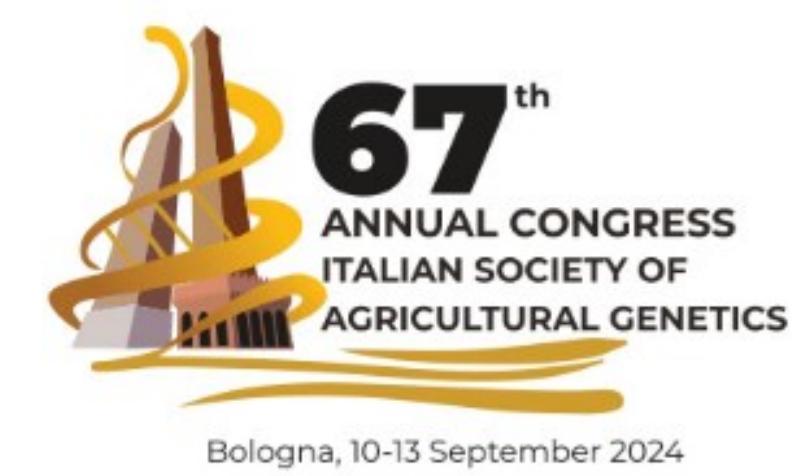


# Thermoprimer as a tool to break dormancy in different varieties and seed lots of *Cannabis sativa* L.

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Hemp (*Cannabis sativa* L.) is an annual, mainly dioecious plant, known for its multiple industrial uses and properties. In recent years, hemp global market is growing fast, thus there is the need to enhance knowledge to ensure high crop yields. One of the key developmental stages that have a high influence on the final yield is the germination and seedling emergency time, that can be delayed by dormancy. This is a mechanism developed by plants to secure that germination takes place only under optimal environmental conditions.

The aim of this work was: (1) to investigate the germinative behavior of Italian monoecious varieties, and (2) to develop treatments to break dormancy. Seed lots from two Italian hemp varieties (Codimono, Carmaleonte) and two breeding lines (CARIFIT1p, CARIFIT2t) have been characterized based on germination performance and seed viability, revealing different levels of dormancy. Seeds were then subjected to a temperature treatment - 45 °C for three days - to break dormancy.

## Plant material

Six seed-lots from four *C. sativa* varieties/lines were used:

- Codimono produced in 2014 (Cod2014) and 2016 (Cod2016)
- Carmaleonte produced in 2017 (Car2017) and 2019 (Car2019)
- CARIFIT1p and CARIFIT2t produced in 2023

