# Seed germination protocol & morphocolorimetric analysis of *Ferula melitensis, Anagyris foetida, Coronilla valentina* and *Euphorbia melitensis.*


University of Malta (PP2)

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# 1. Introduction to the plant material

- 4 species of interest: 2 strict endemic species & 2 indigenous species of Fabaceae:
  - *Ferula melitensis* (Brullo et al., 2018) (Apiaceae).
  - *Euphorbia melitensis* (Parlatore, 1867) (Euphorbiaceae).
  - *Anagyris foetida* (L. 1753).
  - *Coronilla valentina* (L. 1753).
- Purpose of germination & morphocolorimetric protocols:
  - Ecological restoration purpose.
  - Seed quality testing.
  - Accurate seed characterisation.

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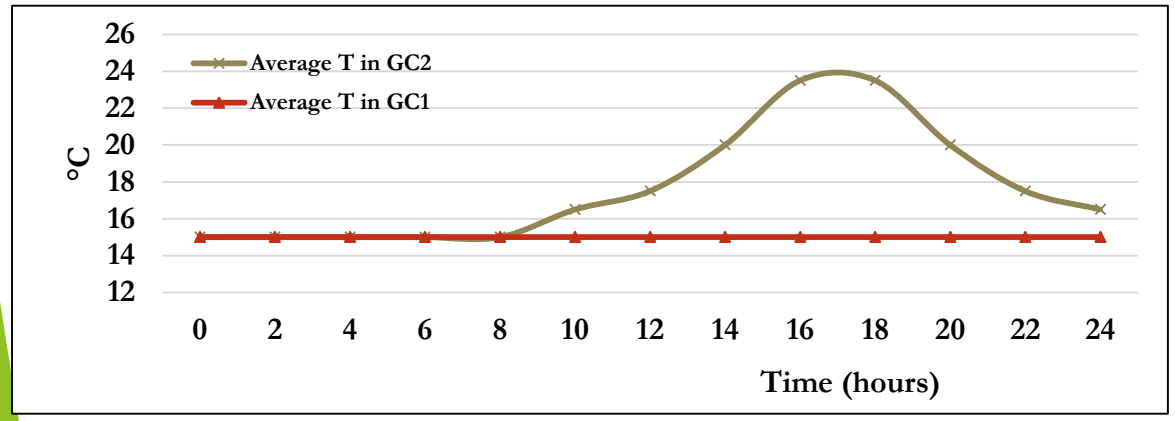
# 2. Material and Method

## 2.1 Germination trial for protocol definition.

- Standardised sowing methodology:
  - 90mm Petri Dish, 25 seeds/replicates, 5ml distilled water, 2 layers of filter paper.
- Light, temperature and hydropriming variables explored:
- Standardised data record for time series analysis:

| Variables | Values |
|---|---|
| Photoperiod (h) | 0, 12/12, 18/6, 16/8 bi-hourly increment cycle. |
| Temperature (°C) | 4°C,15°C,20°C, 25°C, 15-23.5°C increment cycle. |
| Hydropriming time (h) | 0, 24, 48, 72, 96h. |
| Hydropriming Temperature(°C) | 80°C=>25°C (RT), 40°C constant. |
| Substrate | Sterile water, Compost & Perlite 2:1. |




| Species | Collection site | Collection date | Hydropriming T° | Sowing date | Seed | Replicate | BATCH | Saoking time (h) | Germination day | Day to germination |
|---|---|---|---|---|---|---|---|---|---|---|
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 1 | 1 | 1 | 24 | 20/08/2021 | 11 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 2 | 1 | 1 | 24 | 20/08/2021 | 11 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 3 | 1 | 1 | 24 | 23/08/2021 | 14 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 4 | 1 | 1 | 24 | 23/08/2021 | 14 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 5 | 1 | 1 | 24 | 23/08/2021 | 14 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 6 | 1 | 1 | 24 | 25/08/2021 | 16 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 7 | 1 | 1 | 24 | 25/08/2021 | 16 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 8 | 1 | 1 | 24 | 27/08/2021 | 18 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 9 | 1 | 1 | 24 | 27/08/2021 | 18 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 10 | 1 | 1 | 24 | 30/08/2021 | 21 |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 11 | 1 | 1 | 24 | | NG |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 12 | 1 | 1 | 24 | | NG |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 13 | 1 | 1 | 24 | | NG |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 14 | 1 | 1 | 24 | | NG |
| Coronilla valentina | Wied Babu | 04/06/2021 | 75_24 | 09/08/2021 | 15 | 1 | 1 | 24 | | NG |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# 2.2 Morphocolorimetric study using Image Analysis.

