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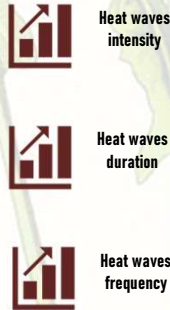
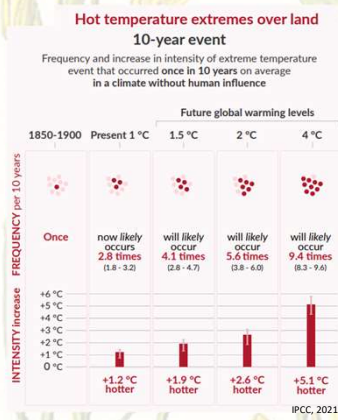
Towards a better characterization of heat stress recurrence: A case study in oilseed rape

Brunel-Muguet S¹, Magno Massuia de Almeida L¹, Delamare J¹, Le Franc L¹, Bernay B², Pontin J², Fremont J¹, Avice JC¹, Mollier A³, Personeni E¹, Soyer J⁴

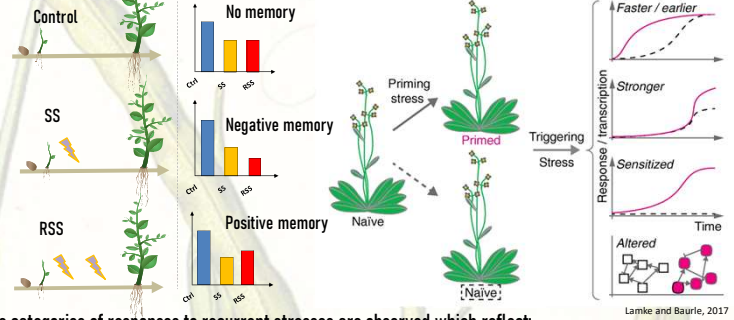
1: UMR 950 Ecophysiologie Végétale, Agronomie et Nutrition NCS (UCBN, INRAE) 2: Plateforme Proteogen, US Emerode (UCBN, INRAE) 3: UMR 1391 Interactions Sol Plante Atmosphère (Bordeaux science agro, INRAE) 4: UMR 1290 Biologie et GEstion des Risques en agriculture, (Univ. Paris-Saclay, INRAE)

Climatic context and plant acclimation

Intensification of on-going climate change Figure 1



Responses to recurrent stresses Figure 2



Three categories of responses to recurrent stresses are observed which reflect:

- An independent response i.e. the response to one stress (single stress, SS) is not modulated by prior stress exposures
- A negative memory effect i.e. when recurring stresses (RSS) lead to amplified (negative) responses
- A positive memory effect i.e. when a first stress exposure (priming event) helps prime the plants and better cope with subsequent stresses.

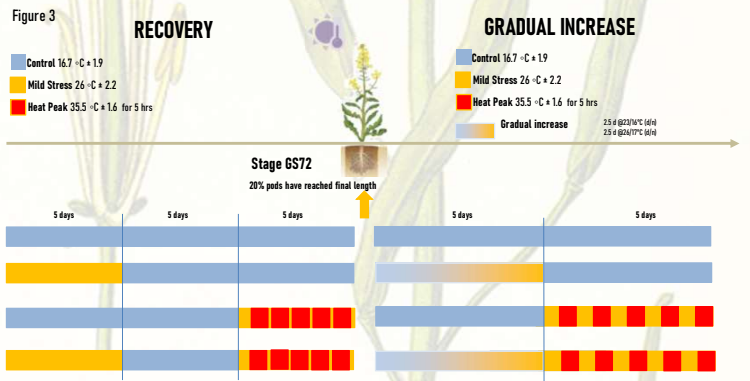
The last category raises the questions of (i) the characteristics of the stressing event to be priming and (ii) the molecular players that account for priming effects and acclimation.

Objectives, Experimental design and Analyses

Our objectives were to (i) induce a thermoprimer effect to alleviate the negative impacts of later intense heat stress on seed yield and quality criteria and (ii) to identify physiological pathways and epigenetic markers that account for positive memory (priming) or negative memory (cumulated effects). Two sets of experiments were designed such that the effects of an early mild stress event followed by a recovery phase (EMS+R) or a gradual increase (GI) in temperature prior to 5 day-intense heat (IH) were tested.

Seed yield and quality criteria were measured to assess any priming effect of EMS+R or GI prior to the IH. In order to consider the age of pods at the time of stress application, measurements were also performed at the cohort level.

For the GI experiment, global and targeted proteomics analyses were performed by Mass Spectrometry following technical adjustments (Le Franc, in prep.) to quantify the abundance of histone post translational modifications (histones PTMs). Clustering and Gene ontology (GO) analyses were carried out to highlight the physiological processes involved in response to GI-induced priming.



