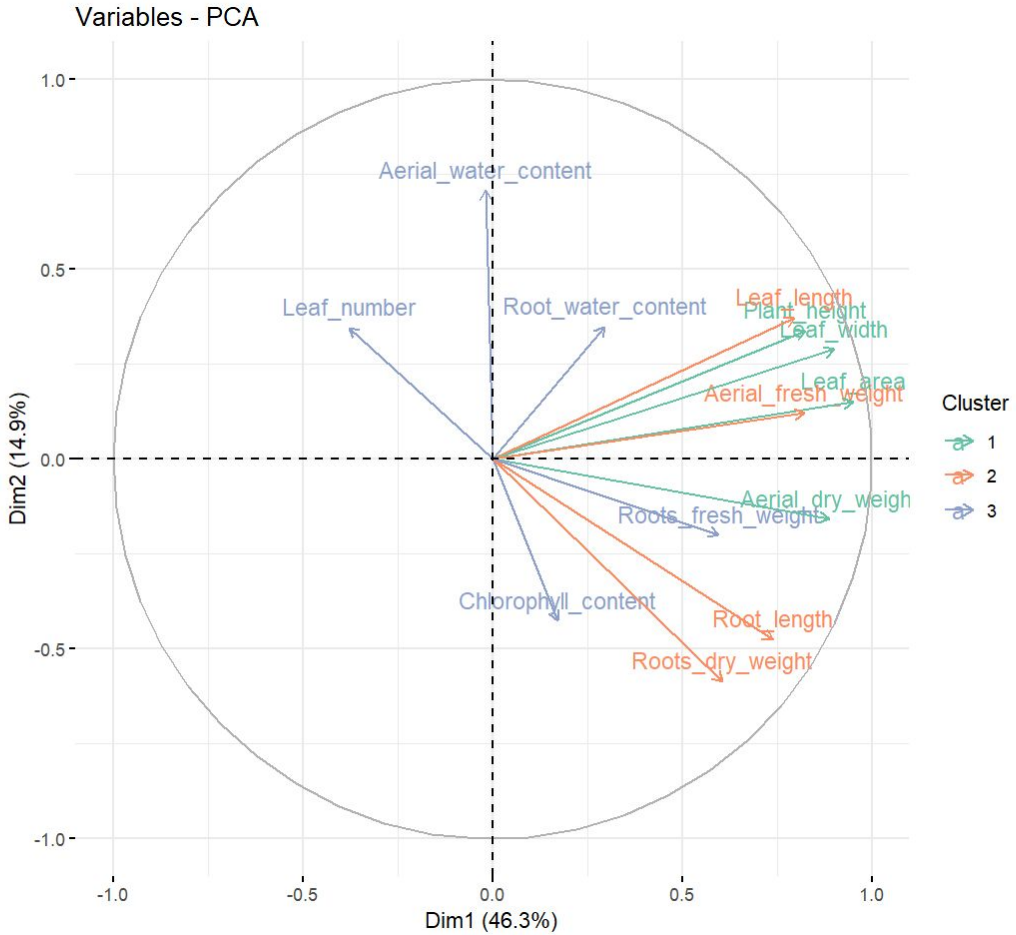
PCA

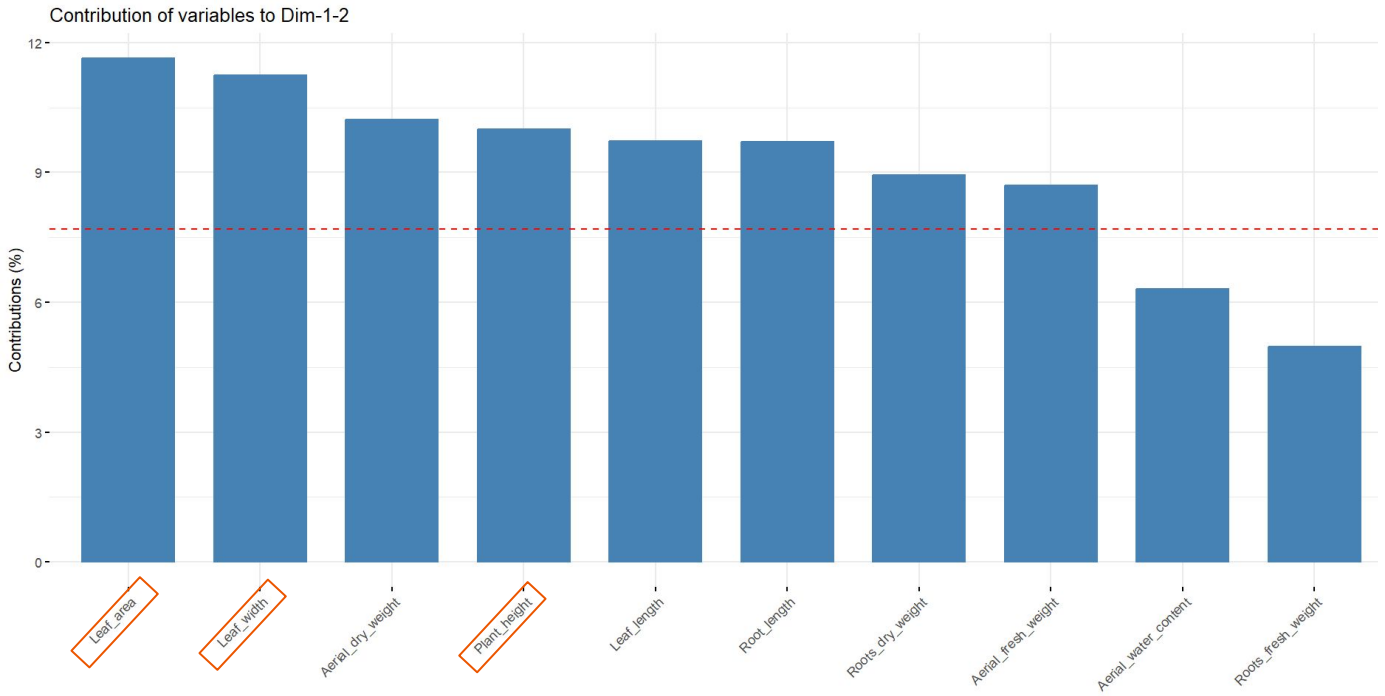
- Carrying out of PCA to reduce the dimensionality of the dataset.
- PCA helped us to identify the variables that better explain variation and growth behaviour of plants under stress.
- The green are more closely related to PC1 and orange to PC2



1

PCA Dim-1-2

- Variables and their contribution (%) to explained variation.
- Red line indicates expected average contribution.
- Identification of most relevant variables.
- Leaf Area, Leaf Width, and Aerial Dry Weight represent more than 70% of the explained variation.



