

Advanced mathematical algorithms to characterize olive varieties through morphological parameters

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Variability of olives



Use of morphological parameters

- ✓ Morphological characters have been widely used for descriptive purposes and are commonly used to distinguish olive cultivars.
- ✓ The systematic utilization of descriptive morphological characters of the tree and various tree organs has enabled the characterization and discriminatory identification of varieties.
- ✓ Morphological characteristics are sometime correlated or associated with disease susceptibility and can be used as markers in breeding.
- ✓ Some morphological characters are particularly useful since they enable discrimination between morphologically different cultivars.
- ✓ These discriminatory characters are very heritable and are little influenced by the environment.

Motivation of this work

It is of great importance to evaluate and characterize the phenotypic diversity of the crop species.

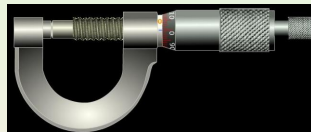
Among them **olive** represents an important case due to its **rich patrimony of varieties** and **wild plants**.

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



- The morphological analysis of olive leaves, fruits and stones may represent an efficient tool for the characterization and discrimination of varieties and the establishment of relationships among them.
- In recent years, much attention has been focused on the comparison of morphological characteristics of the main olive cultivars. Despite this, 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) or other methods not suitable for the handling problem, due to the imposition of some prerequisites (e.g. color of the images background, position of the object, etc.)



Presenting a **semi-automatic methodology** of **detecting various morphological parameters from imaging data**. Applying intuitive mathematical descriptors many fruit, leaf and endocarp features may be quantified.

GOAL OF THIS TALK:

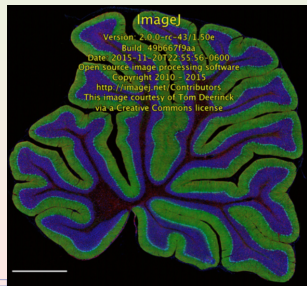
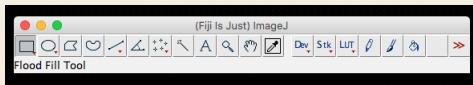


In general, our methodology includes five main steps:

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

Segmentation Step

- The process during which the objects in an image are separated from the background is known as **segmentation**.
- For the segmentation of the images we are using **ImageJ**.
- **ImageJ** is an open scientific image analysis software.
- **Segmentation** is **one of the most important steps**, because from here it will result a **binary image** (black & white) and then we will have the mathematical representation of each shape.



Segmentation Step

- The most commonly used methods for cell segmentation include thresholding, template matching and deformable models among others.
- Here we are using color thresholding techniques, by modifying **manually** the values of "Hue", "Saturation" and "Brightness" of each image.

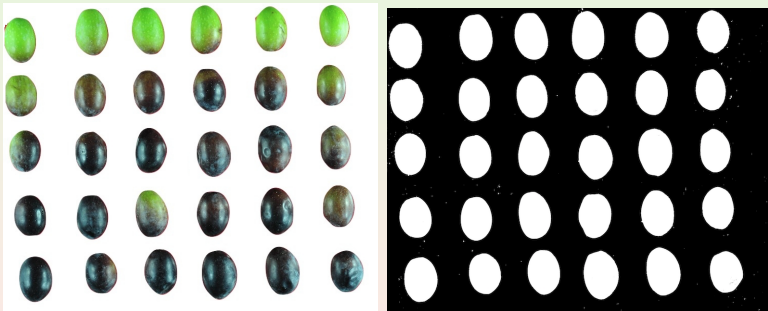
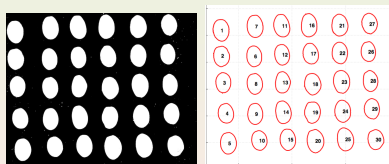


Figure: Raw and Thresholded image from "LECCIO-DEL-CORNO" from the Collection of Olive Germplasm of Marrakech (Morocco).

Morphological Analysis

- Using the Image Processing Toolbox from **MATLAB (R2011b)** we created an automatic algorithm for object contour extraction from the binary images (left figure).
- 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 (right figure).



