



Università
degli Studi
di Cagliari



Centro
Conservazione
Biodiversità



Stazione Sperimentale
di Granicoltura
per la Sicilia



REGIONE SICILIANA
Assessorato Risorse
Agricole e Alimentari

The use of image analysis techniques for seed dormancy and germination studies: the case of *Brassica insularis*

Andrea Santo, Efisio Mattana, Oscar Grillo, Gianluigi Bacchetta



Ensuring the survival of endangered plants in the Mediterranean islands

Catania, 18th - 20th April 2013



The Mediterranean climate is characterized by:

- warm to hot dry summers;
- mild to cool wet winters;
- unpredictability of temperatures and precipitations.

Lower germination rate and the requirement of cool temperatures, suggest that field germination of Mediterranean coastal species is tuned to take place well into the rainy season.

This pattern is generally known as “Mediterranean germination syndrome” and such a “delay mechanism” is often considered an advantageous ecological adaptation towards the unpredictable rainfall pattern under the Mediterranean climate.



Brassica insularis germinated seed



Coastal cliffs are characterized by:

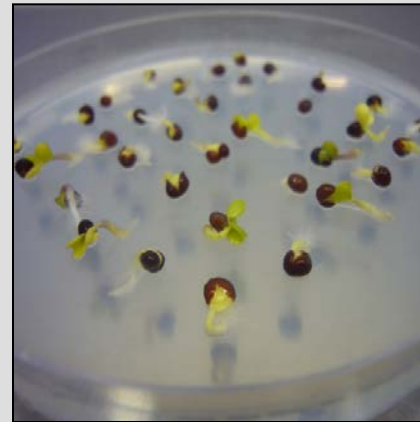
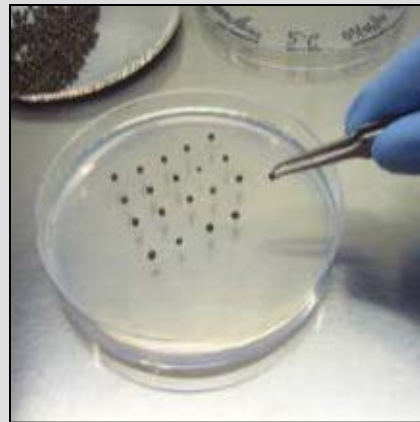
- harsh conditions
- sun exposition
- strong winds
- daily thermal variations
- strong water deficit during dry months

With the high insolation and temperatures typical of summer period, the considerable water quantity present in these habitats tends to become salt crusts on the rocks, reaching salinity values even higher than sea water (ca. 35-37 ‰).



The goals of the work

- to apply image analysis techniques to investigate seed inter-population variability of *Brassica insularis*;
- to characterize seed germination ecology in terms of light and temperature and to evaluate the effect of dry after-ripening on seed germination;
- to evaluate inter-population variability in seed germination.



B. insularis populations



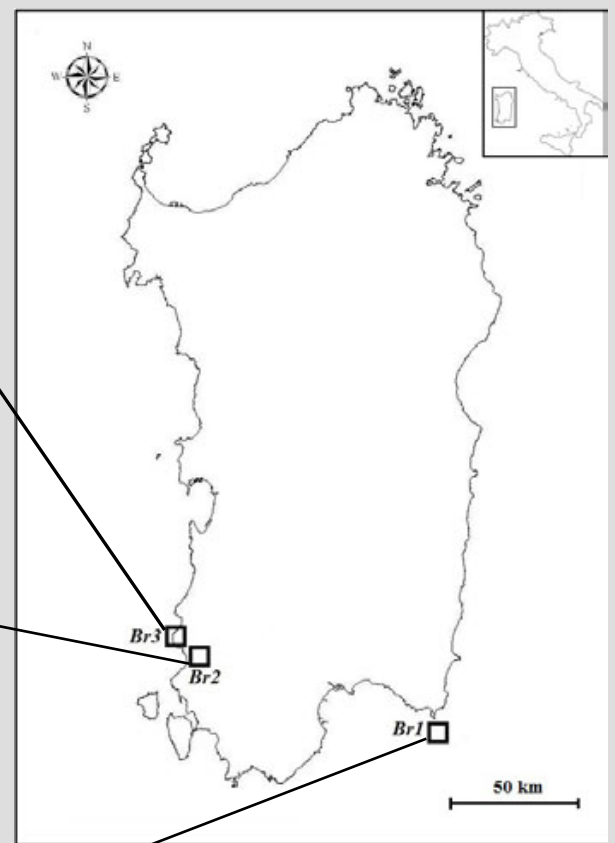
Planu Sartu (CI)



