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### ► To cite this version:

A Sonnet, B Decaux, V Dufayet, C Devaux, B Raffiot, et al.. Tools to help breeding for frost tolerance of winter grain legumes. 4TH INTERNATIONAL LEGUME SOCIETY CONFERENCE, Sep 2023, GRANADA, Spain. hal-04400241

**HAL Id: hal-04400241**

**<https://hal.science/hal-04400241>**

Submitted on 17 Jan 2024

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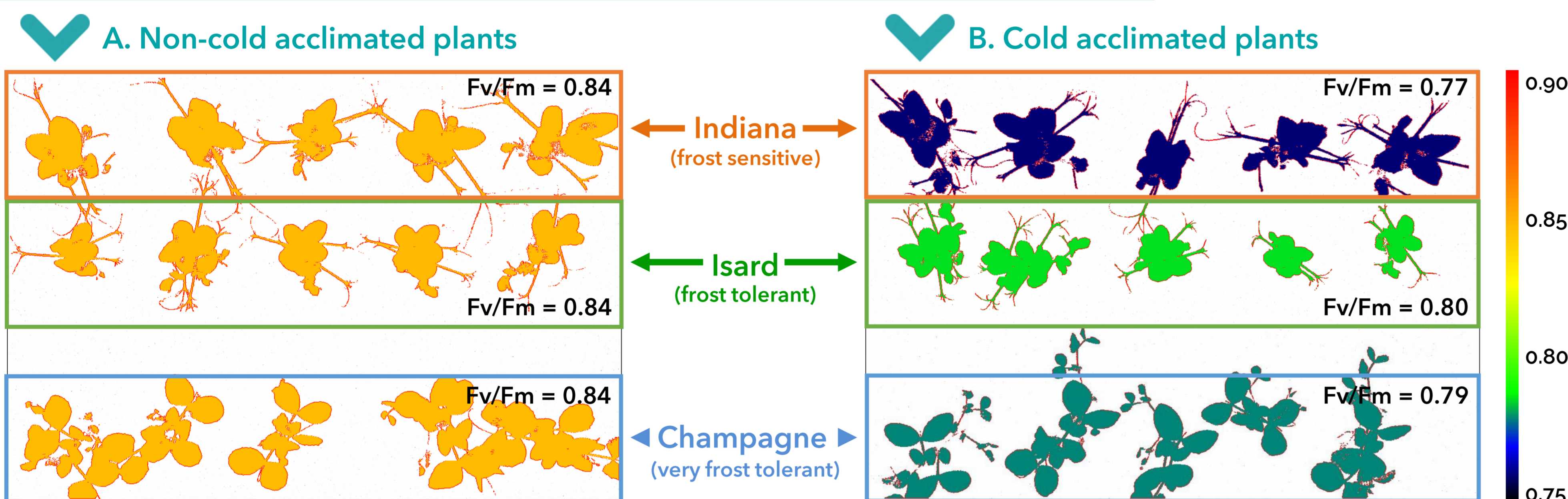
# Tools to help breeding for frost tolerance of winter grain legumes