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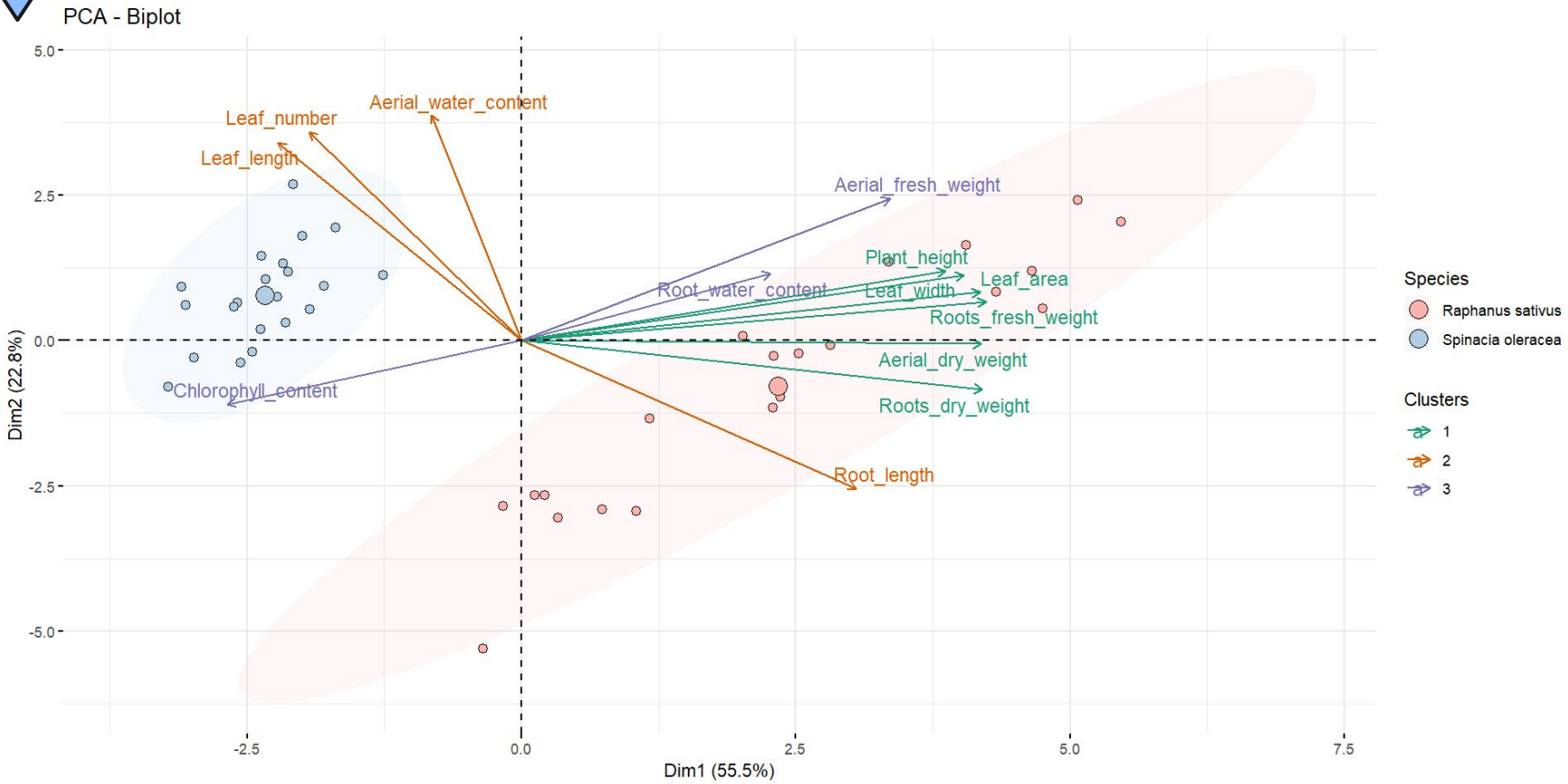
Significant differences at W1 and W6

- ANOVA test showing significant or not significant differences between species and the 3 most important variables (according to the PCA) for week 1 and week 6.
- *Solanum lycopersicum* presented significant differences in week 1, so it has been discarded from further analysis.
- The other species were grouped according to common significant differences in the 3 variables analyzed.

	Species	W1	W6	Variable
1	Amaranthus retroflexus	not significant	significant	Leaf area
2	Amaranthus retroflexus	not significant	significant	Leaf width
3	Amaranthus retroflexus	not significant	not significant	Plant height
4	Beta vulgaris	not significant	significant	Leaf area
5	Beta vulgaris	not significant	not significant	Leaf width
6	Beta vulgaris	not significant	not significant	Plant height
7	Portulaca oleracea	not significant	significant	Leaf area
8	Portulaca oleracea	not significant	significant	Leaf width
9	Portulaca oleracea	not significant	not significant	Plant height
10	Raphanus sativus	not significant	significant	Leaf area
11	Raphanus sativus	not significant	significant	Leaf width
12	Raphanus sativus	not significant	significant	Plant height
13	Solanum lycopersicum	significant	significant	Leaf area
14	Solanum lycopersicum	significant	significant	Leaf width
15	Solanum lycopersicum	not significant	significant	Plant height
16	Sonchus oleraceus	not significant	significant	Leaf area
17	Sonchus oleraceus	not significant	not significant	Leaf width
18	Sonchus oleraceus	not significant	not significant	Plant height
19	Spinacia oleracea	not significant	significant	Leaf area
20	Spinacia oleracea	not significant	significant	Leaf width
21	Spinacia oleracea	not significant	significant	Plant height