Masùa (CI)



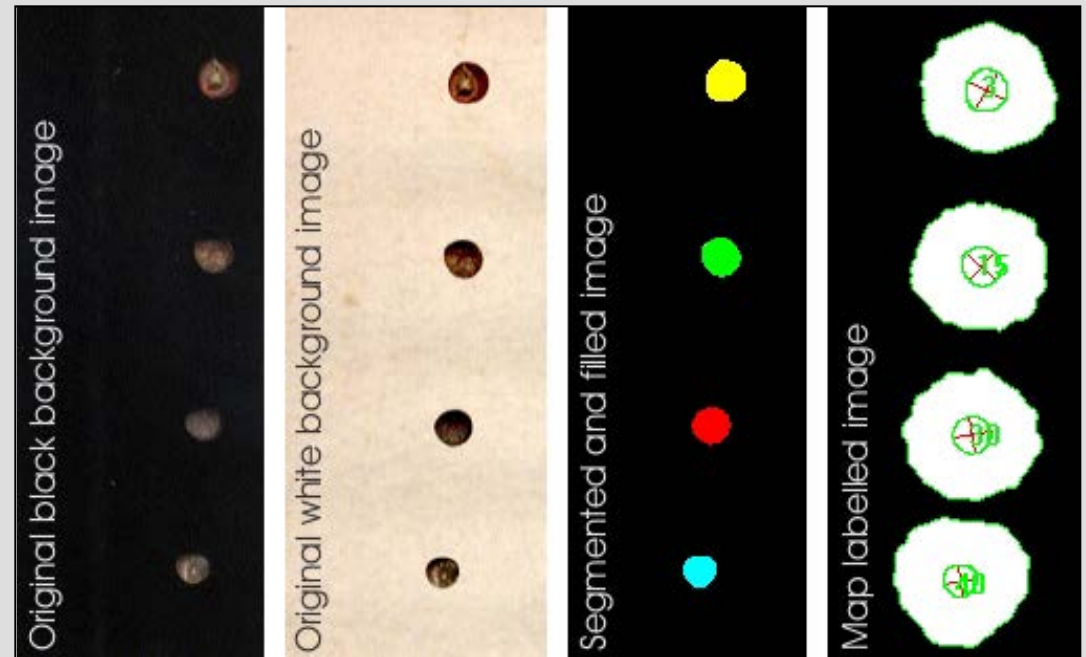
Isola dei Cavoli (CA)



Seed image analysis

Digital images of seed samples were acquired using a flatbed scanner with a digital resolution of 200 dpi on black and white background.

Samples consisting of 100 seeds, randomly disposed on the scanner tray, were acquired and used for the digital image analysis.



A total of 114 morpho-colorimetric quantitative features describing seed size, shape, colour and texture were measured by computer vision.



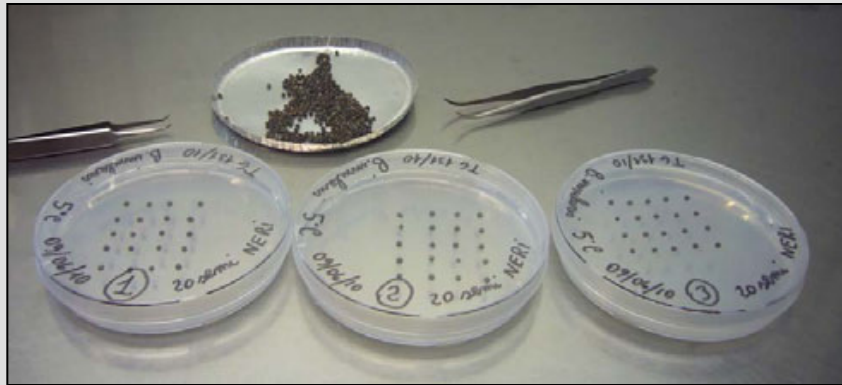
2010 Isola dei Cavoli population seeds



The seed moisture content of each chromatic category was determined by drying at $103 \pm 1^\circ\text{C}$ for 17 hours.