- Image Analysis using standardised, macro based methodology.
- (ImageJ/Fiji (Abramoff et al. 2004) with Particles8 plugin (Landini, 2008).
- Seeds morphometric and colorimetric parameters

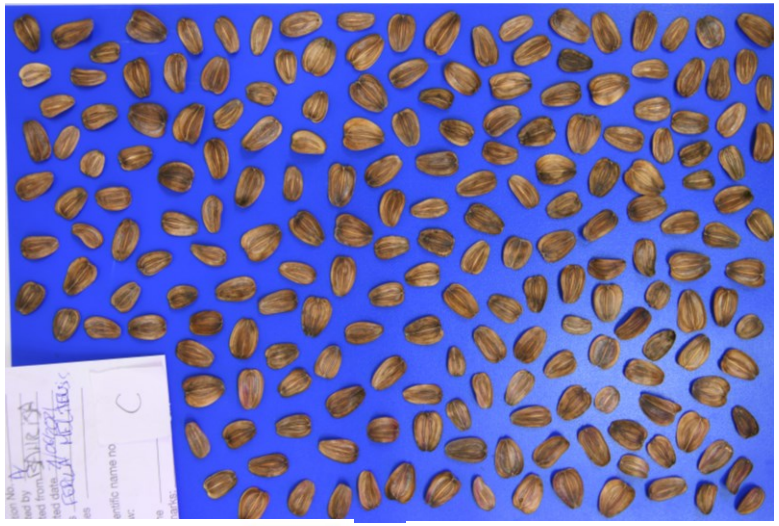

Sample preparation

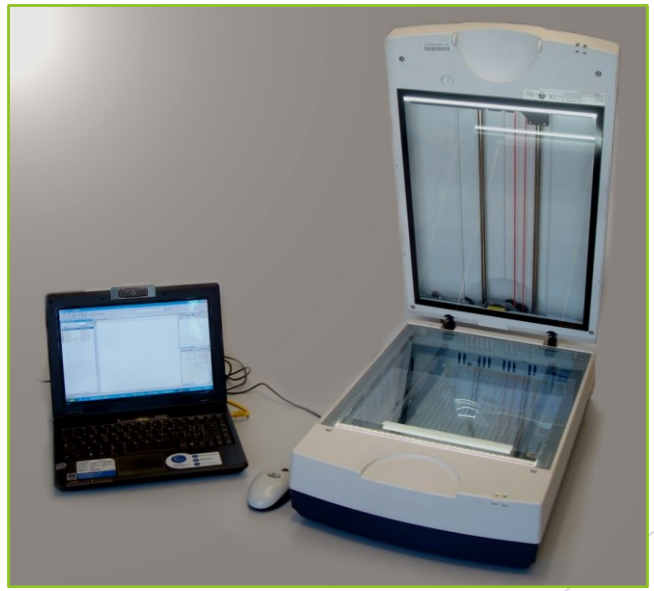

Image aquisition


Data collection


Data analyses and statistics

- Adapted from published and unpublished methodology by Prof. Gianluigi Bacchetta *et al.*





