OliveId: an open access software for describing olive morphological parameters

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Overview

Motivation

Motivation of this work

It is of great importance to evaluate and characterize the phenotypic diversity of the crop species.
Among them **olive** represents an important case due to its **rich patrimony of varieties** and **wild plants**.

- Olive cultivars may strongly differ morphologically and physiologically. Differences can be noticed on tree, leaf and fruit shape and size.

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Use of olive morphological parameters

- ✓ Morphological characters have been widely used for descriptive purposes and are commonly used to distinguish olive cultivars.
- ✓ Morphological characteristics are sometime correlated or associated with disease susceptibility and can be used as markers in breeding.
- ✓ Some morphological characters are very heritable and are little influenced by the environment.

- In recent years, the vast majority of the efforts has been concentrated solely on the molecular genotypization.

⋆ For the calculations of the morphological parameters have been used old fashioned techniques (e.g. screw gauge, millimetre paper) or other methodologies-software (e.g. *Tomato Analyser, LeafAnalyser, LAMINA, SmartGrain*, etc.) not suitable for the handling problem, due to the imposition of some prerequisites (e.g. detailed and intensive labour for the preparation of the samples, color of the images background, position of the object, etc.).

GOAL OF THIS STUDY: is to present **a first integrated semi-automatic methodology** of detecting various olive morphological parameters from imaging data⇒ many fruit, leaf and endocarp features may be quantified.

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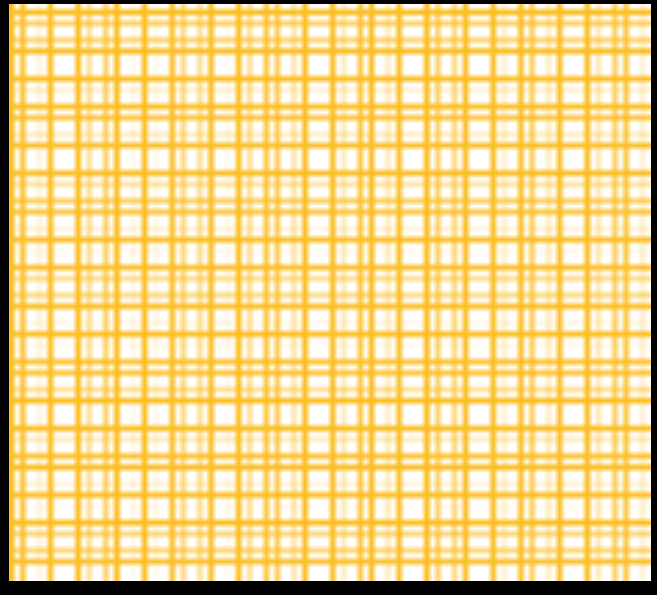

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oliveId

Methodology

In general, our methodology includes five main steps:

- **Collecting** fruit (at two different ripening stages) and leaf samples (mature leaves at 4-5 nodes from the mid-shoot apex) from the fields.
- **Creating imaging data** (by taking photos of a representative number of objects (25-30) under standard conditions of light, distance and resolution).
- **Segmentation step.** Separating fruits, leaves and endocarps from the background.
- **Applying mathematical algorithms** for the calculation of the morphological parameters.
- **Post-processing analysis** of the numerical data for their inference (characterisation and discrimination of varieties, establishment of relationships).

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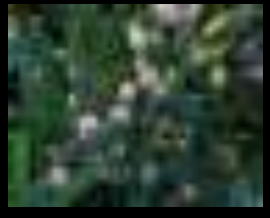
- **Collecting** fruit (at two different ripening stages) and leaf samples (mature leaves at 4-5 nodes from the mid-shoot apex) from the fields. 
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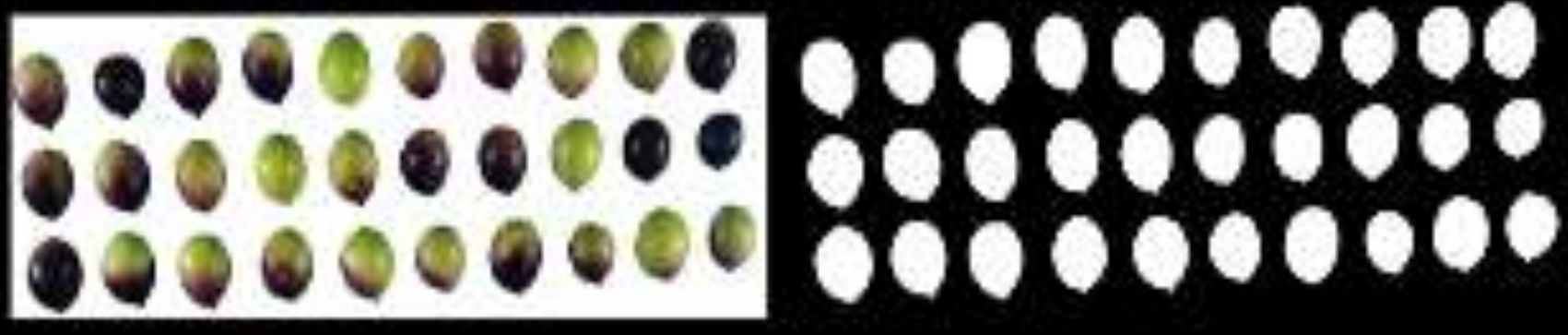
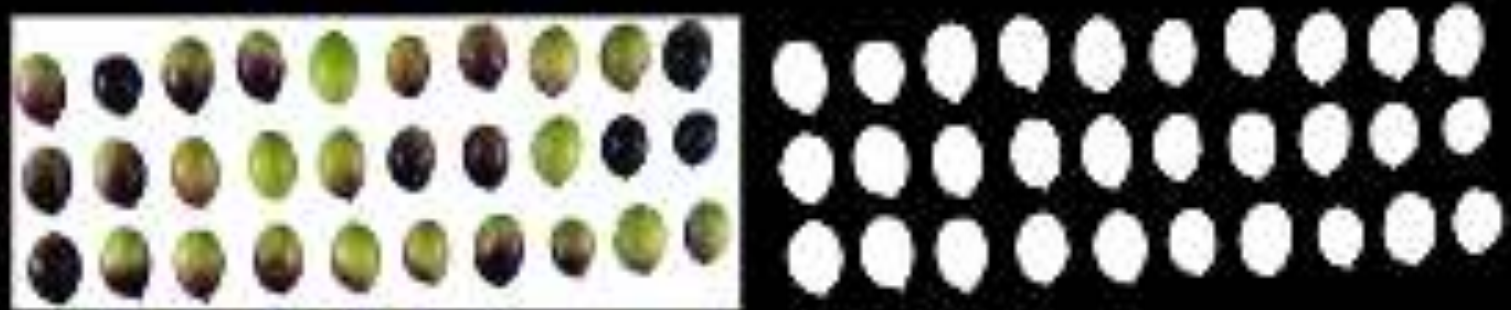
Segmentation Step