- This mathematical representation of the experimental data enables to analyze **quantitatively** and **qualitatively** the morphology of the olives, leaves and endocarps of each cultivar.

Characters of the fruit

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

- Most of the studies on the morphological analysis of the fruits consider two positions for the description of its shape. Position A is where the fruit displays the greatest asymmetry and position B is reached by turning 90° from position A.
- **Our approach:** We select the position of the fruit in such a way that the apex to be always higher than the base.



Characters of the fruit

The following parameters have been taken into account:

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

The characters which are in bold are useful for cultivar discrimination

Characters of the fruit

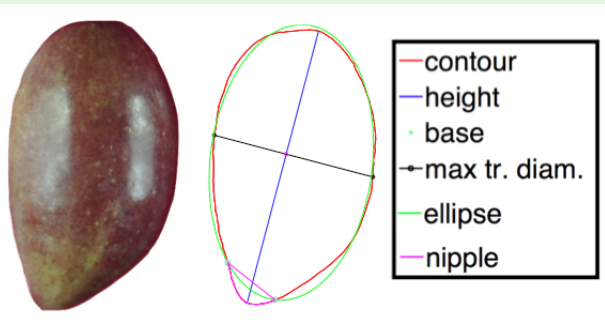


Figure: "CORNICABRA" from the Collection of Olive Germplasm of Marrakech (Morocco).

- **Ellipse:** is the best fit of an ellipse for the given set of points.
- **Nipple:** is the lower part that it is defined between the ellipse and the shape boundary.

- **Contour** is a closed polygonal line, consisting of a set of points.
- **Height:** the distance between the two far apart points.
- **Maximum transverse diameter:** the biggest segment which is perpendicular to the height.

Characters of the fruit

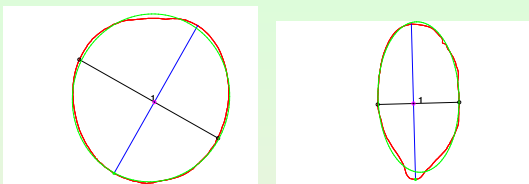


Figure: Shape index. Left: spherical (TOFFAHI) and Right: elongated (MASTOIDIS) fruits.

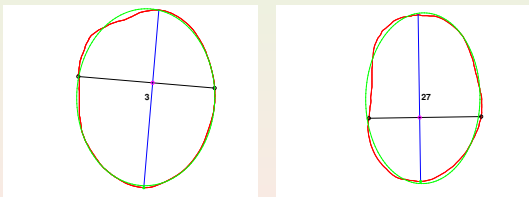


Figure: Position of the maximum transverse diameter. Left: towards the apex (BALADI-ROUMANI) and Right: towards the base (R-KHAMI).

Characters of the fruit

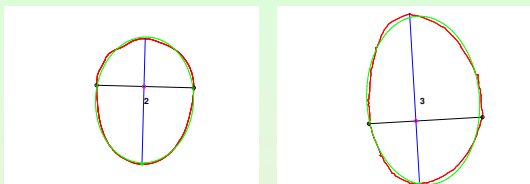


Figure: **Vertical symmetry**. Left: Higher value (GORDAL-SEVILLANA) and Right: lower value (PENDOLINO).

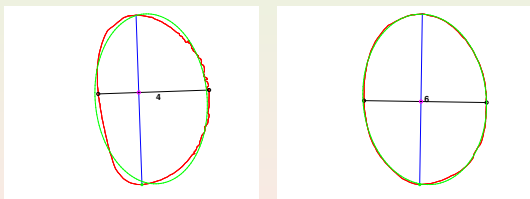


Figure: **Transversal symmetry**. Left: Asymmetric fruit (NOCIARA) and Right: symmetric (LECCIO-DEL-CORNO).

Characters of the leaf

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, height, maximum transversal diameter (width).
- Vertical and transversal symmetry.
- Major and minor axis of a fitted ellipse, **shape index**.
- Height, area of the petiole.