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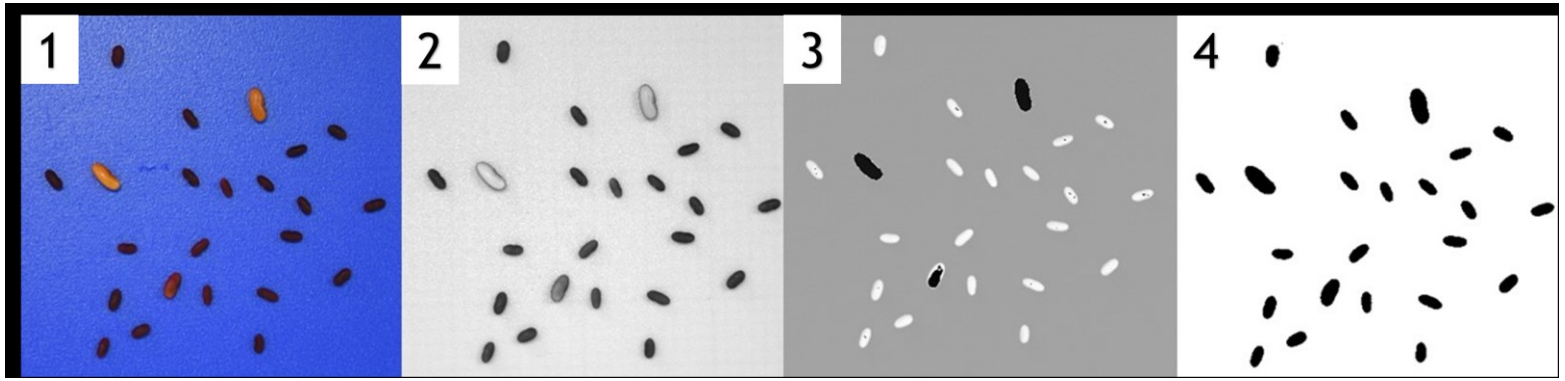
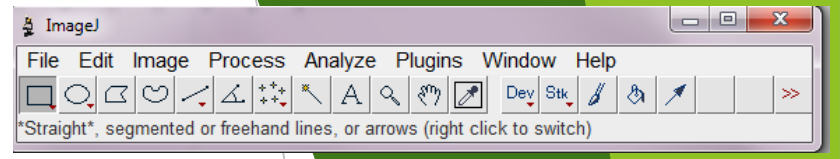
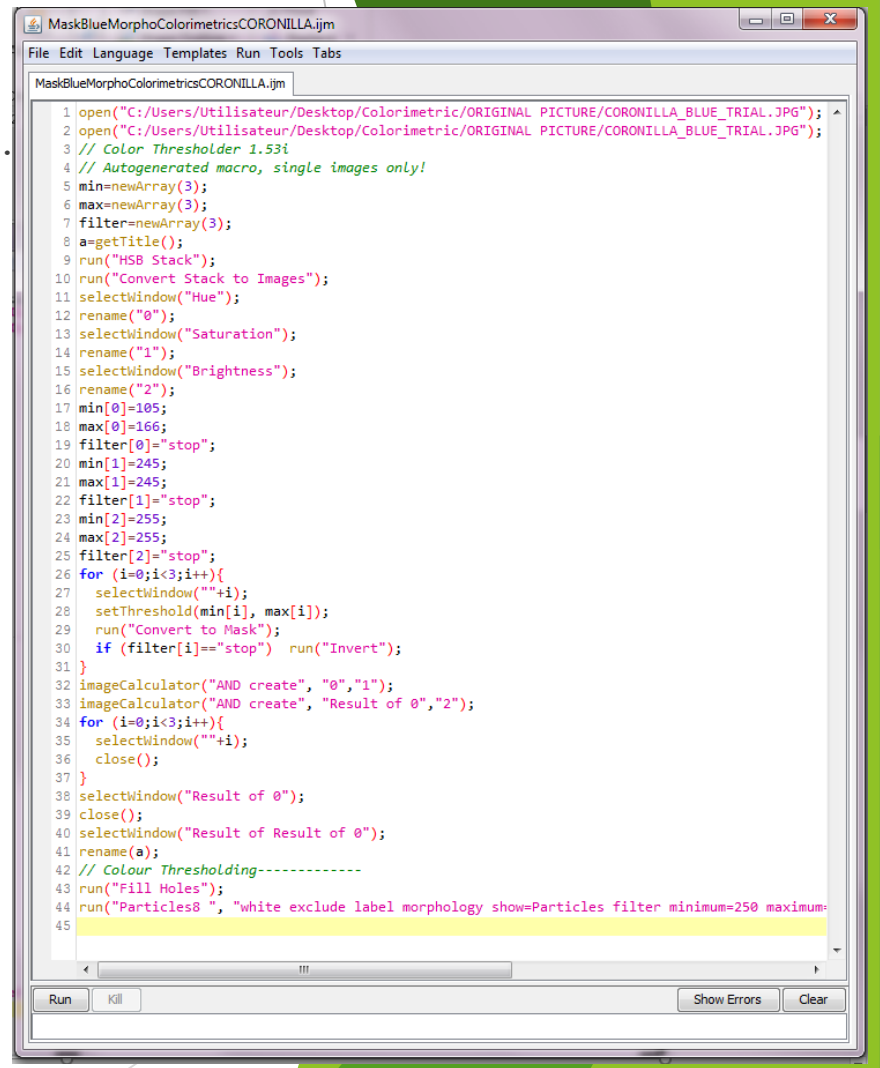
# 2. Material and Method

