**HEATSTOP PROJECT**

**Report on the 1st, 2nd and 3rd two-month periods**

Period: 30/11/2023 – 31/05/2024

**M1 – EFFECTIVE PROJECT MANAGING**

Both RUs are collaborating to the achievement of the first milestone, M1 – EFFECTIVE PROJECT MANAGING, that will be completed at the end of the project, as scheduled in the GANTT. A kick-off meeting took place on 01/11/2023 as a part of the ***A1.1: Administrative project coordination***, during which the two groups planned the research activities envisaged by the HEATSTOP project, and they signed the partnership agreement (***D1.1: Partnership agreement***). The financial matters are being addressed in compliance with MUR requirements, through a constant dialogue between the Universities administrations and the RUs.

The two RUs are both independently and conjunctly addressing all the technical aspects of the project realisation, as part of the ***A1.2: Technical project coordination***, that will be in place for the whole duration of the project, with a bimonthly evaluation of the progresses made, summarised in this report.

**M2 - SELECTION OF TWO CULTIVARS OF THE SAME SPECIES FOR THE STUDY, ONE HS-TOLERANT AND ONE HS-SENSITIVE**

Both RUs are participating in the achievement of M2- SELECTION OF TWO CULTIVARS OF THE SAME SPECIES FOR THE STUDY, ONE HS-TOLERANT AND ONE HS-SENSITIVE. First, a screening of the commercially available cultivars of rapeseed (*Brassica napus* L.) and tomato (*Solanum lycopersicum* L.) was made by the Tor Vergata RU, in compliance with ***Activity A2.1: Screening of commercially available and genetically characterized cultivars of rapeseed and tomato****.* Concerning rapeseed, since seeds of the spring varieties were not immediately available from any supplier, only winter varieties were screened, although their life cycle is one month longer since it requires vernalisation. However, the vernalisation had been planned in the project GANTT, hence it will not impact on the timely realisation of the project. Tor Vergata RU screened a total of 35 winter varieties of rapeseed, ultimately selecting two cultivars, F1 hybrids, produced by Società Italiana Sementi (SIS): Dariot as the heat-stress sensitive, and Phoenix as the heat-stress tolerant. This choice was made according to the features declared by their breeder (*i.e.*, medium dimensions, suitability for indoor cultivation, high yield, different sensitivity to abiotic stresses) and to preliminary lab tests performed by the two RUs (***D2.1: List of the four suitable cultivars***).

Likewise, 15 different cultivars of tomato with determinate growth were screened by Tor Vergata RU, selecting the Dwarf Big Rio (F1 hybrid, Blumen) as the heat-stress sensitive cultivar, and the Ramino Special (F1 hybrid, L’Ortolano) as the heat-stress tolerant, according to the features declared by their breeders (*i.e.*, small dimensions, suitability for indoor cultivation, short life cycle, different sensitivity to heat stress) (***D2.1: List of the four suitable cultivars***).

Seeds of the four cultivars selected by Tor Vergata RU were then bought by UniBo RU and sown according to published protocols, as part of ***A2.2: Selection of the model species***. Briefly, seeds were sterilised in 0.75% sodium hypochlorite for 5 minutes and then germinated in Petri dishes with 100% humidity. Upon germination, the plants were re-potted in a seedbed using draining soil with sand, humus, and ultrafine peat. Plants were grown in standard conditions with 16 hours of light, 8 hours of darkness, and a constant temperature of 22 °C.

After 45 days, rapeseed plants reached the rosette stage (stage 30 on the BBCH phenological scale). Thus, they were transferred into pots with a diameter of 5 cm, using peat soil, and they were vernalised in a growth chamber by lowering the temperature of two degrees every two days, until reaching a constant temperature of 4 °C. The vernalisation lasted 40 days, according to literature, and afterwards the temperature was gradually brought back to 22 °C. 26 days after the end of vernalisation, rapeseed plants entered the flowering stage. Fresh pollen was collected from the anthers using a brush and it was immediately stored at -80°C. Each rapeseed flower was estimated to produce around 0.5 mg of fresh pollen. Brushed anthers were then dried at room temperature for 12h, the dried pollen was collected using a sieve and stored separately at -80°C.

