MINUTES OF THE 4th DARKWIN ANNUAL MEETING

Date	12/11/2024
Hour	09:30 h
Venue:	Virtual attendance: https://conectaha.csic.es/b/ang-bss-mfo-glx
Attendees:	Attendees are listed in ANNEX 1.
End of the meeting	14.00 h

Content:

Adoption of agenda

The agenda was adopted in the form shown in these minutes.

1. <u>Welcome to all attendees:</u> the project coordinator Prof. Francisco Pérez Alfocea welcomed all partners and thank for their presence at the meeting.

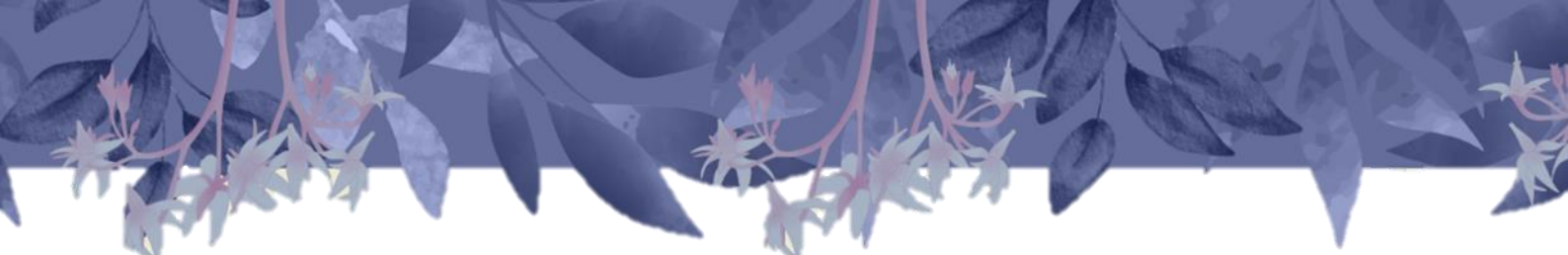2. <u>Introduction of the project DARkWIN by the project Coordinator</u>

   F. Pérez Alfocea recalled the objective of this meeting, that is to check the progress of project tasks and potential problems.

   -Overview of the project: the project is in month 23, succeeded in the first Reporting Period (RP) and Interim Payment, there is an upcoming report on month 24, and a meeting with PO and experts from the EU Comission, scheduled for February 6th, online.

   -Regarding the workflow: WP1 and WP2 have been fully achieved, currently the project is focused on WP3, which is a central work package in the project. WP4 will start next month.

   -Technical objectives have not changed, the first three technical objectives have been completed. WP3 involves three technical objectives and at this moment the team is analysing data obtained during the first trial.

   -About dissemination and exploitation objectives: the objective number four is already achieved, as it consists on dissemination of the project in fairs, congresses and project´s activities are disseminated by the uploading and sharing of videos. It is needed to keep working on contacting potential customers, and to

continue with publications and Intelectual Property plan (at least one patent is expected from this project).

- Deliverable 3.1 sent has been approved by the EU Commission, there are two more pending (D3.2 by CSIC-EBD and D5.9 by DORIANE partners). The coordinator asked for having the corresponding drafts by mid-December for checking them before submission.

- Next Deliverables are 4.1 from WP4 by April 30th (responsibility of CEBAS-CSIC) and D3.3. by the end of the year (from MPIMP).

-Two milestones have been achieved, the third is ongoing.

-The project coordinator reminded the recommendations by experts: to keep budget under control and keep contacting stakeholders for the road to the market.

-Regarding risk assessment and contingency plan: three risks affecting WP3 still active, data is currently on analysis yet.