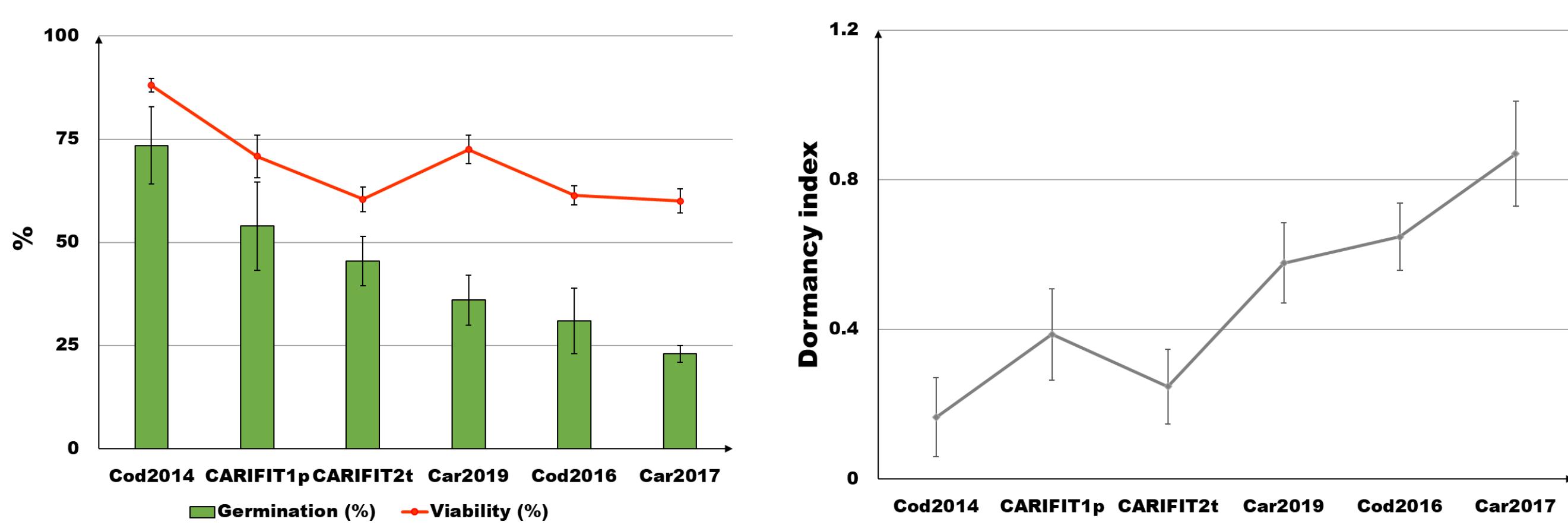
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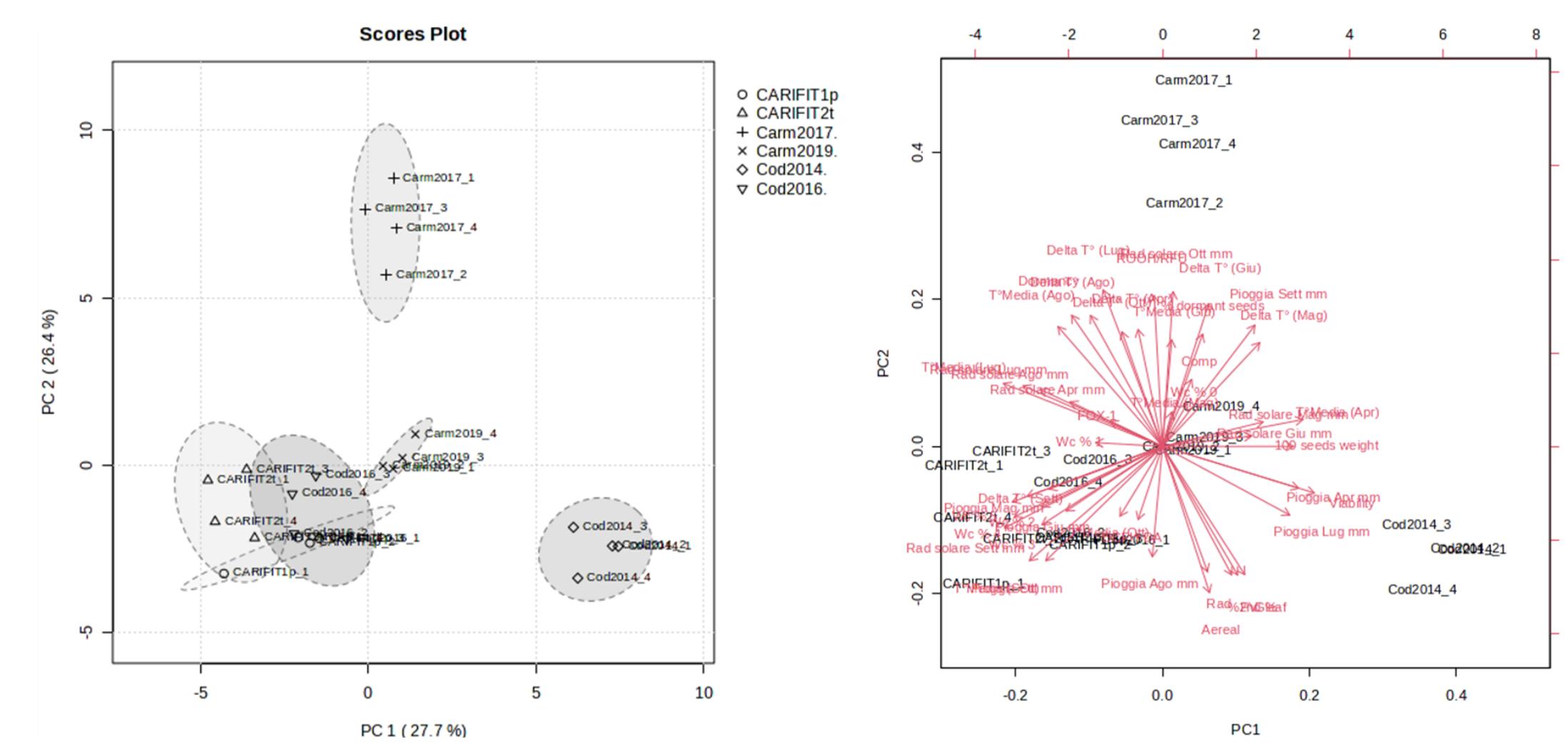
CARIFIT

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## Germination performance and Dormancy Index