## 2.2 Morphocolorimetric study using Image Analysis.

- Use of ImageJ/Fiji (Abramoff et al., 2004) with Particles8 plugin (Landini, 2008).
- Standardised, macro based methodology:
  - Image decomposition using ImageJ using species specific parameters
  - Original (1) Brightness (2) Hue (3) Mask (4).
- Image analysis using Particles8:
  - Large output: 63 variables for each single seed.
  - 36 Colorimetric variables: Red/Green/Blue pixel density, count etc…
  - 27 Morphometric variables: Measures, surfaces ratios and proportions.

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# 3.1 Seed Germination of *Ferula melitensis*

- Optimum germination conditions identified … in nursery pots:
  - Fluctuating temperature 15 to 23.5°C, no light, fluctuating Relative Humidity from 50 to 85%.
  - Average germination: 61%, mean time to Emergence: 41 days.
- Replication in Petri dish gave mixed results due to contamination patterns:
  - Biased MGT due to possible symbiotic organism: Small sample size.



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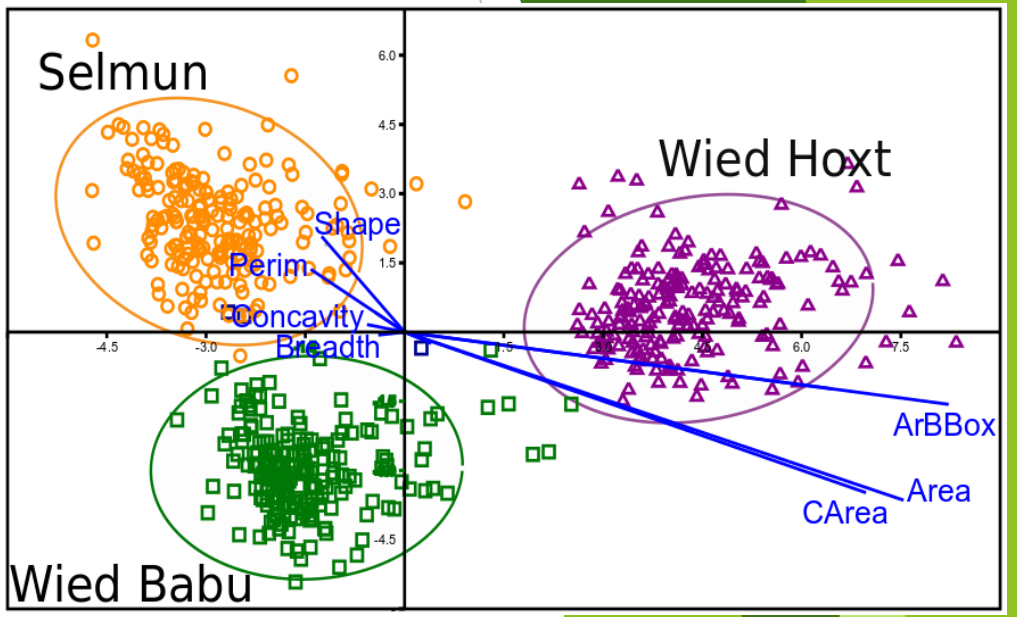
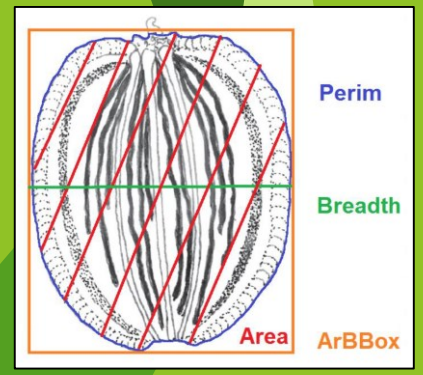
# 3.1 Morpho colorimetric analysis of *Ferula melitensis*

