SOCRATES: reSilience tO reCuRrent heAt sTrEsSes

Abstract

The increased frequency of extreme climatic events, such as heat waves, is a major challenge for agricultural systems in a context of a growing worldwide population. Although there is a large body of literature on heat stress effects on crop performances, little is known about the recurrence of stressing events which effects are likely to reflect stress memory, thus meaning that the response of the plants to subsequent stressing events are modulated by prior stress exposures. Indeed, stress memory can be positive as a consequence of a priming effect upon the first exposure, or negative when the effects are accumulated and amplified. These differences are accounted for by specific -omics signatures i.e. metabolomic, proteomic and transcriptomic. In addition, the few studies that have attempted to analyze the responses to recurrent heat stresses, have faced the issue to analyze memory effects regardless of age effects and compensation effects which can be important in indeterminate species such as oilseed rape (Magno et al. 2021, 2022).

In this context, the PhD project **SOCRATES** aims at analyzing memory effects under controlled conditions (in the greenhouse) that will be designed to prevent these difficulties i.e. the different sequences prior to the intense heat stress will cumulate the same thermal time, meaning that the plants will be at the same phenological stage when the intense heat stress occurs for the several prior pre-stress sequences tested.
The analyses will be performed (i) at seed maturity (seed yield, components, seed quality criteria such as fatty acids, nitrogen, soluble sugars contents, germination behaviors) and (ii) at intermediate stages (on leaves, seeds and/or pod envelops to be defined) throughout the heat stress sequence to measure -omics signatures and identify specific pathways and metabolic processes that reflect positive (priming), negative effects or absence of memory.
In addition to these dynamic analyses, measurements by NIRS (Near Infra-Red Spectrometry) will be performed in order to acquire the reflectance spectrum which will be compared to metabolomic signatures. The underlying objective is to develop an *in situ* detection tool of the metabolic status of stressed plants. Measurements in the field - performed on assays conducted by Terres Inovia (https://www.terresinovia.fr/) – will be used to validate the correlations between reflectance spectrum and metabolomic signatures acquired on plants grown under controlled conditions in the greenhouse.

The objectives of the PhD project will aim at (i) analyzing the memory effects induced by repeated stresses regardless of age and compensation effects (ii) identifying specific metabolomic signatures to further highlight metabolic pathways involved in stress responses and memory effects in the perspective to guide towards the selection of varieties that are more likely to be thermo-sensitized (iii) developing an *in situ* detection tool that would help adjust crop management and anticipate negative impacts on yield and seed quality.

Questions of research

The candidate will focus on three main questions of research:
Axis 1: **Comparison of several thermo-sensitization protocols under controlled conditions** that would differ from the duration of the recovery phase between the mild pre- stress event and the later intense heat stress.
Axis 2: **Identification of -omics signatures (metabolomic, proteomic and transcriptomic) of stress memory along with epigenetic marks that are involved in their regulation.** A focus will be performed on post translational modifications (PTMs) of histones by mass spectrometry. These -omic signatures will be measured in leaves, seeds and/or pod envelops (to be determined) during the whole stress sequence (pre and post- mild stress, post recovery, post intense stress). Then, they will be associated to the final performances of the plants in order to investigate on whether the -omics modifications lead to **positive effects** (priming effect of the pre-stress), **negative effects** (cumulated effects of both mild and intense events and/or too long duration of the recovery phase that might reset the modifications), or **no effects** (no memory).
Axis 3: **Detection of correlations between the modifications of the reflectance spectrum and the metabolic signatures to predict the potential for acclimation of plants subjected to repeated heat stresses.** The calibration equations will be performed in material (leaves, seeds and/or pod envelops) from plants grown under controlled conditions, which then will be validated on material from plants grown in the field.

Working hypotheses

Hyp 1: The comparison of several expected thermopriming protocols that cumulated similar thermal time prior to the intense heat stress should help analyze memory effects solely rather than a combination of these effects with age effects.
Hyp 2: The modifications of metabolic pathways are different whether (1) the memory effects are positive, negative or there is no memory effect (2) post-translational modifications of histones are involved in the responses to repeated heat stresses by regulating gene expression.
Hyp 3: The variations in the reflectance spectrum acquired by NIRS are markers of specific metabolic signatures that determine plant behaviors when challenged to repeated stresses.

Program of research

Axis 1 is dedicated to the cultivation of oilseed rape (greenhouse year 1, field year 2), under several defined thermopriming protocols (for the greenhouse condition). Dynamic non-destructive measurements (gas exchange, NIRS reflectance, fluorescence of chlorophyll) and destructive analyses of the seed quality at the maturity stage will be performed for plants grown both in the greenhouse and in the field.

Axis 2 is dedicated to the characterization of -omics signatures and PTMs of histones involved in heat stress memory by targeted proteomics (for plants grown under controlled conditions only). **For the greenhouse conditions**, the analyses throughout the stress sequence (pre- and post-mild stress, post recovery and post intense heat stress) should allow (i) to indicate to what extent, the different thermo-priming protocols impact the seed development and maturation and whether the sequence prior to the intense stress (i.e. mild stress and recovery) help mitigate the negative impacts of the intense stress, (ii) to identify the most variable metabolites amongst the stress modalities, (iii) to monitor the levels of these metabolites and further determine their role in the stress memory acquisition or reset and eventually (iv) to confirm (or not) the presence of histone marks known to be involved in heat stress memory and responses (i.e. H3K4me3 et H3K27me3, H3K4me1; Lämke et Baurle, 2017, Haider et al. 2021). **For the field conditions**, harvests at relevant times will be carried out according to climatic profiles that will be recorded and analysed in real time.
The objectives are to identify (i) specific metabolic signatures to categorize the responses to repeated heat stresses i.e. no memory, positive memory, negative memory and (ii) epigenetic regulations that account for the different metabolic and proteomic signatures.

Axis 3 is dedicated to the analyses of the correlations between the modifications of reflectance spectrum and the metabolic signatures. These analyses will rely on data acquired under controlled and field conditions from axes 1 and 2. The finalized objectives are to develop predictive models of specific metabolic signatures from modifications of reflectance spectrum (Burnett et al. 2021) in the perspective to use the later as proxy of the specific metabolic signatures associated to the different categories of responses to repeated heat stresses (axis 2). Based on non-destructive measurements (reflectance spectrum), these correlations should indicate whether plants acclimated (priming) or did not (negative memory or absence of memory) upon later intense heat stress. In this work, field data will be used for the validation of models that will be calibrated with data from controlled conditions.

Skills and academic profiles

The PhD candidate must hold a Master degree or equivalent. He/She must have good background in plant (eco)-physioIogy and molecular biology. Motivation, curiosity and open-mindedness are key assets to apply to this multi-disciplinary project which will require to interact with different teams and researchers.
Good command in R and basic French (or willingness to learn) would be much appreciated.

Additional information

PhD grant from INRAE (National Research Institute for Agriculture, Food and the Environment) and ANR (French National Research Agency) about 1400€/month.
Starting date: October 2023 for 3 years
Localization: Research Unit EVA, University of Normandy (https://www6.rennes.inrae.fr/umreva/)
Director and supervisors: Sophie Brunel-Muguet, Jean-Christophe Avice, Benoît Bernay.

Applications should be addressed by e-mail (CV, application letter, recommendation letter) to Sophie Brunel-Muguet (sophie.brunel-muguet@inrae.fr), Jean-Christophe Avice (jean-christophe.avice@unicaen.fr) and Benoît Bernay (benoit.bernay@unicaen.fr) **before 15th of May 2023**.