Effect of light

Three replicates of 20 seeds were incubated in a growth chamber in the light (12h of irradiance per day) and in the dark, at 15°C.



Effect of temperature

Three replicates of 20 seeds were incubated in a range of constant temperatures (5, 10, 15, 20 and 25°C) and at an alternating temperature regime (25/10°C) in the light (12h of irradiance per day) in growth chambers.

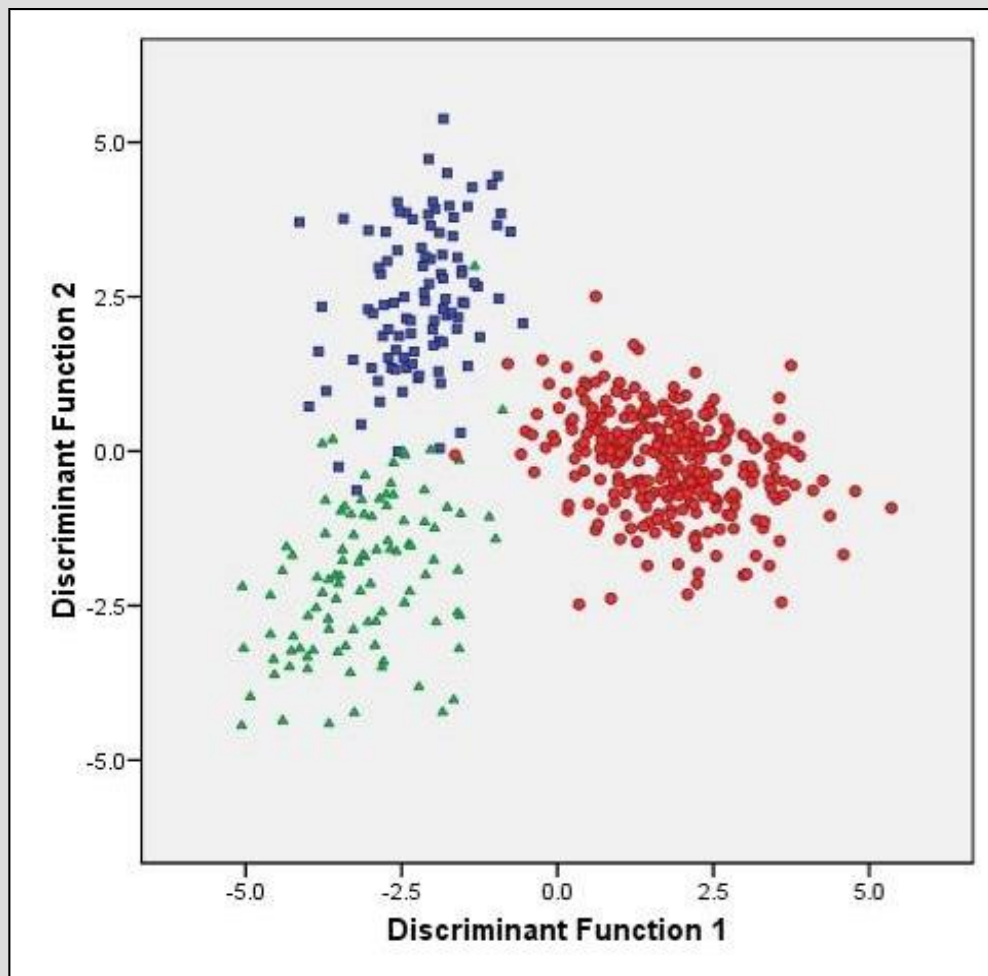


Effect of dry after-ripening

The advancement of seeds drying (15°C and 15% R.H.) was monitored by measuring the activity water (a_w), until reaching $a_w=0.180$.



The jars were incubated at 25°C. After three months, seeds were sown in Petri dishes in the light to the above specified germination conditions.