- Detailed morphometric characterisation of the mericarps of *Ferula melitensis*, complement of recently established it as a distinct species from the more widespread *Ferula communis*.
- Multivariate analysis of the morphometric variables (LDA) of 3 wild populations of *Ferula melitensis* displays important phenotypic variability at small geographical scale.
- Further work in progress, including colorimetrics, 15 more accessions including 3 *Ferula communis* from southern Sicily.





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## 3.2 Seed Germination protocol & Morphocolorimetric analysis of *Anagyris foetida*

- Preliminary analysis showed challenges:
  - Seed colour polymorphism biased the image analysis.
  - Low seed germination % without pre-treatment.
- Significant increase in G% and MGT after 24h hydropriming.
- Ongoing research on characterisation of seed maturity of *Anagyris foetida* for seed quality purposes.

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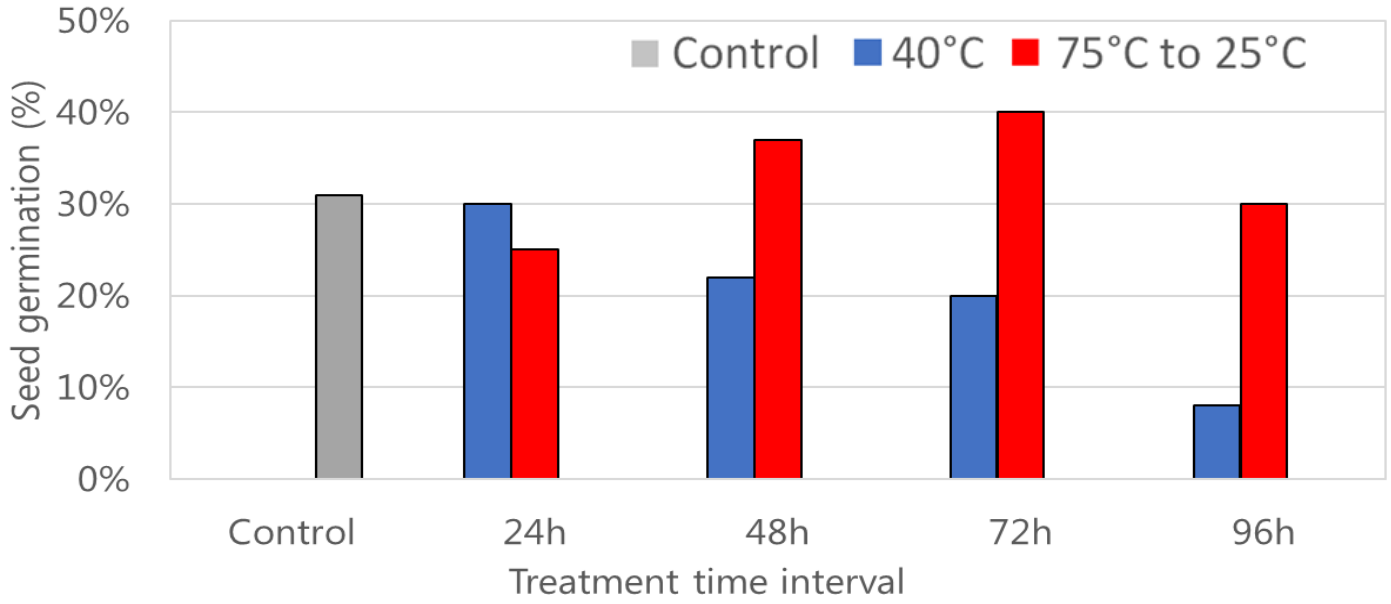
## 3.3 Seed Germination protocol of *Coronilla valentina*