- We are using color thresholding techniques, by modifying manually the values of “Hue", “Saturation" and “Brightness" of each image in ImageJ.



Morphological Analysis

- Using the Image Processing Toolbox from **MATLAB** we created an automatic algorithm for object contour extraction from the binary images.
- The final outcome of the algorithm is the representation of each shape by a discrete sequence with all its boundary points. This sequence with the boundary points represents each shape and it can be considered mathematically, as a **closed polygonal line**, which vertices are the boundary points and its (undirected) edges were defined by subsequent vertices.



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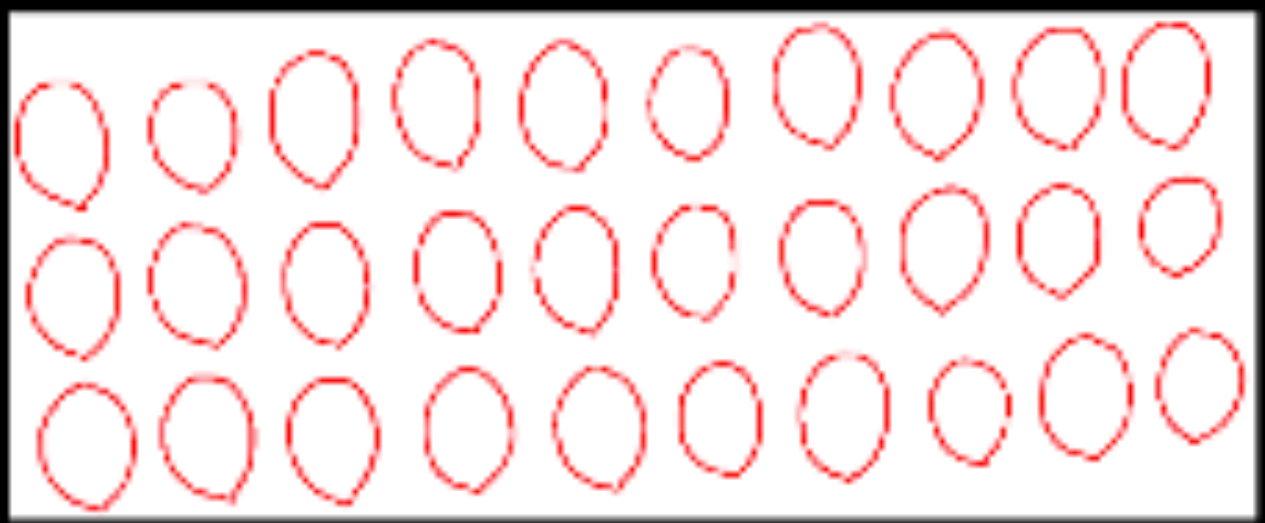

Characters of the fruit

Observed in samples of 30 fruits and taken from the middle section of fruiting shoots chosen from the most representative shoots on the south-facing side of the tree at shoulder level. Very small or very large fruits are discarded from the sample.

- Two imaging positions which are adopted by UPOV and International Olive Council (IOC). Position A refers to fruits or endocarps in which they display their greatest asymmetry; whereas, position B is reached by turning 90^o from position A

Characters of the fruit

The following parameters have been taken into account:

- Area, perimeter, height, maximum transverse diameter, position of max transverse diameter.
- Vertical and transversal symmetry.
- Major and minor axis of a fitted ellipse, shape index.
- Shape of the base and apex.
- Presence or not of a nipple, height, area of the nipple.

The characters which are in red are useful for cultivar discrimination.