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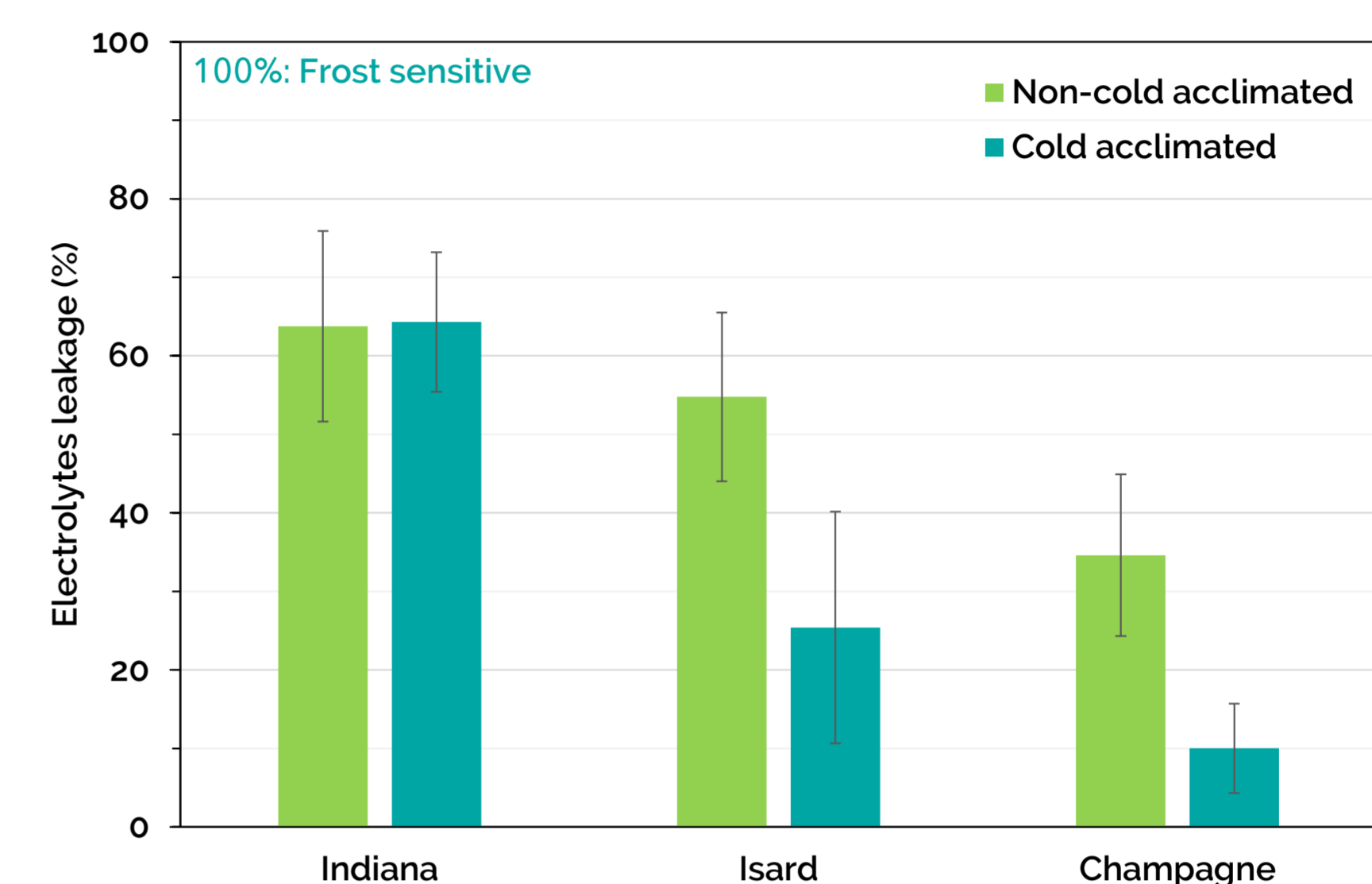
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Frost is a major environmental limitation to crop productivity for a number of species including winter legumes. In the context of climate change, milder autumn temperatures limit the achievement of cold acclimation, i.e. the ability for plants to increase their level of frost tolerance in response to low but non-freezing temperatures. Thus, even if frost events tend to be less severe, screening for frost tolerance remains a concern for breeders, especially since the annual variability of winter climatic conditions may result in unefficient screening in field conditions. Different tools are worth to be developed to help breeding for frost tolerance of winter grain legumes, such as phenotyping in controlled conditions, including use of imaging, or modelling of frost tolerance and frost damages. These tools are illustrated below.