- Identification of *C.valentina* subsp. *glauca* according to the flora of Pignatti.
- Testing the effect of hydropriming conditions on germination of *Coronilla valentina*.
- Optimum hydropriming treatment at 72h with the 75°C to 25°C (Room Temperature).




| Conditions (unit) | Value |
|---|---|
| Hydration Time (hours) | 0 |
| | 24 |
| | 48 |
| | 72 |
| | 96 |
| Temperature (°C) | 40°C constant |
| | 75°C, then left to cool to 25°C |

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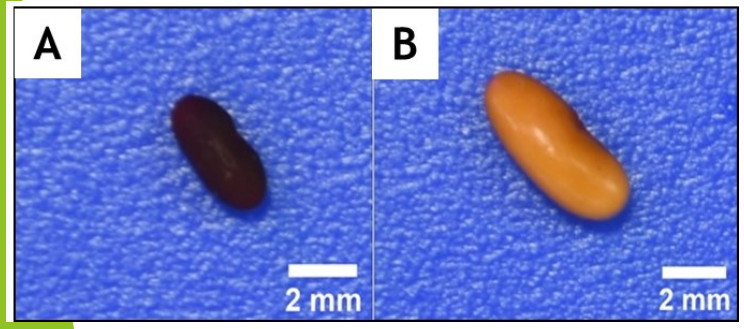
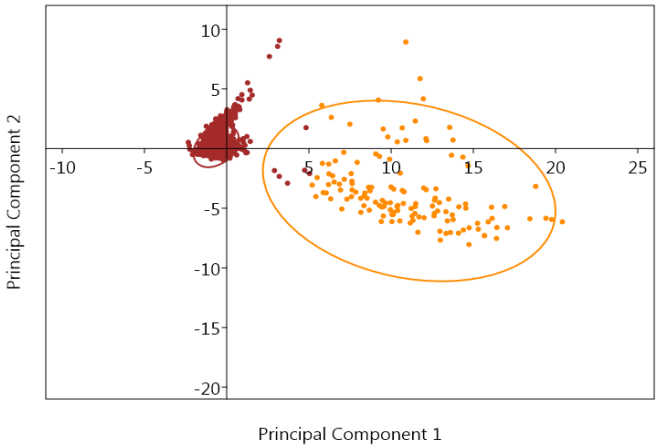
# 3.3 Seed Germination protocol & Morphocolorimetric analysis of *Coronilla valentina*

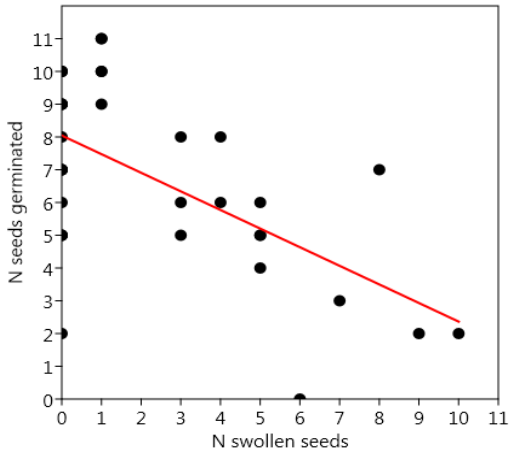
- First detailed morphocolorimetric characterisation of seeds (N=500) of *C.valentina* subsp. *glauca*.