Characters of the fruit

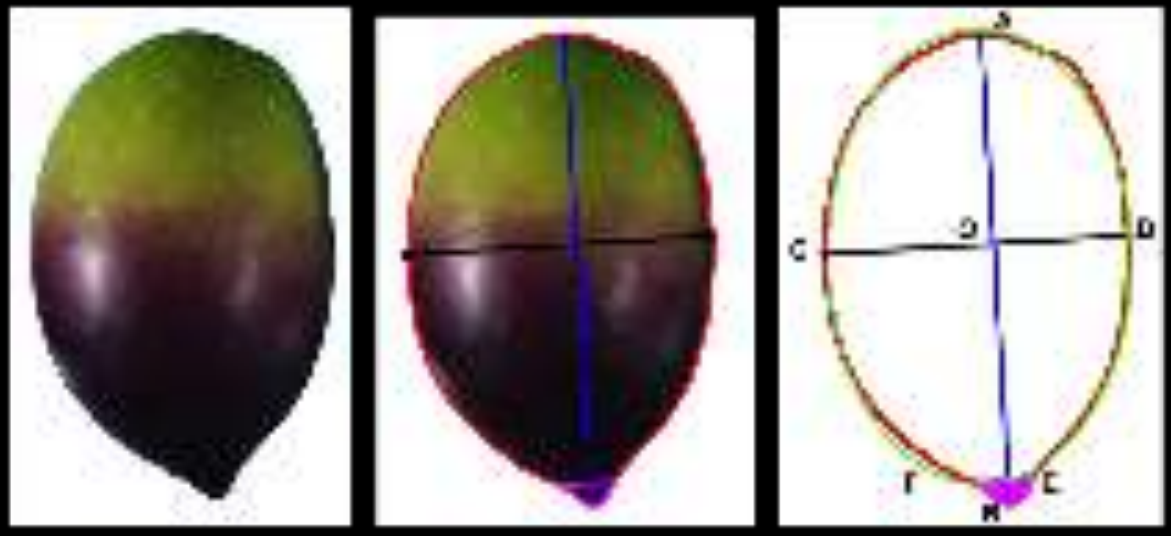

Figure: **Morphological characters of the fruit.** (a) Raw image data. (b) Raw image data with measurements. (c) Morphological measurements. Contour (red line): fruit boundary, height (A-B): (blue line), maximum transverse diameter (C-D) (black line), position of the maximum transverse diameter: segment (O-B), best fit ellipse: green curve, fruit nipple: pink curve (arc (EBF)).

Characters of the fruit

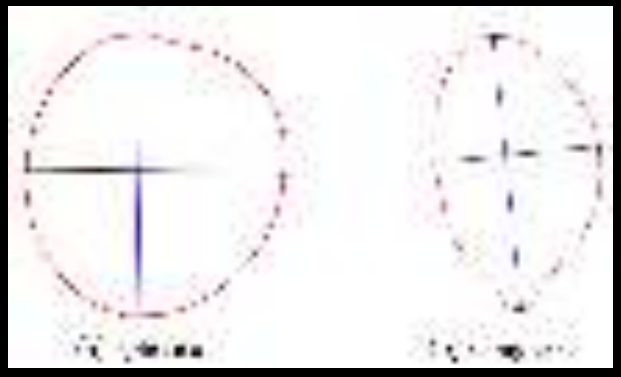

Figure: **Shape index**. Left: spherical (TOFFAHI) and Right: elongated (MASTOIDIS) fruits.

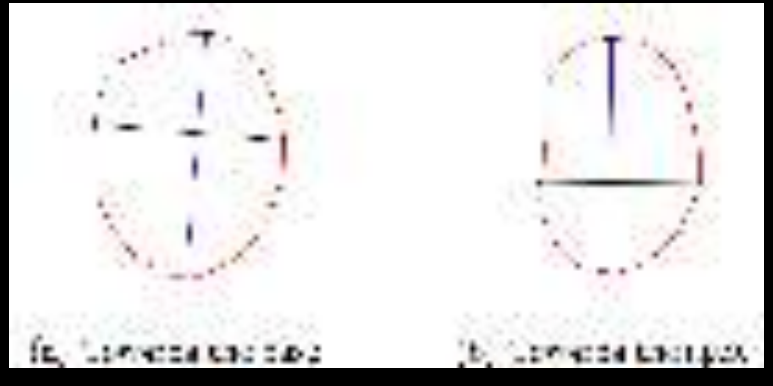

Figure: **Position of the maximum transverse diameter.** Left: towards the base (BALADI-ROUMANI) and Right: towards the apex (R-KHAMI).

Characters of the leaf

Observed in samples of about 25 adult leaves and taken from the middle section of shoots chosen from the most representative shoots on the south-facing side of the tree at shoulder level

The following parameters have been taken into account:

- Area, perimeter, leaf blade height, maximum transversal diameter (width).
- Vertical and transversal symmetry.
- Major and minor axis of a fitted ellipse, shape index.
- Shape of tip.
- Height, area of the petiole.

