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Calorespirometry as a phenotyping tool for selection of genotypes with high plasticity for temperature stress: analysis of pea seeds

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Increasing human population and climatic and edaphic changes impose needs to develop new cultivars with seeds that have greater plasticity for environmental constraints and higher germination rates. Improving seed vigor remains a primary objective of the agricultural industry and the seed/breeding companies that support it. Phenotyping tools to assist breeding programs for phenotyping seeds for resilience and selecting high-quality crop seeds are of interest. Because temperature is the major environmental constraint controlling growth, with a strong impact on crop yield, we have investigated the temperature dependence of respiratory parameters with calorespirometry as an alternative to conventional seed phenotyping strategies. Calorespirometry has been used to assess metabolic and respiratory changes associated with cell reprogramming events, and has been proposed as a screening tool for predicting growth phenotype for temperature stress. Calorespirometry measures metabolic heat rates (R_q) and CO_2 emission rates (R_{CO2}) of biological samples as a function of temperature allowing prediction of growth rates ($R_{struct\ biomass}$) without growing plants under different environmental temperatures. Calorespirometry also allows the determination of the fraction of carbon substrate incorporated into structural biomass, *i.e*. carbon use efficiency (ε), as a function of temperature.

Three pea (*Pisum sativum* L.) cultivars ('Rondo', 'Torta de Quebrar' and 'Maravilha D´América') commonly used in conventional agriculture were selected for calorespirometry measurements and germination trials. The effect of temperature on seed germination was evaluated after an imbibition period of 16 h under dark conditions. Calorespirometric measurements were conducted in isothermal mode in a Multi-Cell Differential Scanning Calorimeter (TA Instruments, New Castle, DE) at 15, 20, 25 and 28°C. A minimum of three measurements were made on each cultivar. The heat rates, R_q, were measured directly and CO_2 rates, R_{CO2}, were determined from the increase in the heat rate in the presence of a vial of NaOH, which reacts with CO_2 in an exothermic reaction (-108.5 kJ/mol CO_2). For data validation, a seed germination trial with the same cultivars was conducted under the same temperatures tested in the calorimeter. Substrate carbon conversion efficiencies and growth rates could not be calculated from the data collected in this study because the calorespirometric ratios, R_q/R_{CO2}, showed that a significant portion of the CO_2 was produced by anaerobic respiration.

At 20, 25 and 28°C, R_q was significantly lower in cv. 'Torta de Quebrar' than in the other two cultivars. At 15°C, no significant differences were detected among the three cultivars. All three cultivars have significantly higher R_q values at 25 and 28°C than at 15 and 20°C (Figure 1). R_{CO2} exhibited lower values for cv. 'Torta de Quebrar' than the other two cultivars. R_{CO2} in cv. 'Torta de Quebrar' was constant from 15 to 28°C. In contrast, cvs. 'Rondo' and 'Maravilha D'América' exhibited an increase in R_{CO2} with increasing temperature. In the germination trial, no significant differences were observed in the percentage of germination at the four temperatures for cvs. 'Torta de Quebrar' and 'Maravilha D'América'. In contrast, cv. 'Rondo' exhibited the highest germination rate at 20 and 25°C, and the lowest germination rate at the extreme temperatures, 15 and 28°C (Figure 1). Cv. 'Torta de Quebrar' presented the highest germination rates, above 90% across the temperature range, while in cv. 'Maravilha D'América' germination was around 70%. In cv. 'Rondo', the maximum germination rate was achieved at 20-25°C (70-80%) (Figure 1). This indicates that cv. 'Torta de Quebrar' has the highest germination rate and the highest resilience in germination across temperatures. In agronomical terms, this result means a high and stable production, which highlights cv. 'Torta de Quebrar' as the most resilient against temperature stress.

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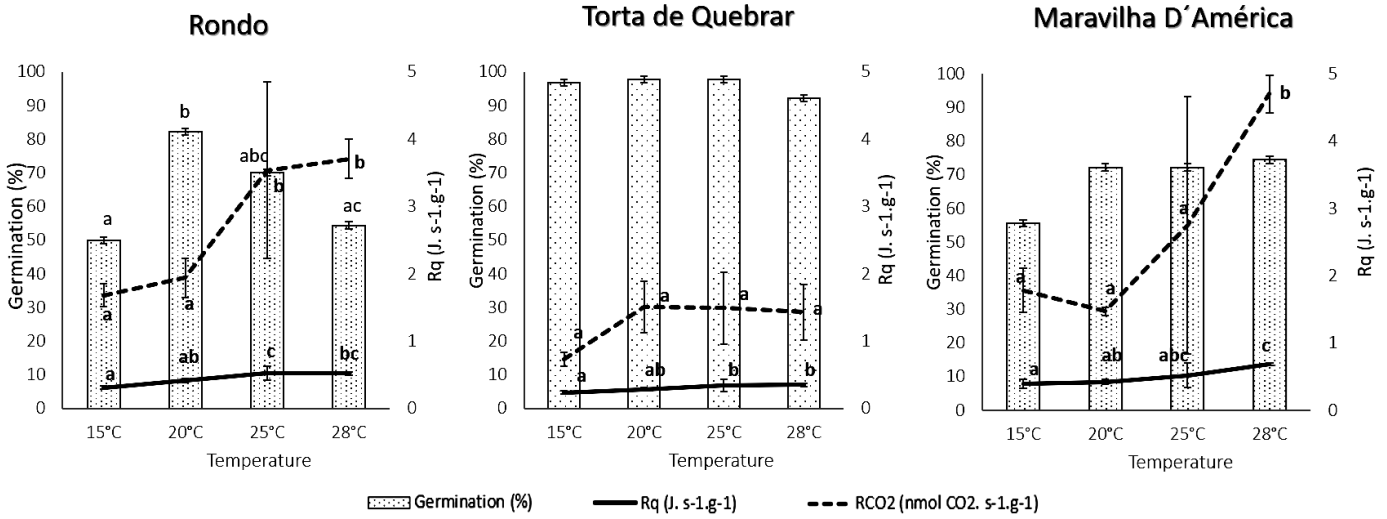

Figure 1 Results of R_q, R_{CO2} and germination rates achieved under different temperatures for the three *Pisum sativum* cultivars

In the calorespirometric measurements, cv. 'Torta de Quebrar' showed the lowest values of both R_q and R_{CO2} and in the germination trial, the highest germination rates, suggesting that early metabolic homeostasis for carbon and energy metabolism further modulates plant cell development and growth. The first hours of seed germination are characterized by the stress generated by water uptake. During the imbibition period, mitochondria, peroxisomes and the plasma membrane NADPH oxidases represent the main sources of reactive oxygen species (ROS) generation. To avoid damage to the embryo that could negatively impact germination, ROS levels must be strictly controlled by antioxidant systems. The reduction of metabolism and efficient mechanisms for ROS scavenging may represent an efficient strategy to overcome the stress imposed on cells. Validation of this hypothesis requires further experiments to evaluate the efficiency of antioxidant mechanisms in seeds of cv. 'Torta de Quebrar' in comparison with the other two cultivars. We propose that low R_q values in seeds during the early stages of germination are correlated with high resilience for temperature stress. The present work demonstrates the applicability of calorespirometry to assess seed vigor at different temperatures, suggesting this method as a reliable phenotyping tool for selecting more resilient genotypes.

Keywords
Germination · *Pisum sativum* · resilience · respiration · stress

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