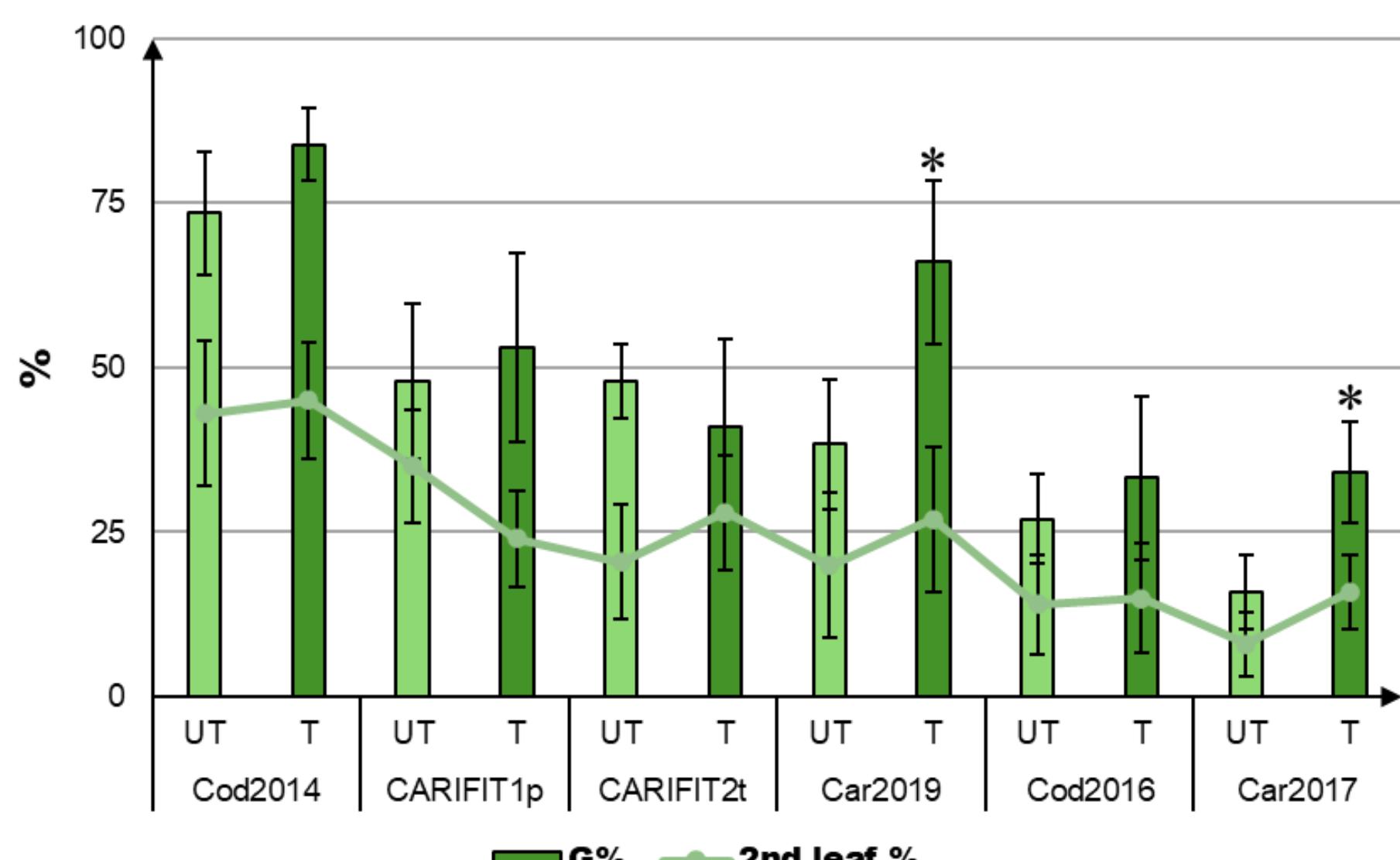
## Principal Component Analysis (PCA) based on germination performances and climatic conditions measured at the seed production sites