Characters of the leaf

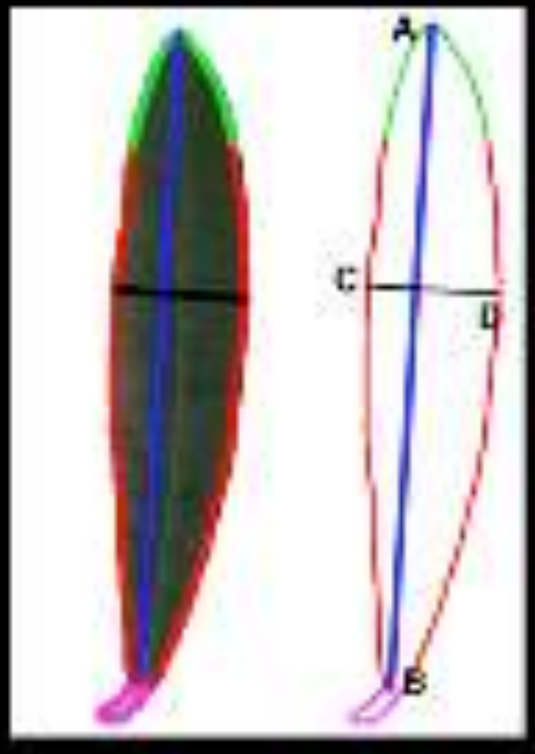

Figure: **Morphological characters of a leaf.** (a) Raw image data. (b) Morphological measurements. Contour (red line): leaf boundary,Tip curve: green line, blade height: segment (A-B)-(blue line), width: segment (C-D)-(black line), petiole: pink curve.

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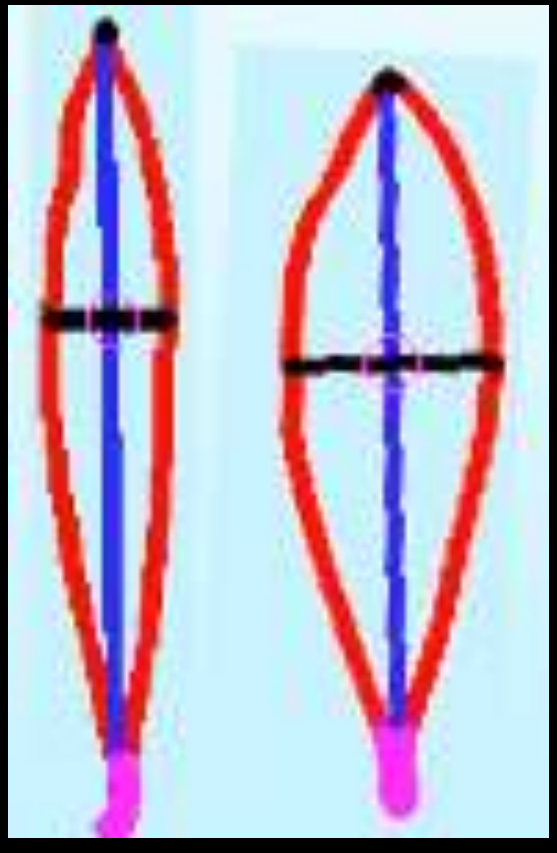


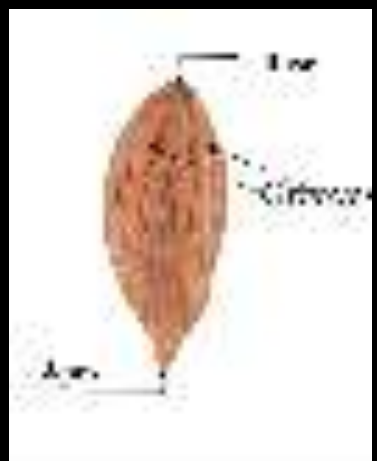
Figure: **Maximum transverse diameter.** Left: Narow leaf (CAROLEA) and Right: Broad leaf (OTTOBRATICA)

Characters of the endocarp

Characters of the endocarp

These are evaluated in the sample of 30 fruits. The endocarp is the internal, woody part of the fruit that encloses the seed. Usually the word stone refers to the endocarp and seed together.

- **In our approach**: we consider an additional position of the endocarp. From the vertical position becomes easier (in an automatic way) the determination of the contour roughness.
- Except the morphological parameters that we took for the fruits and the leaves, here we considered, additionally:
  - ✓ Number of grooves.
  - ✓ Contour roughness.
  - ✓ Apex and base shape.
  - ✓ Average and maximum depth of the grooves.



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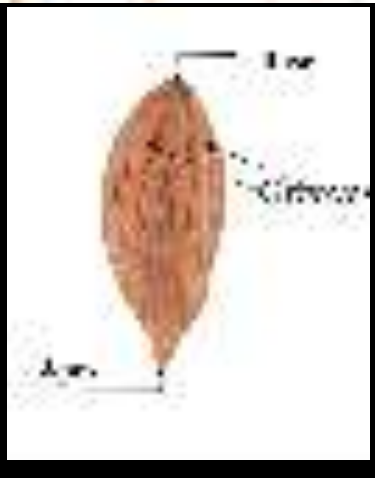

