UNTWIST

UNCOVER AND PROMOTE TOLERANCE TO TEMPERATURE AND WATER STRESS IN CAMELINA SATIVA

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRANT AGREEMENT NO 862524

D6.1

REPORT FROM THE SAMPLING WORKSHOP

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Abstract

The primary objective of the UNTWIST sample workshop was to discuss and provide consensus methods for camelina samples to be handled during the project. The workshop addressed the following subjects: seed cleaning and handling, plant growth conditions in the glasshouse and field, definitions of camelina phenological stages, sampling and storage methods, and sample identification and tracking.

The sample workshop defined and agreed a uniform seed cleaning method for camelina comprising four steps. Several parameters for plant growth conditions in glasshouse and in the field were decided, including sowing density, fertilization, plot design. The workshop further agreed an outline of a pilot experiment for drought stress in greenhouses and growth chambers, as well as for defining the appropriate silique developmental stage for sampling during drought stress. Camelina phenology was also determined and agreed between partners in line with internationally published standards. Addressing the question of sample exchange between partners, the workshop also defined how samples will be collected, named, shipped, and tracked.

The outcomes of the workshop were to establish standardization of camelina culture, but also to provide sample collection, shipping and storage guidelines. These guidelines were adapted to the different collecting and analyzing needs that also defined sample metadata displayed in UNTWIST database. The UNTWIST sample identification and tracking system will provide an unambiguous and versatile method for sample quality control and traceability all along the project. These procedures are essential to define sample handling across partners, and also to establish reference methods for the community.

Disclaimer: The opinions expressed on this deliverable are those of the author(s) only and should not be considered as representative of the European Commission's official position. This public deliverable is a revised version of D6.1 Report from the sampling workshop (submitted by M3), updated as requested by reviewers in the 1st project periodic assessment.

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1. Introduction

An unprecedented amount of new data will be collected within the UNTWIST research programme, comprising genomic, epigenetic, transcriptomic as well as metabolomic data together with phenotypic data under normal and abiotic stress regimes in diverse European locations. The project comprises a highly complementary set of experts in their field, contributing multiple different data sets. As such the project is highly integrative, making use of the analytical and plant growth capabilities of the partners. Consequently, sustainable and robust sample tracking is a major challenge to be addressed in the project. Records will provide details of sample location, sampling time, metrological information and technical versus biological replicates, collectively this will allow for proper statistical evaluation and modelling. Thus, the first task is to deploy sample identification and tracking guidelines.
At the initiation of the project, a sampling workshop was planned to ensure that each laboratory is growing and sampling plants using the same methodology. The workshop took place on 20th October 2020 by video conference and was coordinated by INRAe. The workshop provided agreed methods for: (1) seed cleaning; (2) standard growth conditions; (3) identification of camelina growth stages and seed maturation for sampling; (4) sampling times and storage methods; and (5) sample identification and tracking.

2. Results

2.1 Meeting agenda

The workshop gathered 15 researchers from the UNTWIST partners AIT, INRAe, RRes, FZJ, UNIBO, and CCE. The workshop agenda was developed to discuss and provide agreed methods for: (1) seed cleaning (for Tasks 1.1 and 1.3); (2) standard growth conditions (for Tasks 2.1 to 2.4: glasshouse and field); (3) identification of camelina growth stages and seed maturation for sampling (for Task 2.3) as defined for camelina; (4) sampling and storage methods e.g. snap freezing in liquid nitrogen of samples to be analyzed in WP3; and (5) sample identification and tracking; including the type and amount of meta data (for Tasks 6.3). Several meetings involving two or more partners, either producing samples and/or analysing them, were subsequently held to further detail sample handling rules.

2.2 Seed processing

The seeds of the different camelina accessions used during the UNTWIST project will be processed according to a standard protocol designed by UNIBO with four steps:

- After harvest, plants are left dry for several days and manually or mechanically disrupted to open siliques and release seeds;
- Seeds are manually sifted through sieves of different sizes (typically an aperture range of 3mm to 1.5mm) to remove straw, siliques and weed seeds;
- Seeds are air cleaned to remove siliques debris, dust, and soil particles. Options for air cleaning include a commercial air cleaner, home-made 3D printed device or manually;
- Finally, hand cleaning with tweezers is used to remove the last bits of dust, weed seed or soil.

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Seeds are then stored in a cool and dry room and shipped in sealed paper/plastic bags.