## Phenotyping by fluorescence imaging



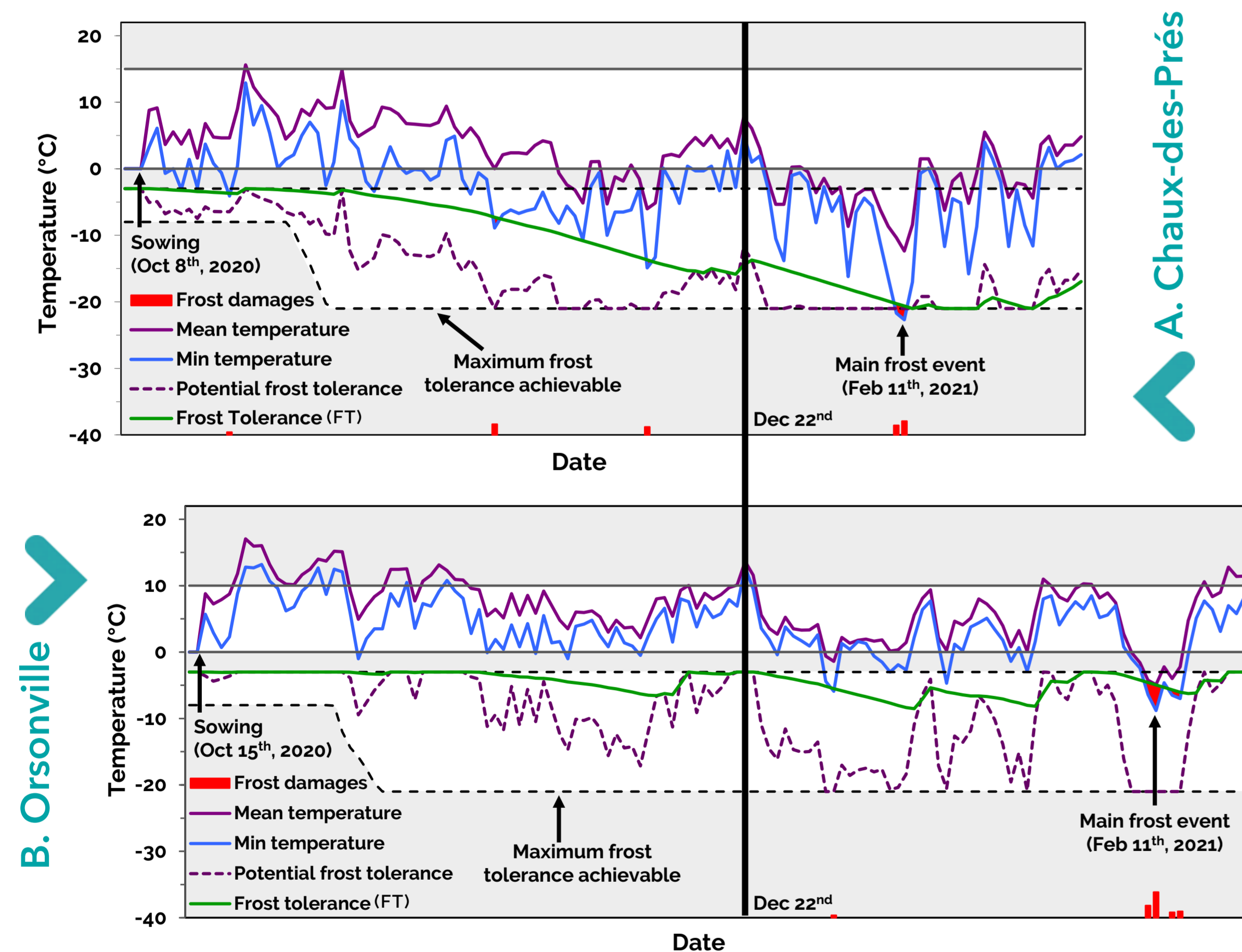
**Figure 1:** Fluorescence imaging on a high throughput phenotyping platform (PlantScreen™). Example of Fv/Fm (maximal quantum yield of PSII photochemistry for a dark-adapted state), for 3 genotypes in 2 contrasted conditions: after a 20-days nursery period, plants were kept at 14/12°C day/night during 1 week (A. non-cold acclimated) or transferred to 4/4°C day/night during 3 weeks (B. cold acclimated). In A and B conditions, plants were measured at approximately the same age, expressed in sum of degree-days since sowing.



**Figure 2:** Electrolytes leakage estimated from plants sampled in 2 contrasted conditions, i.e. A (non-cold acclimated plants) and B (cold acclimated plants) for 3 genotypes: Indiana, Isard and Champagne. The date of sampling is equivalent to the date of fluorescence imaging measurement shown in fig.1. Genotypic variability is greater under cold acclimation conditions.

In a preliminary experiment of the PEAMAGE project, we compared fluorescence imaging data (fig. 1) with frost tolerance data evaluated by electrolytes leakage (fig. 2), in order to identify imaging parameters likely to be correlated to frost tolerance. Fv/Fm is shown as an example among promising fluorescence parameters.

## Frost tolerance model



**Figure 3:** Prediction of frost tolerance (cultivar Spencer, winter 2020-2021) at: A. Chaux-des-Prés (Jura mountain, France, alt: 900m), B. Orsonville (traditional production area, France, alt: 155m). Lower acclimation temperatures at A allowed a complete acclimation. Frost damages are predicted when  $FT > T_{min}$  (Feb 11<sup>th</sup>, 2021; at A,  $FT = -20.6^{\circ}C$  and  $T_{min} = -22.7^{\circ}C$ ; at B,  $FT = -4.9^{\circ}C$  and  $T_{min} = -8.8^{\circ}C$ ). This was confirmed by visual observations.

This prediction model has been developed on wheat (Lecomte *et al.*, 2003), adapted to the pea crop and already used to forecast frost stress evolution in the context of climate change (Castel *et al.*, 2017).

It requires:

- Few input variables: daily mean and minimum temperatures, sowing and emergence dates;
- Genotypic parameters, estimated from observations of control varieties in the site of Chaux-des-Prés: minimum and maximum frost tolerance, cold acclimation rate.