-There are sixty days to present the RP and payments (two months). Doriane and Novedades partners already received 85% of the payment but still have to justify the whole payment.
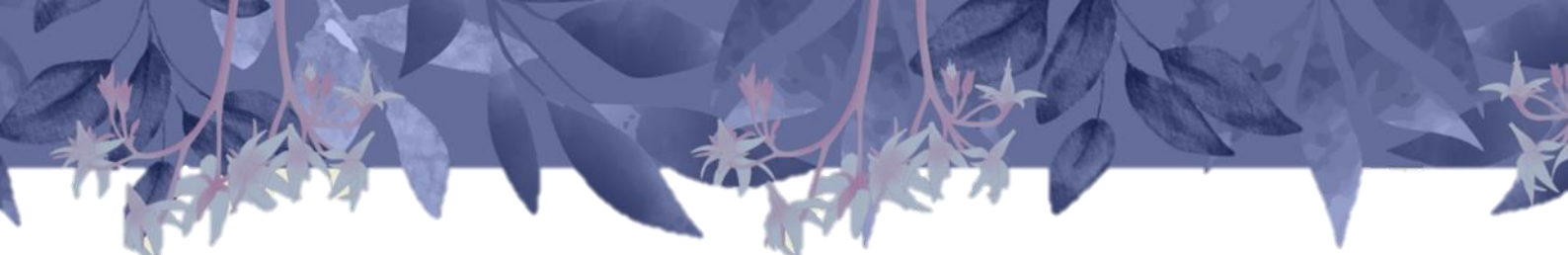
-There has been only one amendment regarding subcontracting that has been approved.

3. <u>Work Package presentations</u>: The work package (WP) leaders presented the completed tasks so far, the state of unconcluded tasks, issues solved and clarified questions to the rest of the partners.

WP2 – Pollinator assisted phenotyping platform under combined stress: *lead partner Juan Pardo Martínez* (NOVAGRIC)

Partner Tristan Duminil from DORIANE presented Task 2.3 from WP2 (and 3.8 from WP3) about data acquisition, notation, and management system integrated into a breeding software:

-Tristan talked about challenges and difficulties found in the analysis of bumblebees' visits, as bugs into algorithms for data transformation that were identified thanks to the help of partner Antonio (CSIC-CAR) and the delay into producing graphics that is faced currently. A meeting for ETL software demo and integration into the breeding software is pending.

-To date, WP3 trial design has been introduced in the breeding software, being structured in three information levels as: Level 1 containing genotypes, Level 2 containing sector information and Level 3 describing each plant. The information of each antenna is found at Level 2 and bumblebees' visits will be stored there as well. The rest of parameters will be located at Level 3 of the software. Finally, information at different levels can be aggregated. Currently, number of replicates per genotype are represented and statistics is ready; next step is centralizing data and finish building the dataset.

-Partner Tristan asked for feedback of the rest of partners and dropped some questions for them, as when flower phenotyping data will be available, dates and experimental design for the next season trial and possible algorithms to apply in the analysis.

-The rest of the partners answered: agro-physiological and pollinator data will be collected during the new experiment and suggested that Doriane could propose new algorithms to analyse pollinator preference, may to be discussed in another meeting.

-The partners agreed to use codified data for genotypes.

Partner Jose Ángel Martín from CSIC-CEBAS talked about Task 2.5, consisting on the training staff for operating phenotyping platform:

-A draft for management of the phenotyping platform, including the regulation of climatic conditions for each sector separately and pollinator managing is being completed in Spanish.
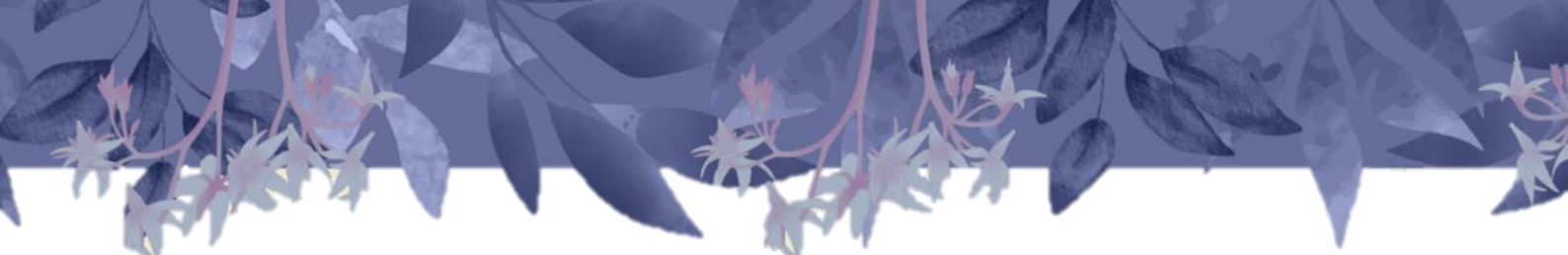
WP3 – Nutritional, metabolomics, transcriptomics, and genetics of GxP under climate change scenario, *lead partner: José M. Jiménez* (CSIC-CBGP)

Partner Antonio Jiménez (CSIC-CAR) talked about Task 3.2, regarding phenotyping by bee choice.