Pollen viability of fresh pollen after freezing and thawing was high (90% on average). The elevated pollen productivity of rapeseed plants allowed us to optimise the germination protocol, reaching a maximum germination rate of 35% for both cultivars. Pollensomes isolation was performed according to literature. The quantification of pollensomes by NTA has not been performed yet due to the delays in order processing that are affecting the administration of the University of Bologna. Nonetheless, we were able to quantify the protein content of such pollensomes to demonstrate their successful isolation.

Tomato plants have been transferred into pots with a diameter of 20 cm in peat soil, and they were grown in standard conditions. After four months, they entered the flowering stage. The collection of fresh tomato pollen using a brush proved impossible, hence the anthers were dried at room temperature for 12 h and stored at -80°C. However, the quantity of pollen per flower produced by tomato was notably lower than the one of rapeseed, and the total pollen isolated proved insufficient to perform any experiment.

For these reasons, we selected rapeseed as the ideal plant model for our study (***D2.2: Germination rate and PS quantitation for all the cultivars***).

**M3 – IMPOSITION OF THE HS REGIME ON BOTH CULTIVARS**

UB is currently working on the activity ***A3.1: Plant cultivation in standard and HS conditions.*** Around 40 plants per rapeseed cultivar were cultivated from seed to the rosette stage (49 days), vernalized for 40 days, and eventually grown in standard conditions until reaching the phenological stage GS61 (54 days). At this stage, half of the individuals from each cultivar were randomly selected for the heat stress imposition (HS plants), while the others were assigned to the control group (CT plants). For both groups, the treatment lasted ten days, in which CT plants remained in standard conditions, while HS plants underwent a gradual increment of temperature (+2.6°C per hour), from 22°C to 35°C, remained at 35°C for four hours, and then gradually returned to 22°C with a temperature decrease of -2.6°C per hour. Photosynthetic efficiency was measured on five leaves per plant with a chlorophyll fluorimeter in five time points, starting from the day before the beginning of the treatment and finishing two days after the end of the treatment (***D3.1: Data on the photosynthetic efficiency and duration of the life cycle for all the samples***). Due to the delays in order processing that are affecting the administration of the University of Bologna, it was not possible to purchase the necessary equipment for the activity ***A3.2: Breeding and seed measurements***, which wasthus postponed to BIM6.

**M4 – EVALUATION OF MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN POLLEN UNDER HS**

To compensate for the delays caused by administrative issues on some activities, UB advanced other activities which did not require to buy additional reagents or equipment, in order to guarantee the timeliness of the project. In particular, UB started to set up an ESEM protocol to observe the morphology of pollen grains without altering their morphology, as a part of ***A4.2: Determination of morphological and physiological signals of stress induction in pollen.***

**M9 – DISSEMINATION OF HEATSTOP MILESTONES AND RESULTS**

Both RUs are working towards the achievement of the last milestone, M9 – DISSEMINATION OF HEATSTOP MILESTONES AND RESULTS, which spans the whole project, as detailed in the GANTT.

In particular, the teams are working on the activity ***A9.1: Dissemination to the public and the stakeholders.*** A website dedicated to the project (<https://site.unibo.it/prinheatstop>) was created by the two RUs on UniBo portal, depicting the milestones and the activities of the project, and portraying the team members. Social pages about the project have been created on Facebook, Instagram, and X platforms. An undergraduate student of the master's degree course in Teaching and Communication of Natural Sciences (University of Bologna) is being enrolled for an internship on the project. The student will be trained on the project milestones, activities, and results, and they will manage the website and the social pages as part of their internship. Aims, methodologies, and the results of HEATSTOP will be presented during the Fascination of Plants Day 2024, a science dissemination event that will take place in the Botanical Garden of Bologna from 18 to 19/05/2024.

The activities carried out in this two-month period did not cause damage (in accordance with the **DNSH principle**) to any of the six relevant environmental objectives (i.e., climate change mitigation, climate change adaptation, sustainable use and protection of water and marine resources, the circular economy including waste prevention and recycling, the prevention and reduction of pollution, the protection and restoration of biodiversity and ecosystems). Furthermore, the Project complies with the relevant EU and national environmental legislation and does not include so-called "brown" research activities in accordance with the EU Commission Communication 2021/C 58/01 "Technical guidance on the application of the DNSH principle".