3

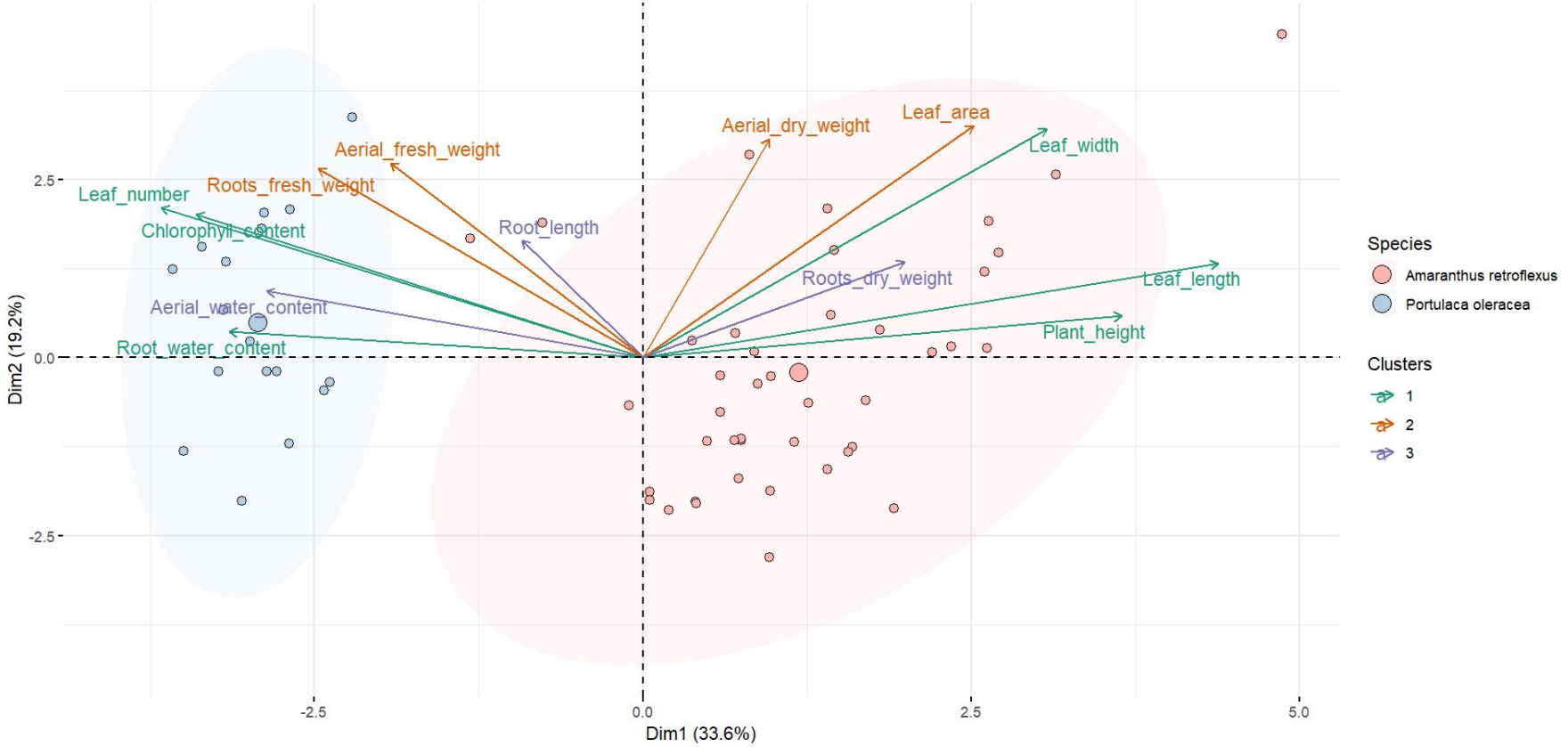
Raphanus sativus and *Spinacia oleracea*