-The outline of this task included: set up (plant-antenna distribution), detections (by sequential reading in each antenna) and preferences (based on spatio-temporal choices). Each antenna is identified by a reader number plus an antenna number and in total 336 plants were monitored.

-RFID detections: All signals were centralized in a computer located at the greenhouse, using a software developed by the CAR team. All data is captured daily in .csv files and sent to Doriane store online. More than 1 million detections were registered.

-Transformation of detections to visits: Antonio developed, in Matlab environment, another version of ETL Doriane Pollinator software to analyse pollinators´ visits to double check information obtained in the greenhouse. Visits are filtered based on different parameters (matching those of Doriane software) based on visit duration, short/long visits, or interval between visits.

-Antonio showed a plot of bumblebees' activity over the 24h daily cycle. Partners discussed the convenience of including nocturnal visits in the analysis, and concluded that data must be explored more extensively.

-Some descriptive data given: average visit duration and most frequent duration, and differences between control and treatment environments.

-Regarding pollinator preference, different metrics were shown and they were used to list genotypes tested in a ranking for visualization of the most preferred genotypes under different environments.

-Partner Antonio reminded that data shown was not exactly the same presented in D3.1, as issues were detected in visit accumulation as mentioned by partner Tristan.

Partner Ángela Sánchez from CSIC-CEBAS presented Task 3.3, about the phenotyping of agronomical and physiological parameters:

- A chronogram is shown to illustrate analysis carried out in parallel to RFID detections during the trial: flower phenotyping, flower sampling, physiological parameters related to photosynthetic capacity in different ways and breeder-like phenotyping of plants and fruits, as well as harvesting, yield recording and soluble-solids content (SSC).

- Number of flowers decreased over time and in response to the treatment, differences between isogenic lines were detected.
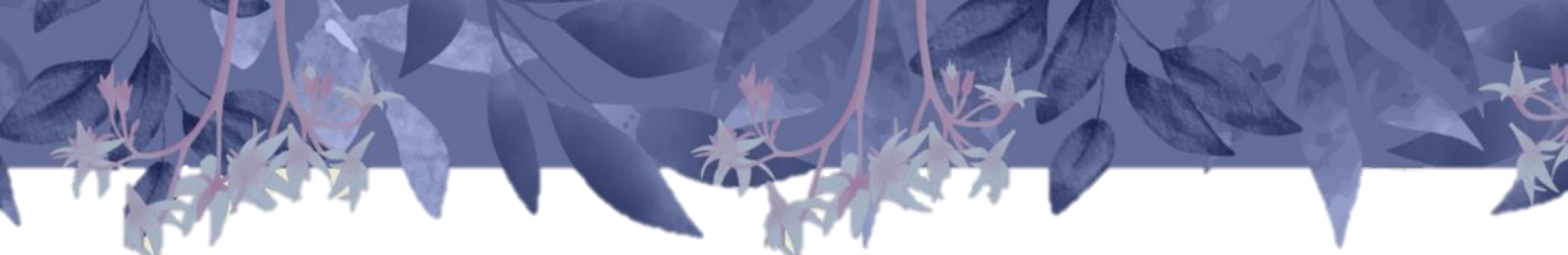
- Correlation analysis indicated that: plants under stress developed less and smaller fruits but with higher SSC, fruit yield evolved in detriment of vegetative growth, evapotranspiration was higher under climate change but other photosynthetic-related parameters varied between control and climate change samples depending on days after treatment.

- All tasks were completed with minor deviations, as fruits from self-pollinated plants could be analysed during the next season.

Partner Ignasi Bartomeus from CSIC-EBD talked about first results obtained in Task 3.4, regarding the phenotyping of flower morphological traits.

-More than 1,000 flowers were evaluated in terms of flower size (petals' diameter an central cone height). Flowers under climate change were smaller for diameter, but not such big differences were detected for cone height upon the treatment. Diameter variations was quite large within the same genotype, although some genotypes were more affected by the treatment.