Characters of the leaf

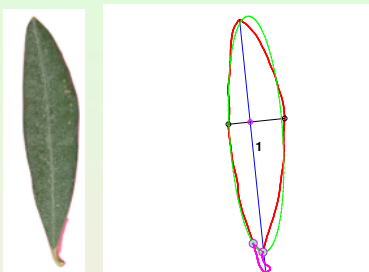


Figure: "NOCIARA" leaf from the Collection of Olive Germplasm of Marrakech (Morocco).

Characters of the leaf

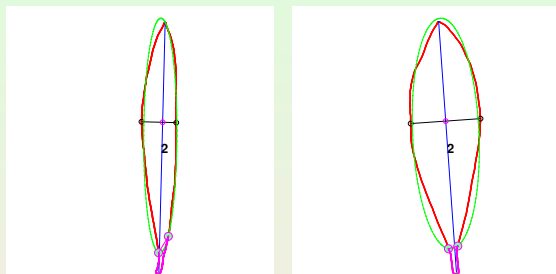


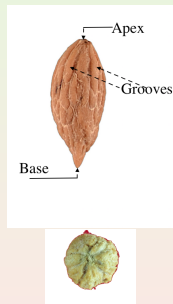
Figure: Maximum transverse diameter. Left: Narrow leaf (CAROLEA) and Right: Broad leaf (OTTOBRATICA)

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 considered 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.**



Characters of the endocarp



Figure: **Number of grooves** (manually). Left: low number (MORAIOLO) and Right: high number (SANT'AGOSTINO).

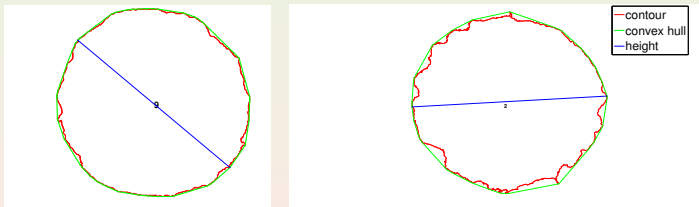


Figure: **Contour roughness**. Left: Smooth surface (MORAIOLO) and Right: scabrous (SANT'AGOSTINO).

Preliminary results from the Collection of Olive Germplasm of Marrakech (Morocco).

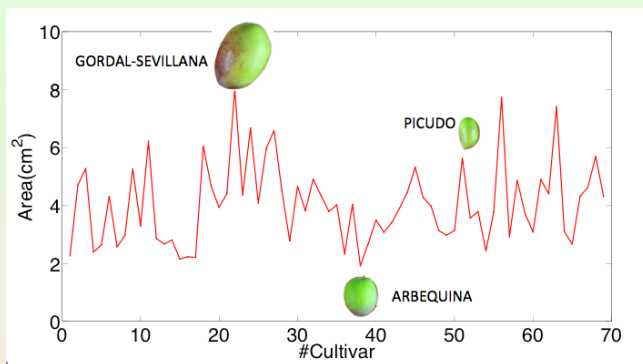


Figure: Plot with the average area of the examined samples from the Collection of Olive Germplasm of Marrakech (Morocco). Three cultivars are reported.

Preliminary results from the Collection of Olive Germplasm of Marrakech (Morocco).

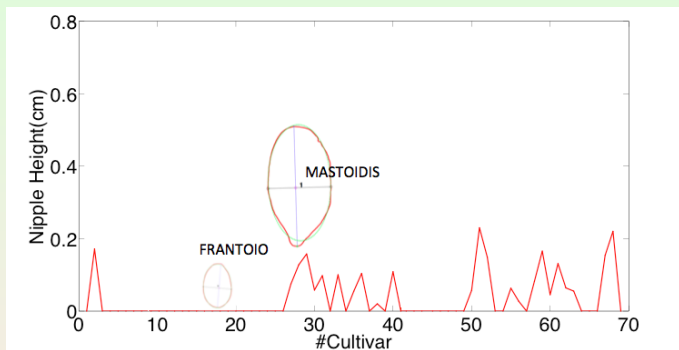


Figure: Plot with the average “nipple height” of the examined samples from the Collection of Olive Germplasm of Marrakech (Morocco). Two cultivars are reported-Zero corresponds to the absence of nipple.

- 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 varieties.
- A key area for future work is to investigate improvements in the efficiency of the algorithms.

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