2.3 Plant growth

RRes provided an outline of the greenhouse culture set up for inducing drought stress. Drought will be assessed by measuring Soil Water Content (SWC). Soil probes will be tested as a means of tracking water content. An experimental set up will be developed such that each plant will correspond to a biological replicate. Sampling will be carried out in scintillation tubes (Polyvials - natural HDPE) that should not be filled more than 1/3 and kept partially unscrewed for snap freezing and storage in liquid nitrogen. A pilot experiment is underway at RRes to define the precise experimental condition that will be used during UNTWIST project drought experiments. The pilot experiments will determine the appropriate standard soil composition, the soil water content for control plants e.g. 50% field capacity, the soil water content defined as drought, the time it takes to reach the drought condition, the time (in days) between growth stage BBCH15 and 18, the mass of aerial tissue available for harvesting/sampling, and the impact of abiotic stress on leaf mass.

UNIBO defined an outline for field trials that should be kept free from weeds with shallow sowing (0.5-1cm deep) at 500 seeds/m^2. Nonwoven fabrics could be used to cover the plots for 1-2 weeks to enhance emergence. Soil analysis will have to be carried out for each field trial condition, subsequent adjustments will need to be made for fertilization and irrigation. It was decided that no pre- or post-emergence herbicides or pesticides will be used during the trials as the camelina genotypes might respond differently.

2.4 Plant phenology

Camelina phenology will be defined as previously published [1] and an operative atlas describing the different developmental stages was provided and circulated by UNIBO.

The consortium agreed that the vegetative stages BBCH 15 and 18 are described by the number of internodes (excluding cotyledons, leaves >1cm) and that 50% flowering means that 50% of the plants have produced at least 1 open flower (petals fully developed outside the sepals).

A pilot experiment to analyze the effect of drought stress on seed maturity will be carried out by RRes by analyzing fatty acyl profile of the different siliques along the stem.

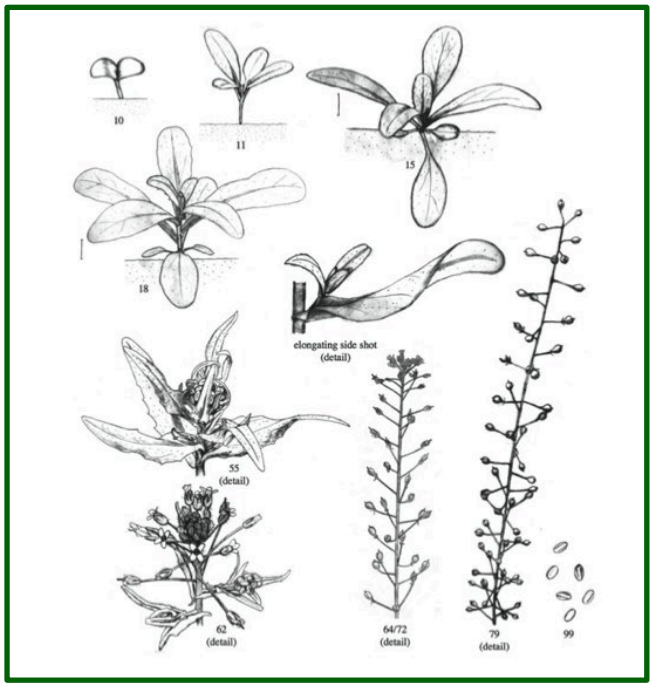

Figure 1 Examples of phenological stage and BBCH number[1]

[1] Martinelli and Galasso, Ann Appl Biol 158 (2011) p 87 – 94.