| Variables | Measures | | | | Shape index | | | | | | | Colorimetrics parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Perim | Area | Feret | Breadth | Circ | Roundness | Compactness | Solidity | Concavity | Convexity | Shape | RedAverage | GreenAverage | BlueAverage |
| unit | mm | mm² | mm | mm | N/A | N/A | N/A | N/A | N/A | N/A | N/A | None | None | None |
| Min | 7.28 | 2.87 | 2.55 | 1.16 | 0.42 | 0.37 | 0.61 | 0.93 | 160.50 | 0.74 | 16.24 | 41.63 | 28.76 | 45.15 |
| Max | 11.12 | 5.47 | 3.75 | 2.06 | 0.77 | 0.70 | 0.84 | 0.99 | 807.00 | 0.95 | 30.20 | 93.89 | 52.82 | 69.64 |
| Mean | 8.87 | 4.09 | 3.27 | 1.57 | 0.65 | 0.49 | 0.70 | 0.98 | 321.25 | 0.90 | 19.34 | 55.60 | 37.19 | 57.39 |
| Stand. dev | 0.56 | 0.44 | 0.20 | 0.13 | 0.05 | 0.05 | 0.03 | 0.01 | 83.31 | 0.03 | 1.78 | 7.92 | 2.50 | 4.04 |

- Testing the effect of hydropriming conditions on germination of *Coronilla valentina*.
- Characterisation of the swelling (A and B) of the seeds caused by hydropriming.
- Hypothesis testing: Correlation between swelling and germination success.
- H1 accepted: Kendall's τ shows a negative correlation between count of swollen seeds and germinated seeds ($r(34) = -.347$ , $p < .05$ ).



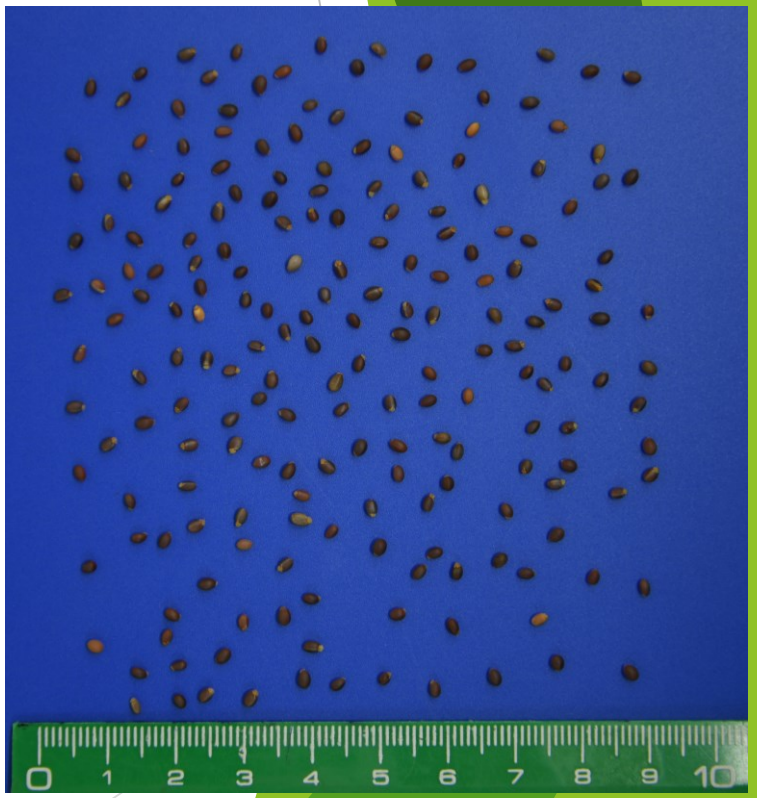



# 3.4 Seed morphocolorimetric analysis of *Euphorbia melitensis*

- First detailed morphocolorimetric characterisation of seeds of *Euphorbia melitensis*.
- Part of a student project with Ms Courtney Farrugia.

| Variables | Measures | | | | Shape index | | | | | | | Colorimetrics parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Perim | Area | Feret | Breadth | Circ | Roundnes | Compactness | Solidity | Concavity | Convexity | Shape | RedAverage | GreenAverage | BlueAverage |
| Unit | mm | mm² | mm | mm | N/A | N/A | N/A | N/A | N/A | N/A | N/A | None | None | None |
| Min. | 6.18 | 2.35 | 2.12 | 1.37 | 0.56 | 0.50 | 0.71 | 0.94 | 89.50 | 0.80 | 15.23 | 46.05 | 33.99 | 33.16 |
| Max | 8.81 | 4.37 | 3.15 | 1.98 | 0.83 | 0.75 | 0.87 | 0.99 | 363.50 | 0.94 | 22.56 | 139.44 | 102.17 | 80.13 |
| Average | 7.37 | 3.16 | 2.54 | 1.66 | 0.73 | 0.62 | 0.79 | 0.98 | 156.65 | 0.90 | 17.26 | 69.11 | 51.26 | 45.63 |
| Stand. dev. | 0.49 | 0.39 | 0.18 | 0.13 | 0.05 | 0.05 | 0.03 | 0.01 | 38.63 | 0.02 | 1.22 | 15.61 | 12.22 | 7.30 |