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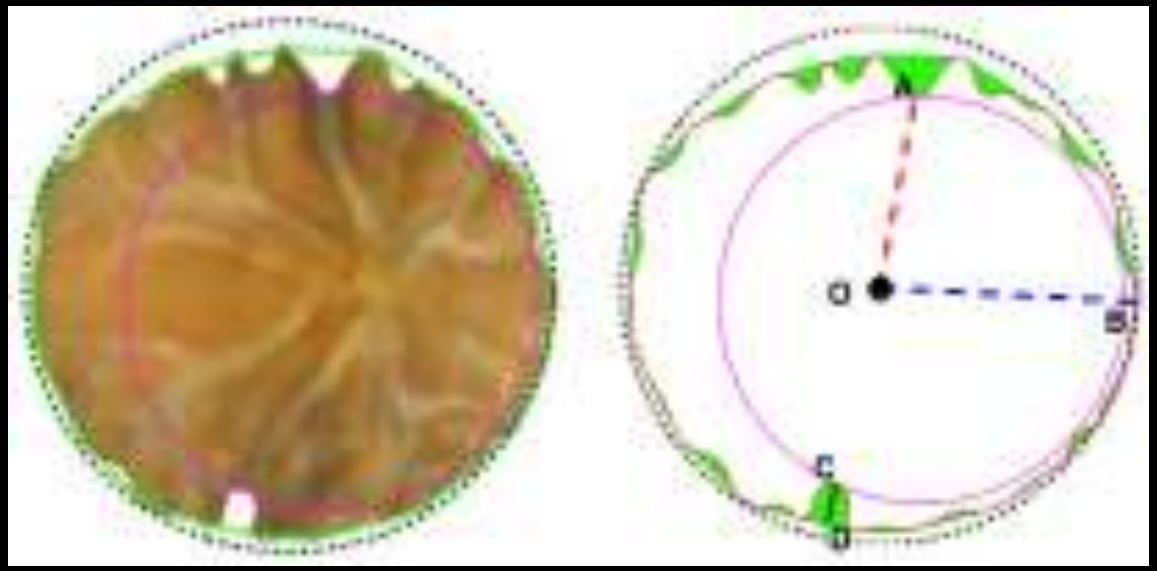


Figure: **Morphological characters of the endocarp in vertical position.** (left) Raw image data. (right) Morphological measurements. Convex hull polygon (green line), maximum depth of the grooves (CD) (black line vertical to the polygonal edge), shortest distance from the centre to the endocarp boundary segment (OA)-(red dashed line), longest distance from the centre to the endocarp boundary segment (OB)-(blue dashed line), inscribed circle the largest circle inside the endocarp boundary (pink dashed circle), minimum bounding circle (black dashed circle)

Characters of the endocarp

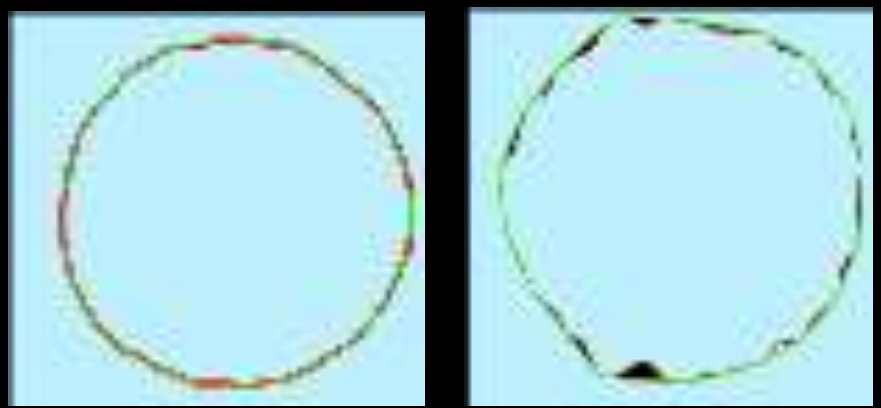


Figure: **Contour roughness**. Left: Smooth surface (BALADI) and Right: scabrous (ASCOLANA).

Morphological analysis of Greek cultivars.