3

Amaranthus retroflexus and *Portulaca oleracea*

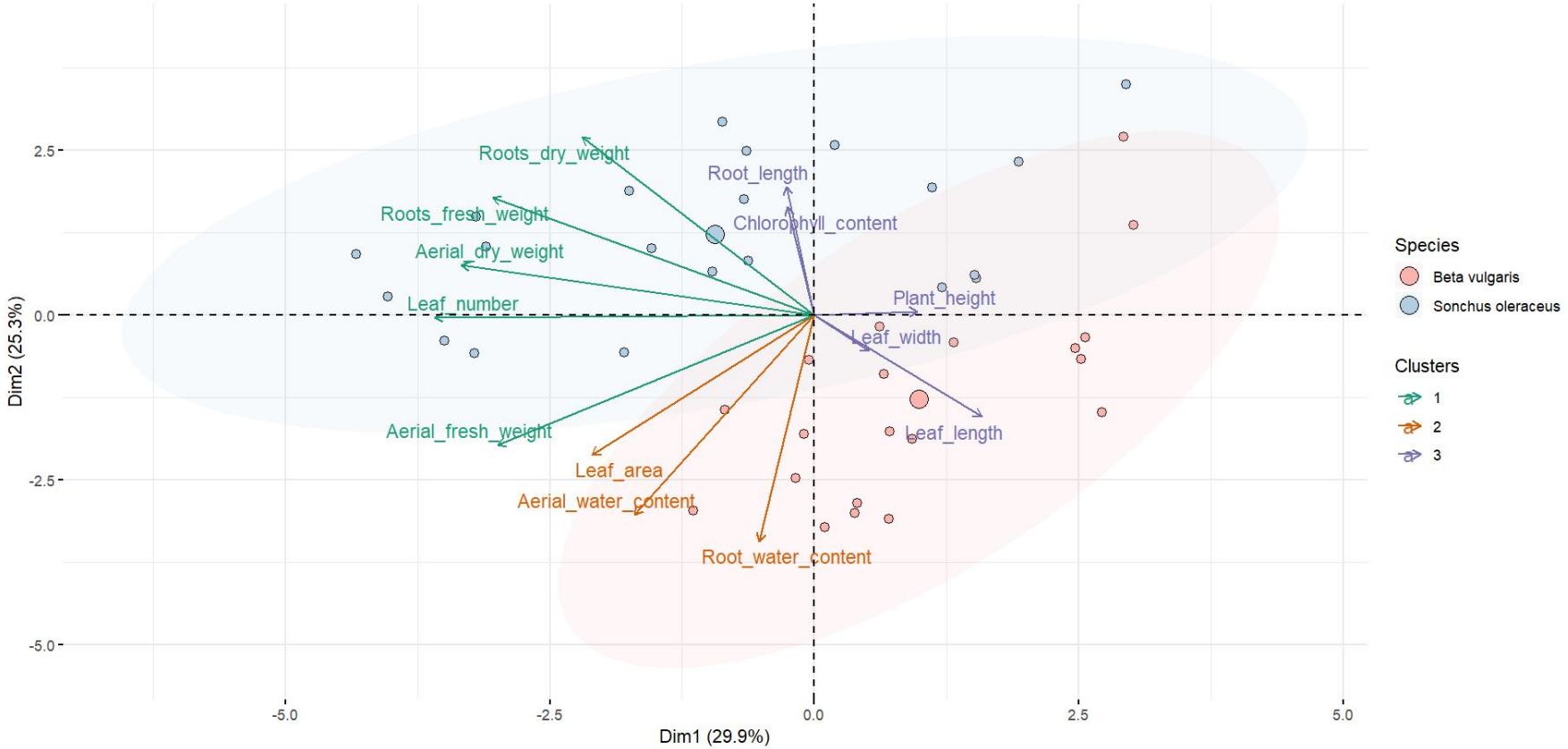
PCA - Biplot

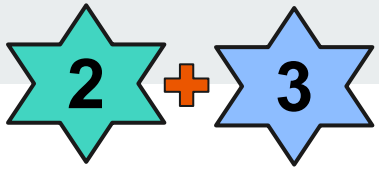


Beta vulgaris and *Sonchus oleraceus*

3

PCA - Biplot





Most important variables for each species

Species	Variables
Amaranthus retroflexus	Plant_height
Amaranthus retroflexus	Leaf_length
Beta vulgaris	Root_water_content
Beta vulgaris	Aerial_water_content
Portulaca oleracea	Leaf_number
Portulaca oleracea	Chlorophyll_content
Raphanus sativus	Roots_fresh_weight
Raphanus sativus	Roots_dry_weight
Sonchus oleraceus	Leaf_number
Sonchus oleraceus	Aerial_fresh_weight
Spinacia oleracea	Leaf_number
Spinacia oleracea	Leaf_length

3

Bonferroni test

Post-hoc multiple comparison test to show differences between treatments.

Color indicates a significant result.

-Less responsive species to WS:
Portulaca oleracea, *Spinacia oleracea*, and *Amaranthus retroflexus*.

-Most responsive species to WS:
Raphanus sativus, *Beta vulgaris*, and *Sonchus oleraceus*.

Species	Variables	Control.Intermediate	Control.Stress	Intermediate.Stress
Raphanus sativus	Roots_fresh_weight	0.000	0.000	0.001
Raphanus sativus	Roots_dry_weight	0.227	0.001	0.079
Spinacia oleracea	Leaf_number	0.882	1.000	0.500
Spinacia oleracea	Leaf_length	1.000	0.018	0.054
Amaranthus retroflexus	Plant_height	0.637	1.000	1.000
Amaranthus retroflexus	Leaf_length	0.031	0.382	0.784
Portulaca oleracea	Leaf_number	0.199	1.000	0.679
Portulaca oleracea	Chlorophyll_content	0.255	1.000	0.091
Beta vulgaris	Aerial_water_content	1.000	0.019	0.043
Beta vulgaris	Root_water_content	0.396	0.158	1.000
Sonchus oleraceus	Leaf_number	0.008	0.000	0.233
Sonchus oleraceus	Aerial_fresh_weight	0.000	0.000	0.035