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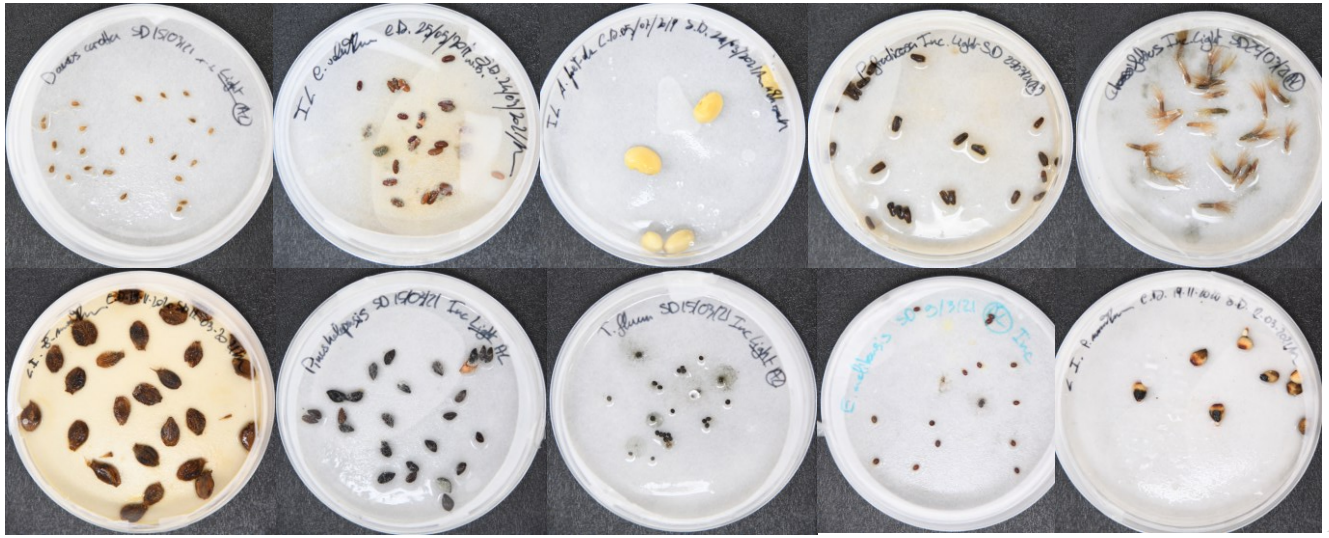
## 3.5 Further results…

| Plant species tested | | |
|---|---|---|
| *Tetraclinis articulata* | *Euphorbia melitensis* | *Teucrium flavum* |
| *Eryngium maritimum* | *Euphorbia dendroides* | *Phlomis fruticosa* |
| *Pancratium maritimum* | *Pinus halepensis* | *Ceratonia siliqua* |
| *Prasium majus* | *Thymbra capitata* | *Laurus nobilis* |
| *Coronilla valentina* | *Drimia marittima* | *Ferula melitensis* |
| *Fagonia cretica* | *Periploca laevigata subsp. angustifolia* | *Anagyris foetida* |
| *Quercus ilex* | *Cheirolophus crassifolius* | *Achantus mollis* |





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# 4. Discussion & Output

- Purpose of germination study:
  - Definition of germination protocol for plant propagation in nursery.
  - Development of seed quality testing.
  - Improvement of seed ecology and physiology.
- Purpose of morphocolorimetric study:
  - Taxonomic contribution.
  - Development of seed quality testing.
- Standardised method allows both high accuracy and replicability.
- Consistency across studies and target species.


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# Thanks for your attention!

… any questions ?

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