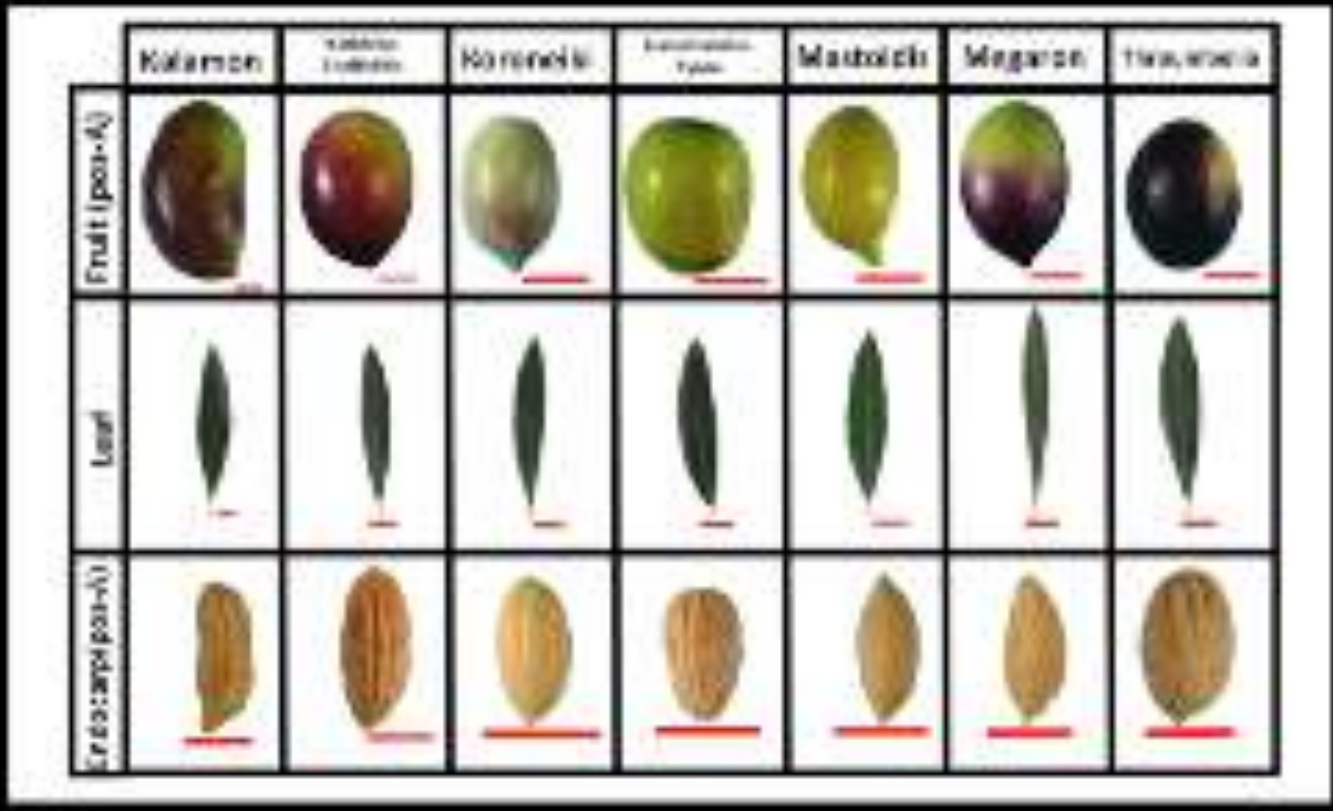


Figure: Greek olive-samples. Representative olive fruit, leaf and endocarp of the Greek olive cultivars charaterized in this proof-of-concept morphological analysis. Red line represents $1cm$.

Morphological analysis of Greek cultivars.

- The proposed methodology is based on robust mathematical descriptors which could provide more **accurate**, **rapid** and consistent results regarding the shape description.

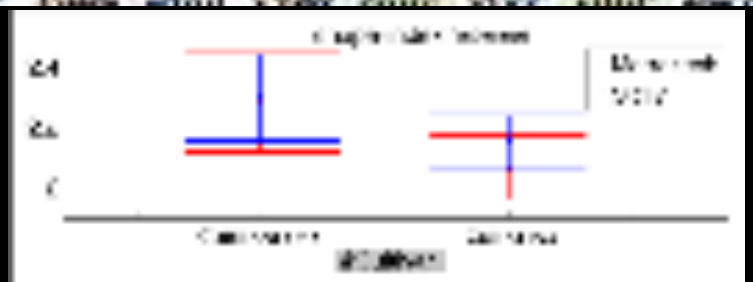
Figure: Morphological analysis of seven Greek olive cultivars.

Can we identify olive varieties?

- By the Tukey-Kramer multiple comparisons post hoc statistical test, all the parameters discriminated either all or some of the seven olive cultivars.
- For example, the multiple comparisons of the fruit shape index in position A, appeared to discriminate successfully six out of the seven olive cultivars.