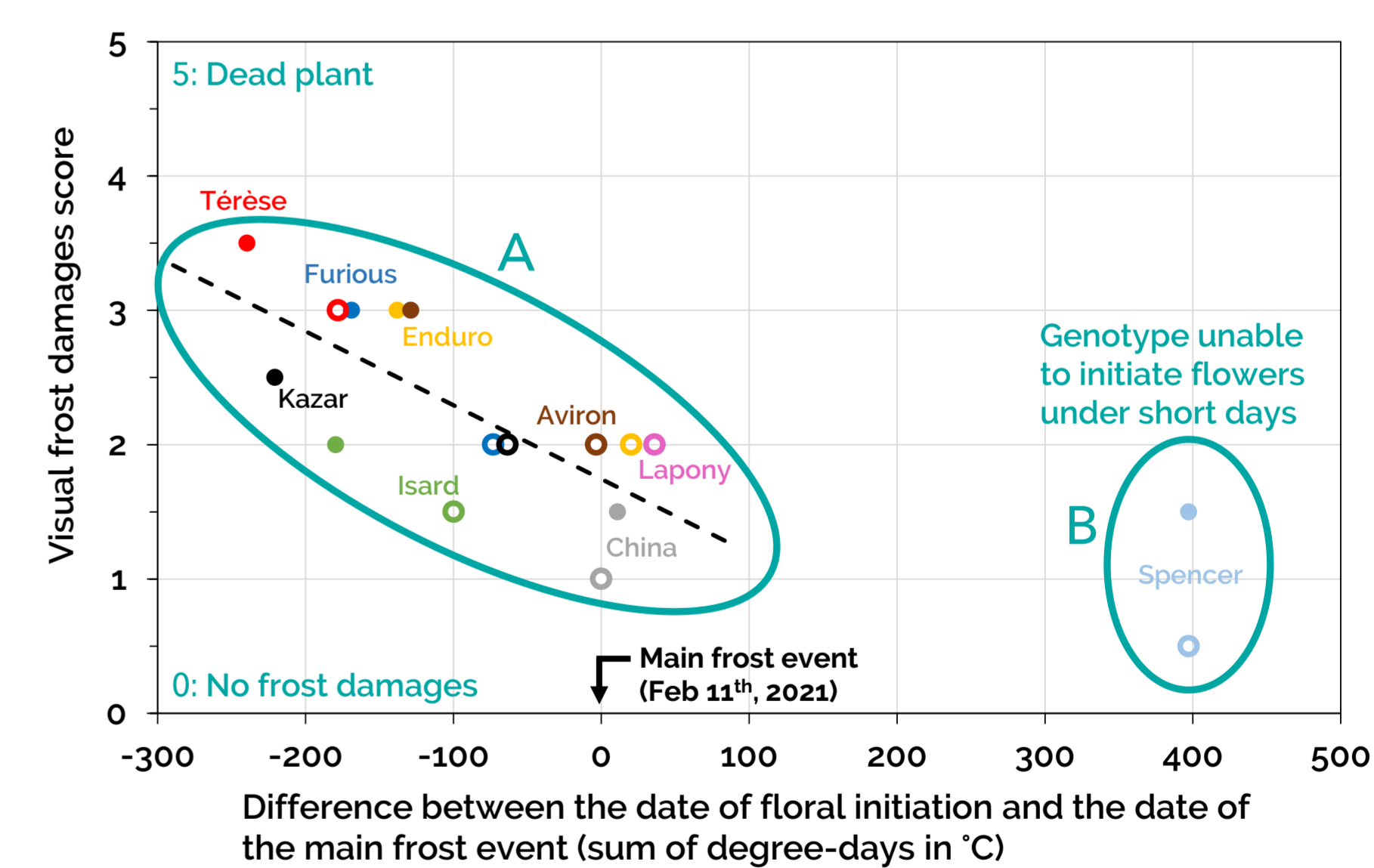
It predicts daily frost tolerance (fig. 3, green curve) and frost damages occurrence and intensity (fig. 3, red bars).

➤ The model highlights a poor cold acclimation in a traditional production area (fig. 3B), comparatively to the high-altitude site (fig. 3A), due to milder autumn temperatures.

➤ The model could be improved (ongoing project LEGHIVER) by taking into account:

- the incident global radiation, suspected to be less favourable to cold acclimation in low-altitude sites;
- the advancement toward floral initiation, suspected to reduce the effect of cold acclimation (fig. 4).

➤ The model could help to manage field sampling of plantlets, according to their level of cold acclimation, and to choose the most appropriate frost temperature to be applied in a programmable freezer, in order to allow a mixed field/controlled conditions screening.



**Figure 4:** Relationship between floral initiation earliness and visual frost damages (Orsonville, 2020-2021, 9 genotypes, 2 sowing dates: Oct 15<sup>th</sup>, 2021 [●] and Oct 24<sup>th</sup>, 2021 [○]). For genotypes of the group A, frost damages are negatively related to the earliness of floral initiation.

## References

- Castel T, Lecomte C, Richard Y, Lejeune-Hénaut I, Larmure A (2017) Frost stress evolution and winter pea ideotype in the context of climate warming at a regional scale, OCL, 24(1) D106, doi:10.1051/ocl/2017002.
- Lecomte C, Giraud A, Aubert V (2003) Testing a predicting model for frost resistance of winter wheat under natural conditions, Agronomie, 23:51-66, doi:10.1051/agro:2002068.