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2.5 Sample handling

2.5.1 UNTWIST sample flow

The UNTWIST project is characterized by an intricate flow of samples that are provided by participating partners who grow, sample and ship material to the analyzing partners. UNWIST gathers five partners producing and collecting samples (RRes, UNIBO, AIT, INRAE Versailles and CCE) and six partners involved in the analysis of the samples (RRes, UNIBO, AIT, INRAE Versailles, FZJ and INRAE Bordeaux). Some partners are involved in both processes like RRes for lipid analysis or UNIBO for carbon isotopic studies. A total of eight different types of analyses will be carried out: genomics (FZJ), epigenomics (FZJ), transcriptomics (AIT), proteomics (RRes and AIT), metabolomics (INRAE Bordeaux), lipid quantification and profiling (RRes and INRAE Versailles), cellular redox status (AIT), carbon isotope discrimination (UNIBO). Considering the complex nature of UNTWIST sample and analysis organization, several meetings gathering partners involved in plant production/sample collection and sample analysis were setup to define how the samples will be identified, collected, stored and shipped. The actual flow of samples between partners was structured according to the analysing partners specifications, defining the quantity and type of tissues, the collecting method and the type of shipping. In most cases, the sample flow was rather simple with plant producing partners sending the samples to one or several analyzing partners. To minimize the number of samples to be collected and shipped, samples for metabolomics and cellular redox status were first shipped to INRAE Bordeaux to be ground and aliquoted for metabolomics then reshipped to AIT. The characteristics of the different samples were structured according to the nature of the analysis carried out, the type of analysis, sample description (including sample name, origin, type of culture, plant stage, sample type, amount needed), harvest & processing method, harvest date & time of day, way of shipping and storage.

2.5.1 Sample identification and tracking

The samples were identified differently according to the plant producer location and whether it concerned the core collection involving large number of samples (>500) or the focus lines (<200). Since, there was no printable solution (including “freeze resistant labels”) that would be resistant to liquid nitrogen, small enough to fit sample tubes and easily accessible to all plant producing partners, the consortium decided to use hand written human readable codes that would be simple enough to avoid writing errors but that could provide minimal information for identifying the sample.

For large set of samples (>500, core collection), it was however decided that single number would be kept for the sake of simplicity and time saving (minimizing collecting times). One plant defined one biological replicate per line and treatment). Each plant was for instance individually numbered 1 to 810 and the same labelling was used for the vials and whole leaf dried material. Together with the samples, an excel file (email, print out) was provided to the analyzing partner with the information linking each number 1-810 with on the line, treatment, replicate number.

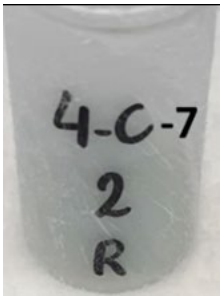

Figure 2 : Example of sample labelling on a scintillation vial indicating the focus line number (4) – the treatment (control condition, C) – days of stress (7) – the replicate number (2) – the destination partner (R, RRES). Source RRES

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For smaller of samples (<200, focus lines), UNWTIST consortium agreed that more information should be associated with the sample code for easier identification. The characteristics of the different samples were structured according to the nature of the analysis carried out and gathered in a document (table) detailing type of analysis, sample description appeared as a very important information as it defines sample origin but also its destination (*e.g.* F, A, I, R). Other important feature was focus line name, the culture conditions (*e.g.* well-watered vs drought), sampling time (*e.g.* 7 day, 15 days from start of stress), sampled tissues (*e.g.* silique, top, middle, bottom), experimental position (*e.g.* lysimeter number), and the replicate number. Hence the location is kept not only in the short sample name but also usually in freezer boxes etc. In addition, detailed sample tables accompanied samples in a physical form (*i.e.* a printout) as an additional measure to make sure that samples are not confused and that data in the database can thus be rectified in an updated version.

Sample metadata also included the developmental stage, quantity in each replicate, whether it is pooled tissues, the harvest and processing method, harvest time, storage and shipping conditions. All information were gathered and will be available in UNTWIST database.

2.5.2 Sampling methods, storage and shipping

Several sampling handling methods were used according to the type of analysis carried out. For carbon isotope discrimination, fresh plants were dried at 60°C, finely ground and immediately analyzed. For genomics, epigenomics transcriptomics, proteomics, metabolomics, lipidomics, cellular redox status, samples were snap frozen in liquid nitrogen and stored at -80°C. Oil content (NMR) and profile (FAMES) were carried out on dry mature seeds were stored in the dark in low humidity at 4°C.

Samples stored at -80°C were shipped with dry shipper while seeds were sent at room temperature by express mail.

3. Conclusions

The partners agreed the following protocols at the sample workshop: (1) methods for seed cleaning and for defining camelina phenology; (2) definitions for sample and tissue collection; (3) the development and implementation of different sample identifying system; (4) the challenges of applying drought stress and the need to define, using a pilot experiment, the parameters for the drought stress experiment in greenhouses including the appropriate silique developmental stage at which the stress will be applied; and lastly (5) the amount and type of meta data to be collected. The overall outcome of the workshop was to provide a defined set of operational procedures for experimentation and exchange within the consortium.

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