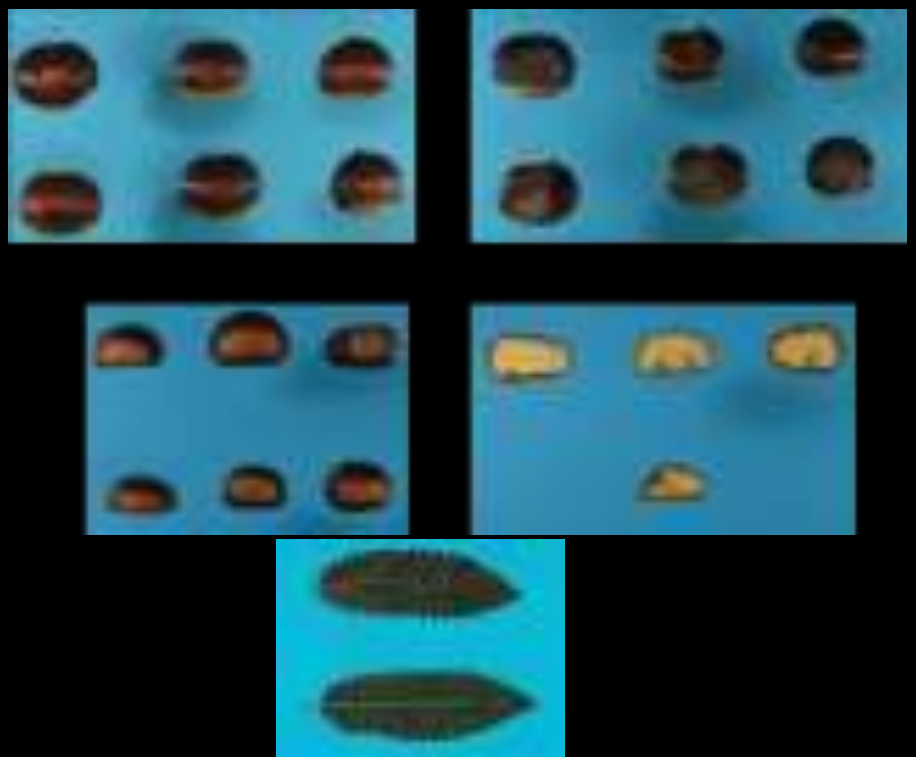
Identification of olive varieties

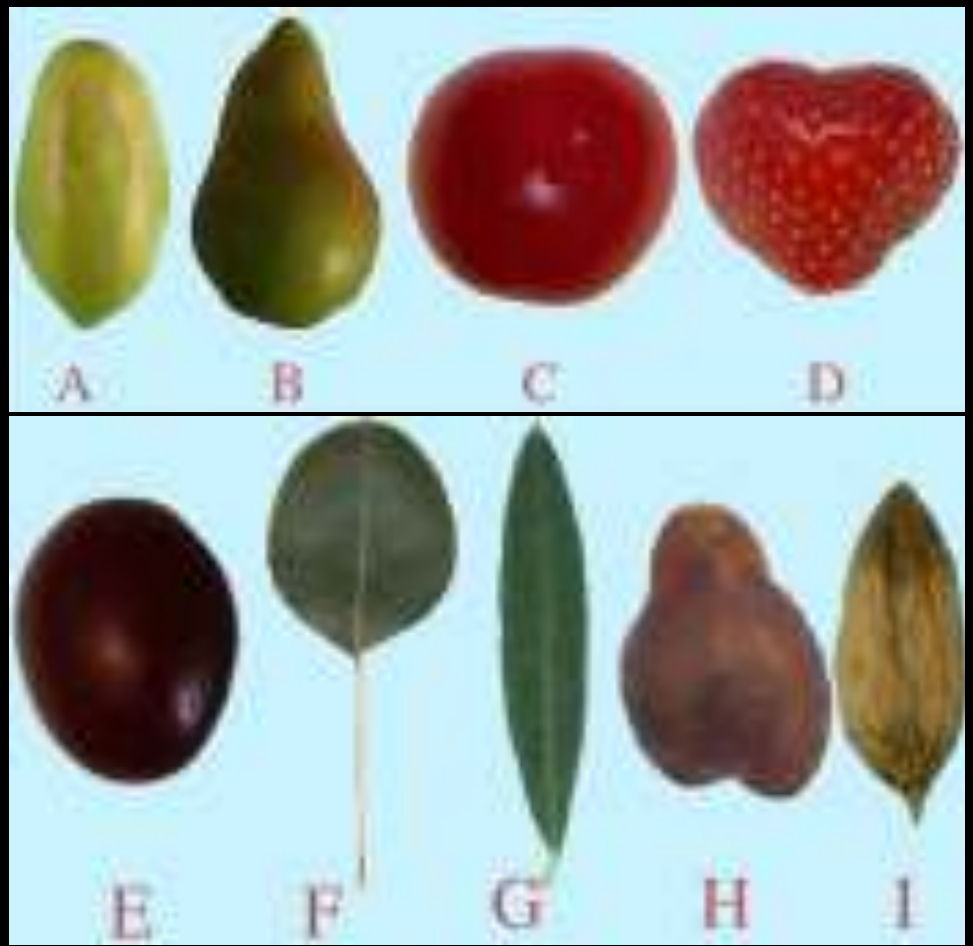
- Do the different environmental conditions-climate affect the morphology?
- Some morphological characters are particularly useful since they enable discrimination between morphologically different cultivars.



Morphological analysis of crop species

- The proposed methodology was also applied to describe other crop species morphologies.





Experiments MAICh-Effect of different climate conditions

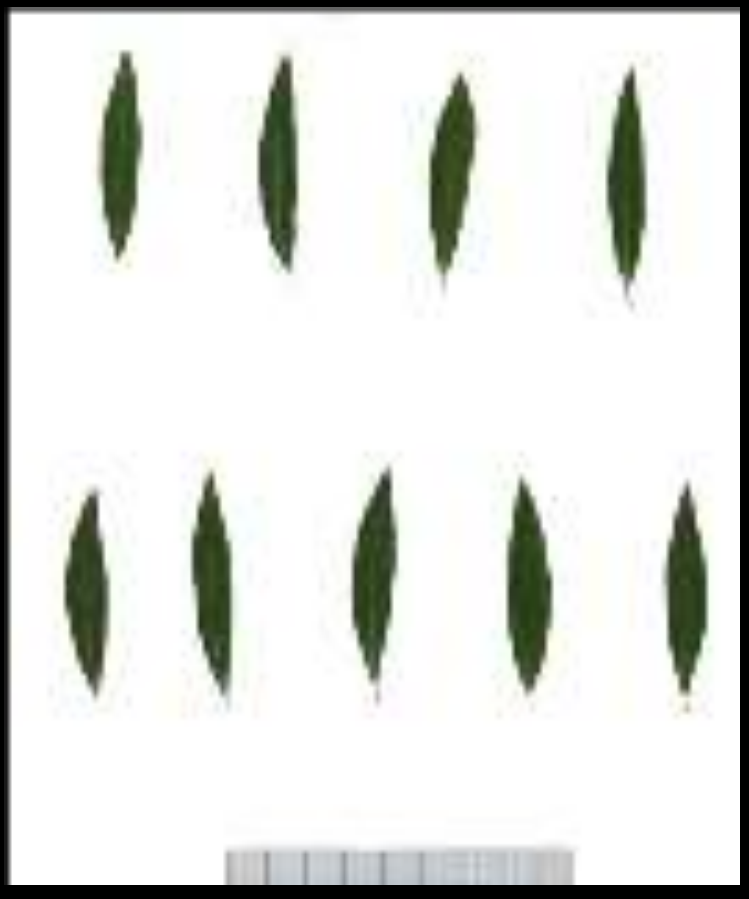
- Koroneiki, Gaidourelia, Leukolia Serron and Arvanitolia olive cultivars are under investigation in different watering conditions.

