Calorespirometry as a phenotyping tool for selection of genotypes with high plasticity upon temperature stress – a focus on the analysis of pea seeds

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Introduction

The availability of phenotyping tools to assist breeding programs in the selection of high quality crop seeds has an obvious interest with consequences for both seed producers and consumers. Calorespirometry, simultaneous measurement of heat and CO_2 rates, has been used as a screening tool to assess metabolic and respiratory changes associated with cell reprogramming events. Considering that seed germination involves the activation of several metabolic pathways, including cellular respiration to provide the required energy, this technique appears to be a promising tool to assist breeding programs, since assess seed viability and demonstrate the link between seed viability and cellular respiration associated with the germination process.

The objective of this work was to prove the usefulness of calorespirometry method to assess seed viability by monitoring cellular respiration associated with the germination process.

Methodology

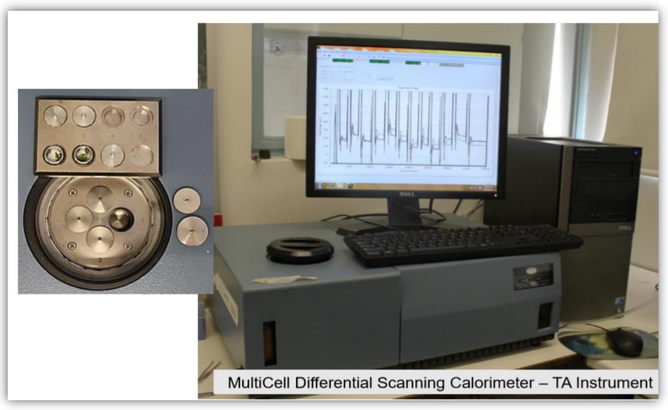
Evaluation of temperature effect on seed germination – Germination trial

- Three commonly used pea cultivars (*Pisum sativum* L.) were used: cv. '**Rondo**', '**Torta de Quebrar**' and '**Maravilha D´América**'.

- 30 seeds of each cultivar was covered with cotton wetted with sterile tap water and incubated under total darkness at different temperatures: 15, 20, 25 and 28°C.

Calorespirometric measurements

- Calorespirometric measurements were performed in a 4100 Multi-Cell Differential Scanning Calorimeter (TA Instruments, USA).
- Calorespirometric measurements were taken by running isothermal calorespirometric experiments at 15, 20, 25 and 28°C.
- Seeds were previously soaked in water for 16 hours.

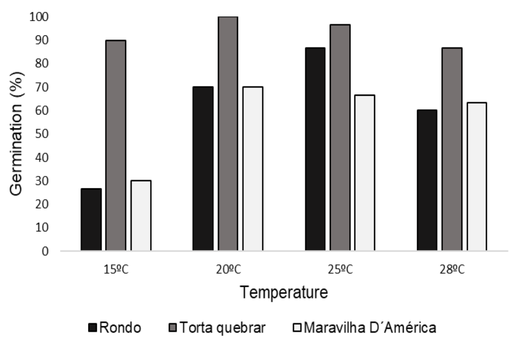


- The values of heat rate (µW) emitted by seed during calorespirometric measurements, allowed the calculation of the following parameters: **Respiratory heat rate (Rq)**, **CO_2 production rate (R_{CO2})**, **Structural biomass formation rate ($R_{structural\ biomass}$)** and **Carbon use efficiency (ε).**

Results

Effect of temperature on seed germination - Germination efficiency

- Optimal germination rates depended on temperature and cultivar.

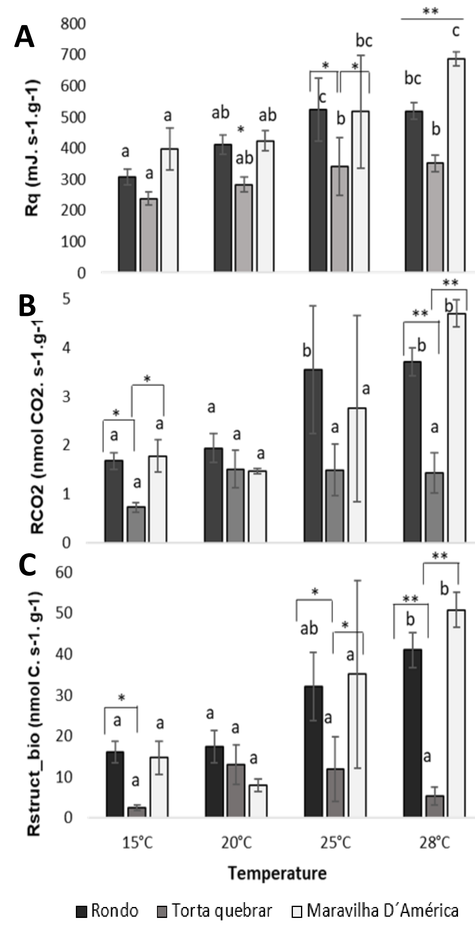


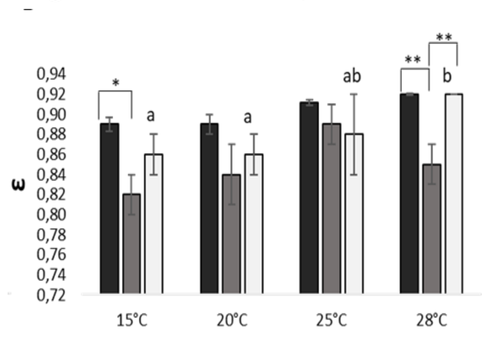
- Cv. 'Torta de Quebrar' and cv. 'Maravilha d'America' show the higher germination rate at 20°C.
- Cv. 'Rondo' show the higher germination rate at 25°C.
- The temperature of 15°C were associated with low germination rates for cv. 'Rondo' and cv. 'Maravilha D'America'.
- The cv. 'Torta de Quebrar' showed significantly higher germination rate in comparison with the other two cultivars under the three temperatures tested.

'Torta de Quebrar' appear as the most resilient, able to more efficiently overcome temperature stress.

Effect of temperature on seed germination – evaluation through calorespirometry

- Significant differences in **Rq**, **R_{CO2}** and **Rstruct_bio** (graphs A, B and C, respectively) among the three cvs. were detected at temperatures that were not optimal for germination.
- Rq was lower in cv. 'Torta de quebrar', compared to the other cvs., that could means a reduction in metabolism (heat rate, Rq) to be more focused in acclimatization.
- The cv. 'Torta de quebrar' exhibited R_{CO2} values highly homogeneous under 20, 25 and 28°C





- Results achieved for *ε* (graph D), showed significant differences among cultivars at the extreme temperatures, 15°C and 28°C, in the same way as observed for R_{CO2}.

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Conclusions

✓Calorespirometry appears to be a promising tool to assess seed viability and to assist breeding programs in the selection of high quality crop seeds, without establishing lengthy germination tests, commonly used in viability analysis.

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