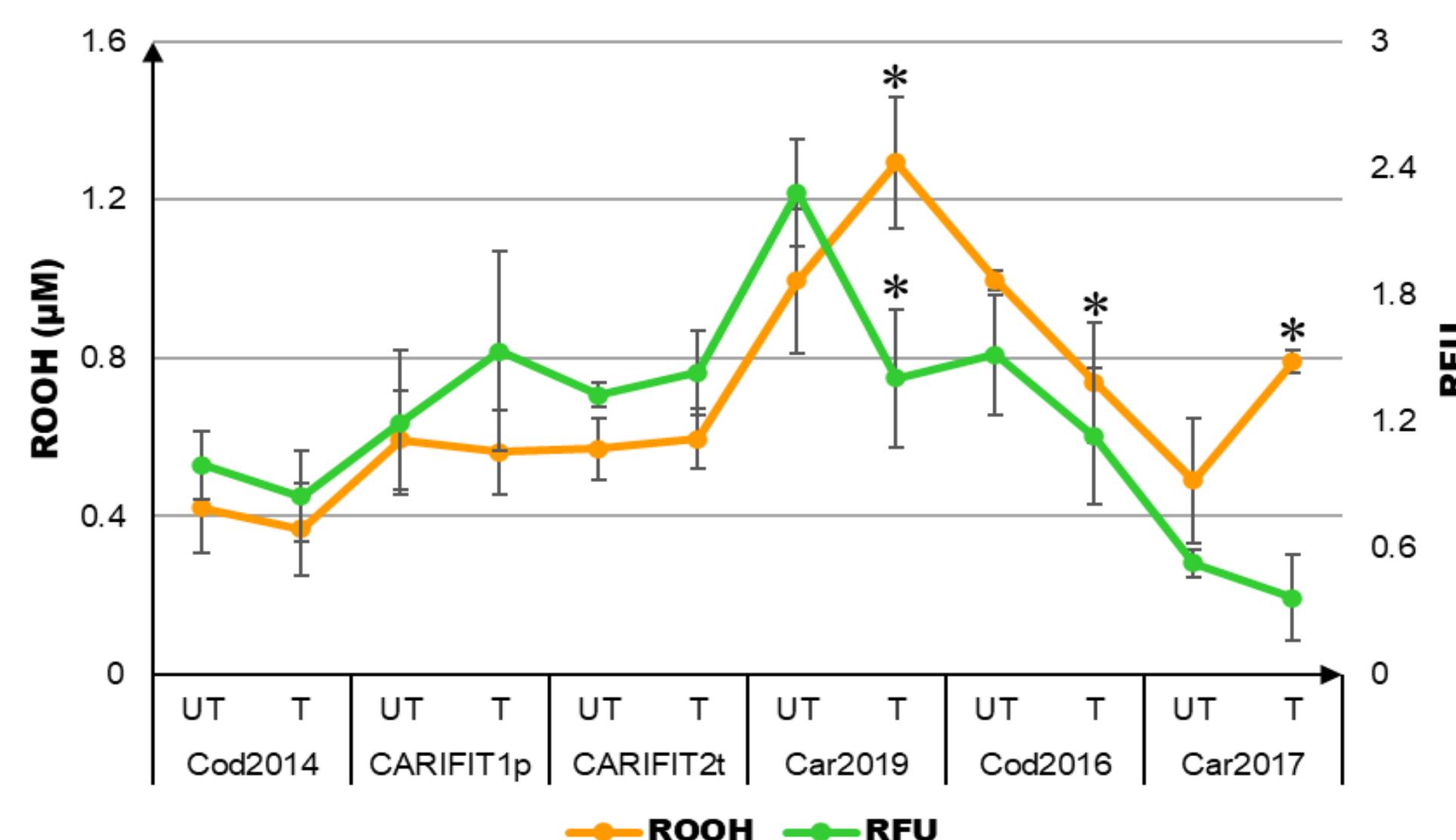
- Cod2014 (73.5±9.3 %) and CARIFIT1p (54±10.7 %) show germination (G%) above 50% while the remaining seed lots have low germination with the lowest for Car2017 (23±2 %).
- Seed viability (V%), measured through the TTC - triphenyltetrazolium chloride – test, show a decreasing trend with the decrease in germination
- Dormancy index, calculated as 1-(G%/V%), indicate high dormancy levels for Car2019, Cod2016, Car2017.
- Given that dormancy can be influenced by the climatic condition during development, climatic data from the production sites were recovered and used to do a PCA analysis; the formed clusters are representative for the production years.

## Thermoprimer applied to *C. sativa* seeds influences germination and the seed oxidative state

### Germination (G) % and second leaf (2nd leaf) % after thermoprimer



### Release of peroxide radicals (ROOH) and Relative Fluorescence Units (RFU)



- Thermoprimer is effective in breaking dormancy in *C. sativa*, and, improving seed germination % and seedling development in the Carmaleonte seed lots (Car2017 and Car2019), up to 2-fold compared with the untreated (UT) controls
- Seed oxidative levels were quantified through biochemical assays: DCFH-DA (dichloro-dihydro-fluorescein diacetate, expressed in RFU) and FOX-1(ferrous oxidation-xylenol orange, expressed in ROOH).
- The collected data show significant increase in ROOH concentrations and decrease of RFU for Carmaleonte seed lots (Car2017, Car2019)
- The positive effect of thermoprimer on Carmaleonte can be attributed to the influence of high temperature on the oxidative status.