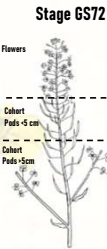
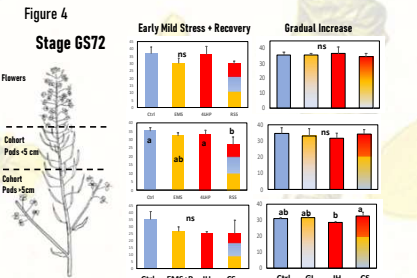
Plant performances: seed yield and quality criteria

Effects at the whole plant scale Table 1

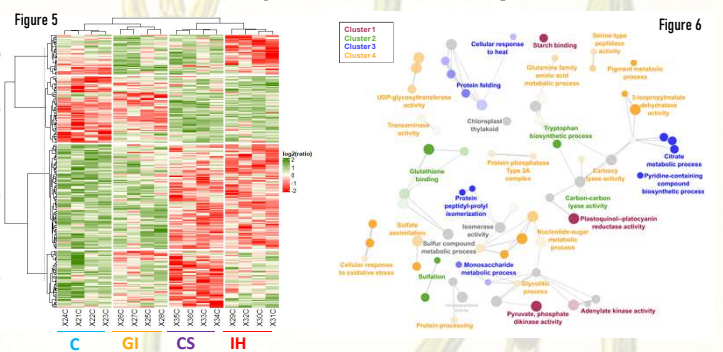
Plant Variables	Ctrl	CS	EMS	IH
Seed yield (g.pl ⁻¹)	7.66	-21%	-38%	+1%
Lipids content (%DW)	35.81	-21%	-17%	-7%
Unsaturated Fatty acids (%DW)	32.97	-22%	-17%	-7%
Saturated Fatty acids (%DW)	2.84	-16%	-13%	-1%
Nitrogen contents (%DW)	2.62	+32%	+21%	+12%

Plant Variables	Ctrl	CS	GI	IH
Seed yield (g.pl ⁻¹)	9.40	-3.6%	-8.5%	-10.0%
Lipids content (%DW)	33.84	+0.2%	-3.9%	-8.5%
Unsaturated Fatty acids (%DW)	31.28	+0.1%	-3.9%	-8.7%
Saturated Fatty acids (%DW)	2.56	+2.1%	-4.2%	-8.9%
Nitrogen contents (%DW)	3.3	+1.5%	+6.0%	+8.2%

Effects at the cohort level: Fatty Acids (FA) concentration (%DW) Figure 4



Global and Targeted (histone PTM) proteomics



Priming effects were mostly observed in the Gradual Increase, modality while negative memory effects (cumulated) were observed in the Early Mild Stress + Recovery modality (Table 1).

Most differences among modalities observed at the whole plant scale are accounted for by a given cohort of pods: the intermediate cohort of pods at the time of pre stress application for the EMS+R experiment and the oldest cohort for the GI experiment (Fig. 4)

These observations lead to conclude that (i) there is a narrow phenological window (in relation with the age of the seeds) for the pre stress to be priming which reflects the need to synchronize the "priming" event and the building processes of the seed storage compounds and (ii) there is a specific duration of the recovery phase over which the mild stress induced memory mechanisms are reset (Lamke and Baurle, 2017).

Clustering (Fig. 5) and GO (Fig. 6) analyses reveal 4 clusters of proteins that were differentially represented according to our modalities.

- Cluster 1 proteins are responsive to the duration of stress : CS vs. [control, GI, IH]
- Cluster 2 proteins are responsive to GI (alone or combined with IH). They involved in stress tolerance acquisition and are involved in S metabolism
- Cluster 3 proteins are more abundant in modalities that include IH (alone or combined with GI).
- Cluster 4 is a mirror of Cluster 3. It gathers proteins that are less abundant under intense heat stress (CS and IH) and that are involved in oxidative stress.

In addition, significant enrichment of three histone PTMs by MS was highlighted : H3K27me1, H3K9me1 and H3K4me1, the later being more abundant under IH that is, in proteins of cluster 4 (not shown).

Conclusions and Perspectives

Thermo-sensitization protocols are difficult to picture in indeterminate growth species due to mixed age organs (and hence different extent of stress sensitivity) and compensation effects (plasticity). Designing thermo-sensitization protocols to maintain seed quality are likely to be criteria specific as a consequence of a required synchrony between the priming event and the quality-related building processes. S metabolism is involved in thermo-sensitization acquisition which raises the question of methylation mediated regulation with S-Adenosylmethionine (-CH3 donor). Several PTMs of histones were associated to heat stress responses and more specifically H3K4me1 which was found to be more abundant upon intense heat. Chromatin Immuno Precipitation sequencing (ChIP seq) analyses are in progress which will allow heat stress (memory) responsive genes to be associated with the over/under abundance of targeted histone PTMs.