Discussion & Conclusions

- **Null hypothesis was rejected**
  - There were significant effects of the water stress treatments on the evaluated plant species.
  - Fixed number of variables could not be found to explain the behavior under water stress conditions for all plant species.
    - **Note:** Not all plant parameters are as relevant for all species. They differ depending on the intensity and duration of water stress, specie, plant growth, and phenological stage (Lisar *et al.*, 2012).

- Chlorophyll content is important in determining plant growth (Li *et al.*, 2018). However, the results do not show any significant differences between treatments for this parameter.
    - **Note:** probability that water stress level was not severe enough to change the chlorophyll content in leaves.
- The results do not show any trend regarding differences between cultivated or wild species. Similar outcomes were observed among similar plant families.

For future analysis

- Improve the experimental design in order to:
  - Include all species.
  - Increase the amount of stress induced in the water treatments.
  - Replicate the results with other species.

- Yield can represent a relevant parameter in plant growth, and could be considered in future studies.

Thank you very much

References

- Lisar SY, Motafakkerazad R, Hossain MM, Rahman IM (2012). Causes, effects and responses. Water stress 25(1), 33. https://doi.org/10.5772/39363

- Li Y, He N, Hou J, Xu L, Liu C, Zhang J, Wang Q, Zhang X, Wu X (2018). Factors influencing leaf chlorophyll content in natural forests at the Biome scale. Frontiers in Ecology and Evolution, 6. https://doi.org/10.3389/fevo.2018.00064