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Emilie Hascoet, Corinne Devaux, Sana Beji, Odile Jaminon, Valérie Dufayet,
Christophe Lecomte, B. Delbreil, Isabelle Lejeune-Henaut

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Development of a freezing test in controlled conditions for *Pisum sativum*

Hascoët e1, devaux c2, BEJIS3, Jaminon o1, BANCOURT L1, KERGROAC'H N1, HÛ J-F4, DEPTA F4, LECOMTE C5, DUFAYET V5, DELBREIL B3, LEJEUNE-HENAUT I1

1 : USC Institut Charles Viollette - Adaptation au Froid du Pois, INRA, Estrées-Mons, 80203, Péronne, France
2 : Terres Univia, Estrées-Mons, 80203, Péronne, France
3 : USC Institut Charles Viollette - Adaptation au Froid du Pois, Université Lille1, 59655, Villeneuve d'Ascq, France
4 : UE GCIE-Picardie, INRA, Estrées-Mons, 80203, Péronne, France
5 : UMR Agroécologie, INRA, 21065, Dijon, France

Freezing is a major environmental limitation to crop productivity for a number of species including legumes. In the context of global climate change, winter crops will experiment milder autumn periods that could interfere with the achievement of cold acclimation, i.e. the ability for plants to increase their level of frost tolerance in response to low but non-freezing temperatures. For the pea crop, a climate analysis coupled to a modelling approach has shown that climate warming will increase the occurrence of freezing damage events, even if these latter will be less severe (Castel et al., 2014). Thus, breeding for frost tolerant winter peas requires not only to improve their frost tolerance threshold, but also to raise their cold acclimation rate. In order to evaluate the genetic variability of this last trait, we are adjusting (optimizing) an experimental protocol in controlled conditions which provides an indirect evaluation of frost tolerance by the measurement of tissues' electrolyte leakage.

Material and Methods



Figure 1. climatic chamber



Figure 2. programmable freezer

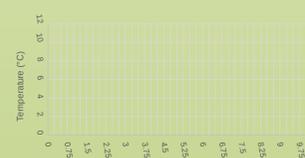


Figure 3. Rate of temperature in the programmable freezer

Results

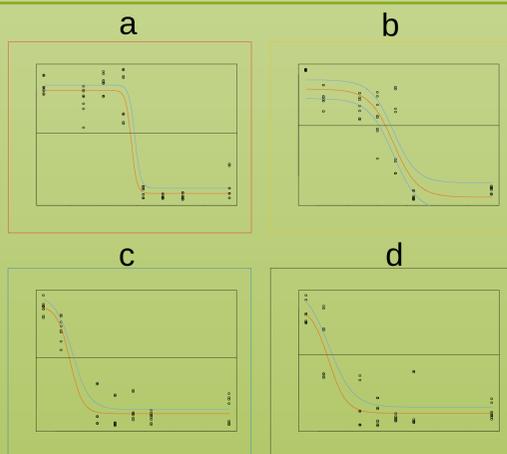


Figure 4. Electrolyte leakage (%) vs temperature (°C) for the pea lines Térèse (a), Isard (b), China (c) and Champagne (d). Data were fitted to a sigmoidal regression model (red line) and 95% confidence interval (blue line) was also represented.

Electrolyte leakage was measured after 14 days of cold acclimation for the pea lines Térèse, Isard, China and Champagne tolerant ones. Eight temperatures were tested ranging from +4°C to -27°C. For each line and each test temperature, the percentage of Electrolyte Leakage (EL) was calculated as:

EL values were fitted to a sigmoidal regression model with the R program (Fig.4) and TEL50, the temperature corresponding to 50% EL, were calculated for each pea line as follows :

The TEL50 values showed significant differences between Térèse, already known as a frost sensitive line (TEL50 = -11°C), Isard, known as an intermediate line (TEL50 = -13,7°C) and both tolerant lines China (TEL50 = - 22,7°C) and Champagne (TEL50 = - 23,6°C).

This ranking is also consistent with that observed for freezing tolerance at the Chaux-des-Prés field platform(Fig.5).



Figure 5. TEL50 and 95% confidence intervals, compared to visual frost damages observed in 2012 at the Chaux-des-Prés field platform. a, b, c : SNK grouping of the TEL50 values

Perspectives

Next improvements of this freezing test under controlled conditions should lead us to rank pea lines not only for their frost tolerance level but also for their cold acclimation rate. This protocol will also help for the phenotypic validation of frost tolerance QTL that requires to detect slight variations among QTL-near isogenic lines.