Experiments MAICh-Effect of different climate conditions

control-90DAYS

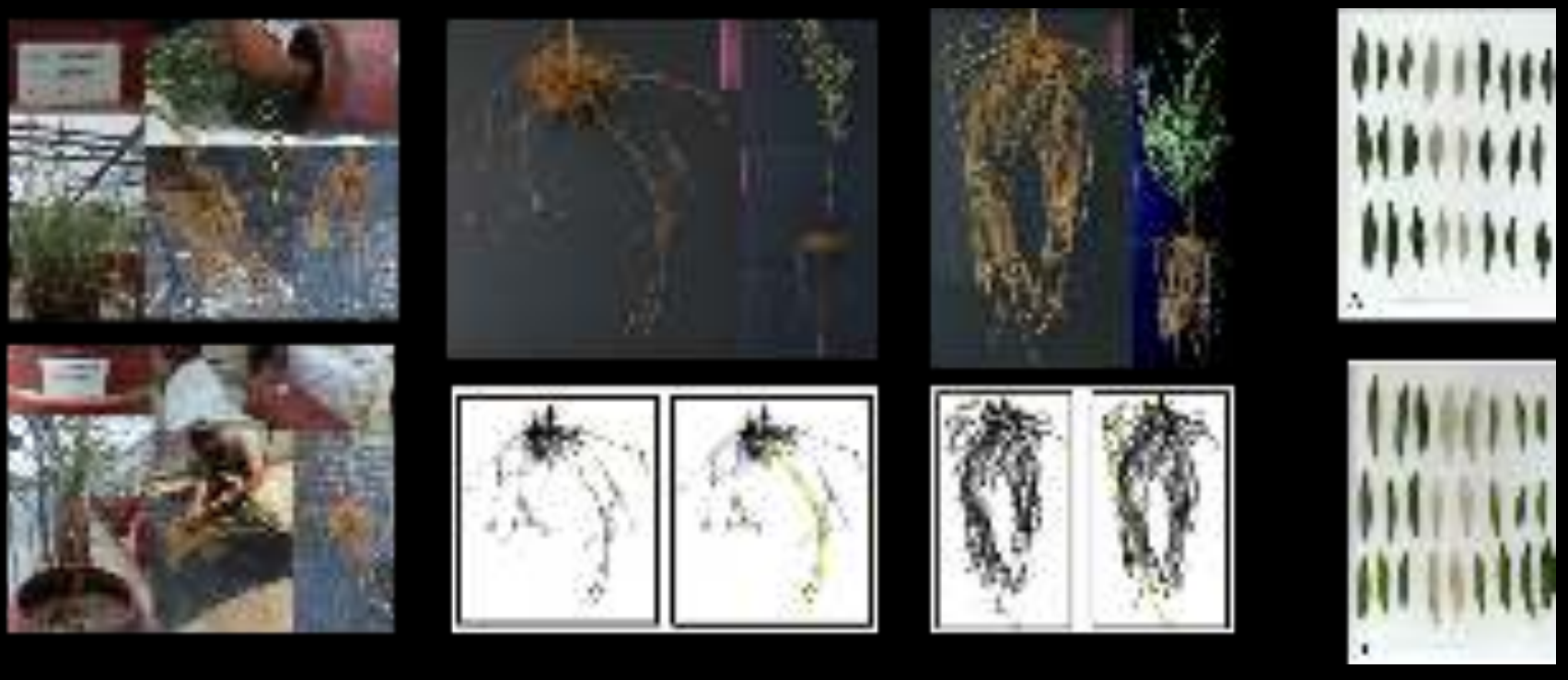
salinity-90DAYS

Experiments MAICh-Effect of different climate conditions



Experiments MAICh-Effect of different climate conditions

- Development of the rooting system in the barrels before the initiation of the salinity treatment.



Conclusion-Work in progress

- The morphological and molecular characterizations (elaiographic cards) are efficient for olive germplasm management, including the characterisation of varieties and the establishment of relationships between cultivars.
- We will construct a new international data base that can be used to make a reference collection of olive germplasm by comparing the morphological and molecular pattern of each identified variety.
- Extension into three-dimensional setting.
- Development application for smartphones and tablets.

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- 2nd APPN & EPPN2020 Meeting featuring Root Phenotyping Technologies & Hands-on Workshop on Hyperspectral Root Phenotyping.