● Isola dei Cavoli	98.7% of c.i.
▲ Masua	97.0% of c.i.
■ Planu Sartu	95.0% of c.i.
Overall	97.6% of c.i.

The morpho-colorimetric analysis revealed high inter-population variability, showing an high morphological seed differentiation among populations.

This pattern is probably due of the presence of some ecologic barriers, which may obstacle inter-population gene flow.

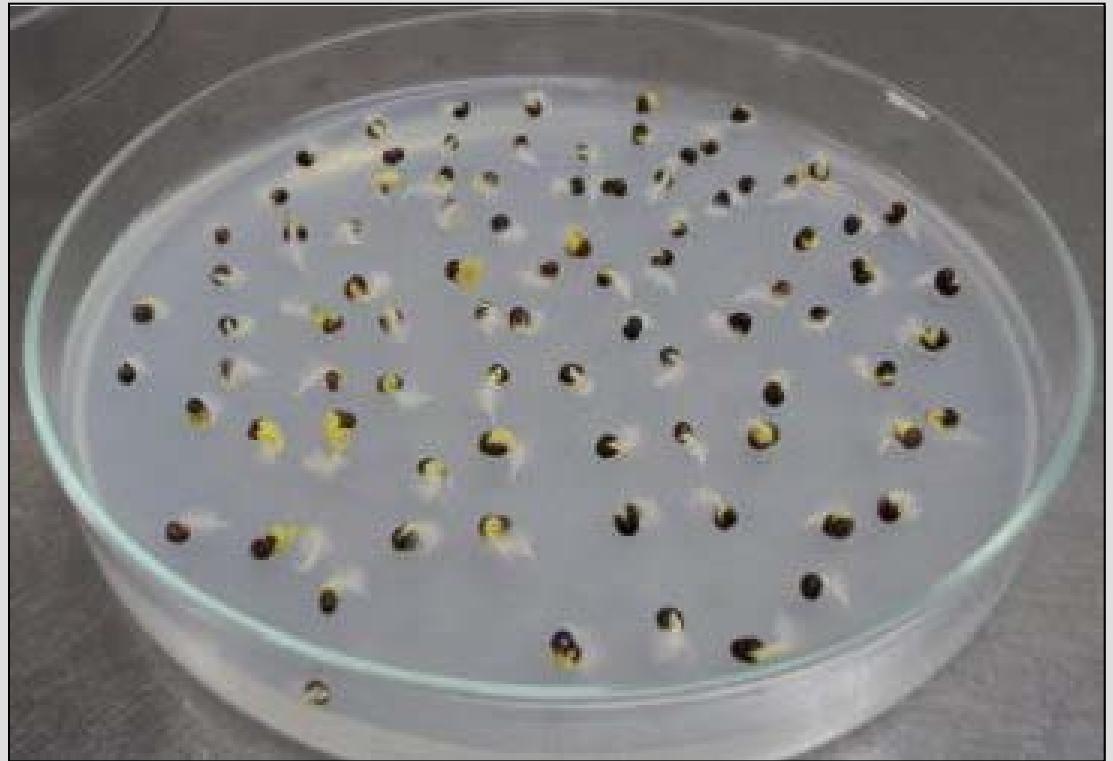
Effect of light

The one-way ANOVA showed the indifference of light on seed germination ($p > 0.05$) at 15°C.

Germination percentages:

Light-incubated seeds
 $35.0 \pm 13.2 \%$

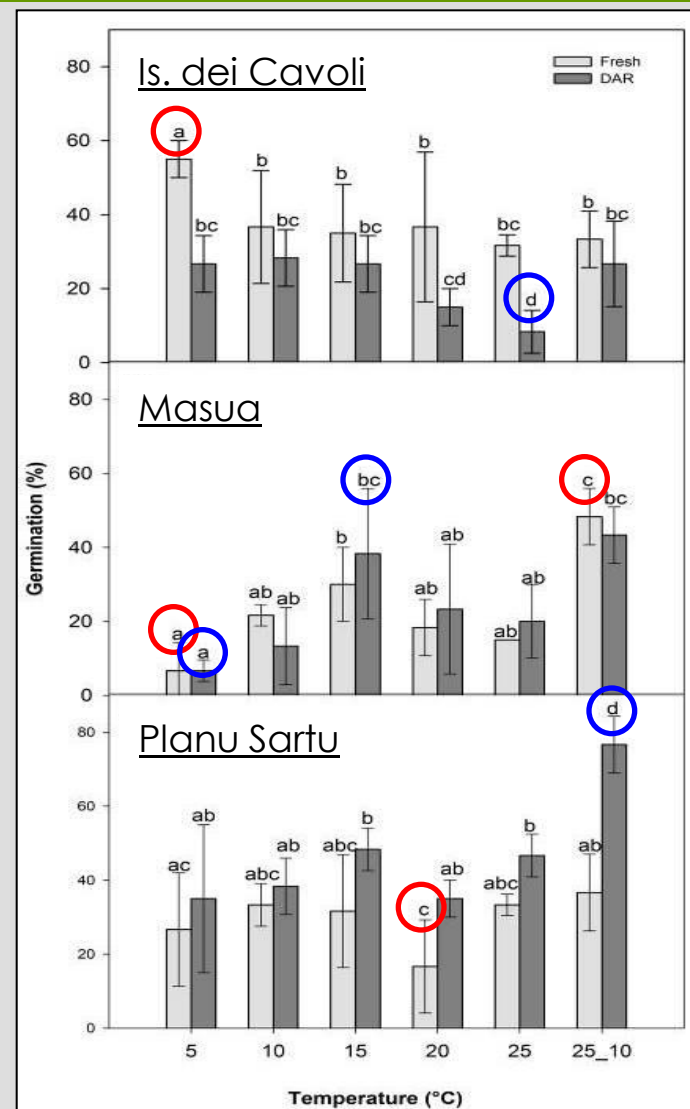
Dark-incubated seeds
 $21.7 \pm 5.8 \%$

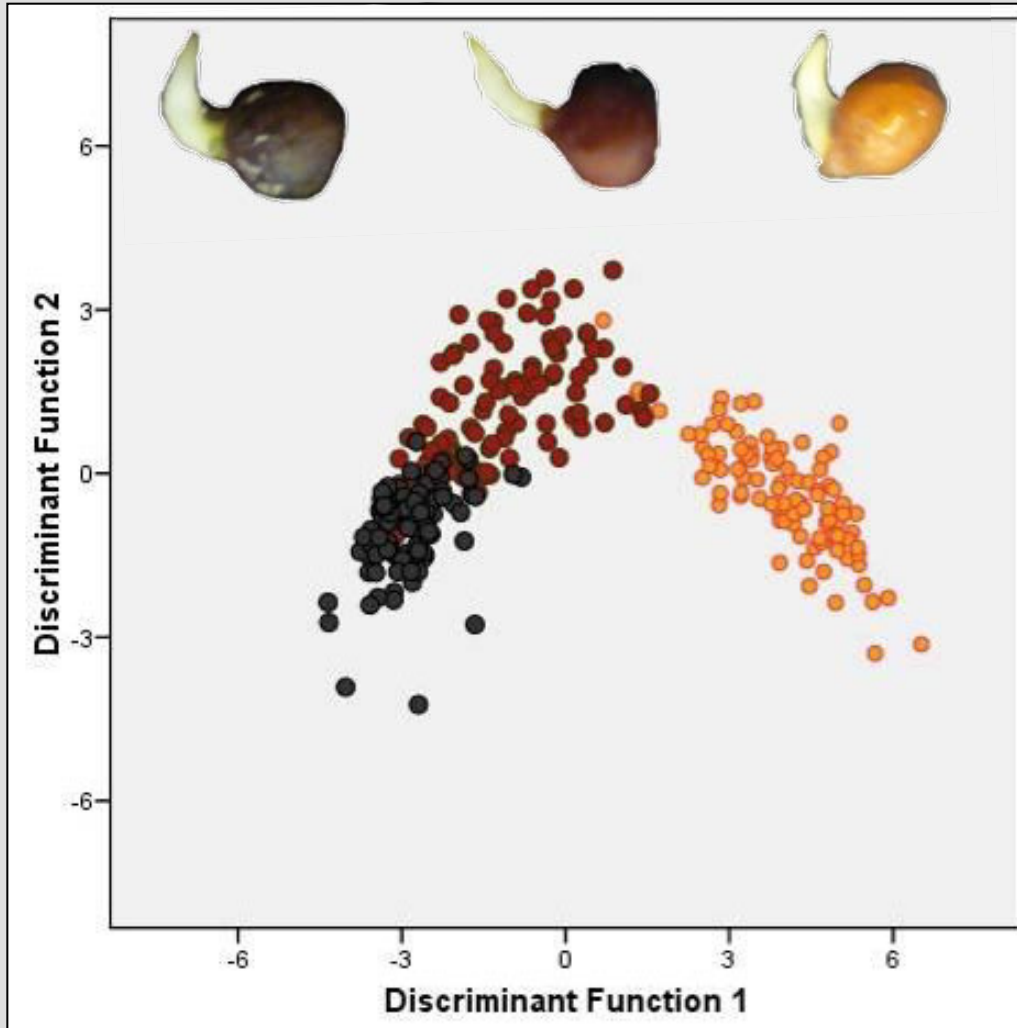


Effect of temperature and dry after-ripening

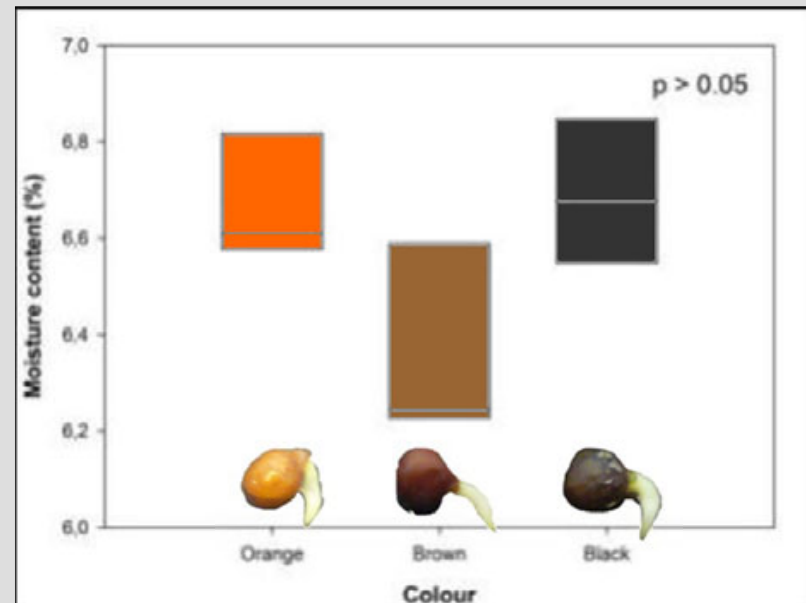
Three way ANOVA

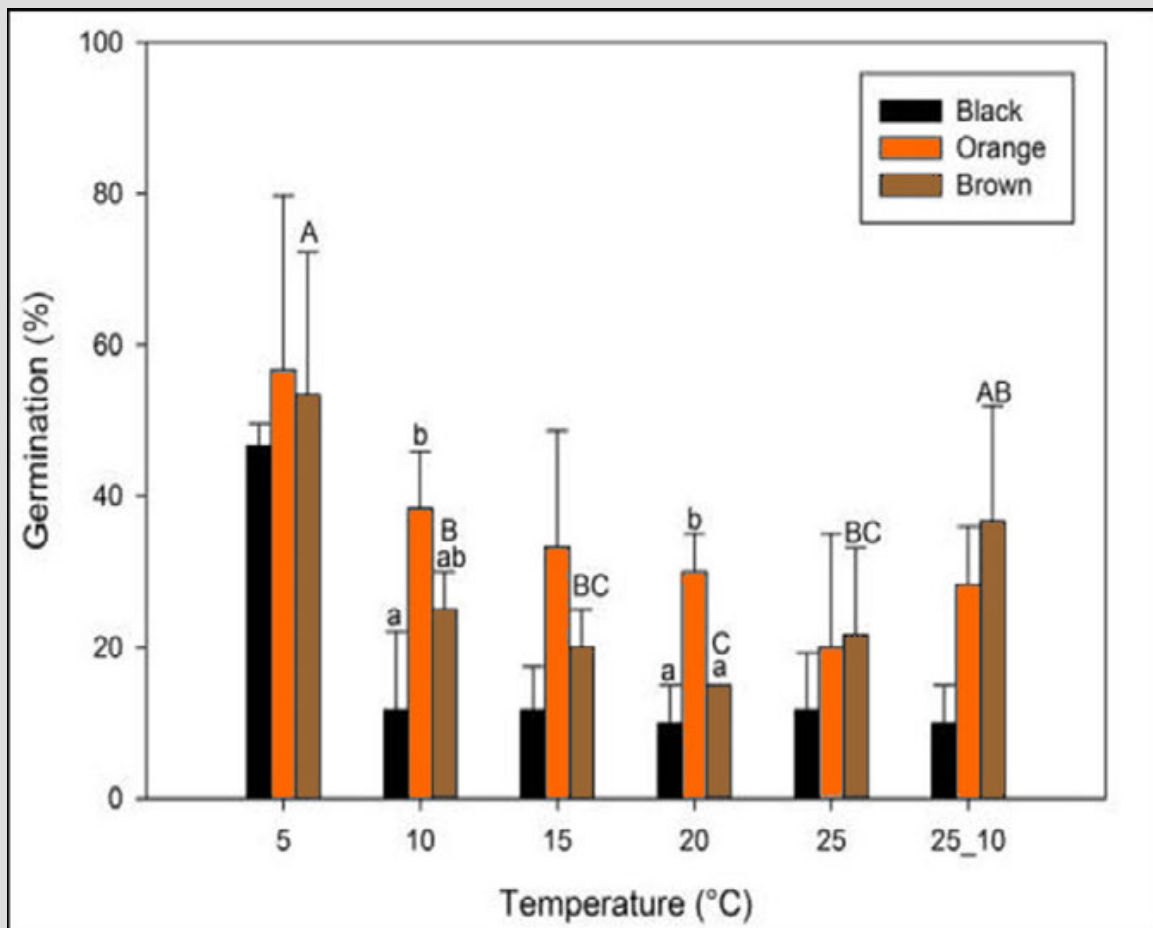
Temperature (T)	highly sig.	($p < 0.001$)
Pretreatment (PT)	non sig.	($p > 0.05$)
Population (Po)	highly sig.	($p < 0.001$)
Po x PT	highly sig.	($p < 0.001$)
Po x T	highly sig.	($p > 0.001$)
PT x T	non sig.	($p > 0.05$)
Po x PT x T	non sig.	($p > 0.05$)





- Black seeds 95.0% of c.i.
- Brown seeds 80.0% of c.i.
- Orange seeds 95.0% of c.i.
- Overall 90.0% of c.i.





The maturation stage of seeds may influence germination behavior. Germination requirements and percentages of immature seeds may be different from those of mature seeds of the same species.

Light did not affect germination percentages highlighting that seeds were not photo-inhibited for germination, contrary to other Mediterranean coastal species.

Brassica insularis differed from other “typical” Mediterranean plants, for which germination at low temperatures is a widely extended trait, demonstrating that germination of this species may occur in a wide time window during the year, as highlighted by Thanos *et al.* (1991) for another species of rocky coastal habitats (*Crithmum maritimum*).



Low germination ($\leq 60\%$) was observed at all tested conditions for fresh seeds of all populations sampled in 2010. The inter-population variability identified through morpho-colorimetric analysis was also observed in germination behavior.

The application of the dry after-ripening treatment had not a significant effect on *B. insularis* seed germination, although inter-population differences were detected. Therefore, *B. insularis* seeds do not need of a dry summer period which forego germination.

In this study, seed heteromorphy was adequately investigated for the first time in this species and only in 2010 for Isola dei Cavoli population seeds, which is clearly the population farthest and isolated from others, growing on a little island (Isola dei Cavoli - SE Sardinia). It may be argued that the geographical isolation of this population may have determined independent evolutionary divergence processes respect to the other Sardinian populations.