-Pollinator marks were also scored in sampled flowers and results pointed to a preference of bumblebees for larger flowers.

-Next step is to analyse pollen quantity and viability by flow-citometry, and also the pollen delivery under a vibration frequency equal to that of the bumblebee while it is extracting pollen from the flower. Also pictures taken could be analysed for assessing differences in flower color.

-Partner Ignasi answered some questions from the rest of the partners, as that the anthers/pistil ratio could be calculated from phenotyping data, and independence can be assumed between each bumblebee's foraging decision, but learning and communication within and out the nest is important.

-CEBAS and EBD partners will keep coordinated for preparing the Deliverable 3.2.

Partner Guillaume Decros from MPIMP presented the scheduled plan for Task 3.5: flower metabolic, hormonal and nutritional signatures.
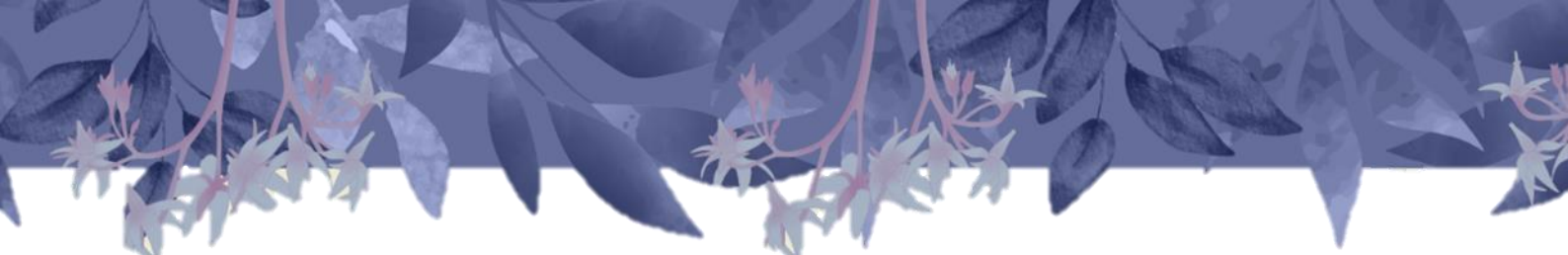
-There are two main axes in this task: Gas/Liquid Chromatography coupled to Mass Spectrometry (GC/LC-MS) for primary and secondary metabolites, which is not started yet, and volatiles (VOCs) analysis, whose preliminary analysis were made in collaboration with project partner Juan José Guerrero (CSIC-CEBAS).

-Samples were received on month 22 of the project and were subjected to Head Space Solid Phase Micro Extraction (HS-SPME). Three conditions were tested: 300 mg of intact flowers, 100mg of intact flowers and 100mg of grinded flowers diluted in calcium chloride. Also influence of the incubation time was checked. The conclusion was 100mg of intact flowers are enough and better results are expected after a 2h incubation time.

-As molecules in leaves can be potentially involved in bumblebees' attraction leaf samples were also tested, for which partner Guillaume did not recommend to run samples directly after thawing. An increase in the number of signals was detected under treatment (salt) conditions.

-Next step is the annotation of the detected compounds, to know if there are known or unknown: a fully untargeted strategy will be applied at MPIMP to measure as much compounds as they can to see how much of them can be identified with high confidence afterwards.

-Saved material will be sent back, so the rest of WP3 partners could have a backup copy of flower samples. Draft of Deliverable 3.3 is expected to be ready during the first half of the next year.

Finally, partner José Jiménez (CSIC-CBGP), WP3 leader, presented the Task 3.6 and 3.7 plan for transcriptional and genetic analysis of the mapping population subjected to climate change conditions:

-The partner José Jiménez considered good news to see variation in the population phenotype for detecting QTLs in the genetic analysis.

-Flower samples arrived at CBGP and RNA has to be extracted. RNA-extraction kits have been tested and a kit was selected that works well. The estimation is that in a month or month in a half RNA samples will arrive to the sequencing company and in three months he could start the analysis.

-Results depend on the quality of tomato annotation. The reduction in number of lines involves lower resolution, which means more genes to look at. Confirmation of phenotypes during next season is highly recommended, and backcrosses with cv. Moneymaker parental are needed for fine mapping task.
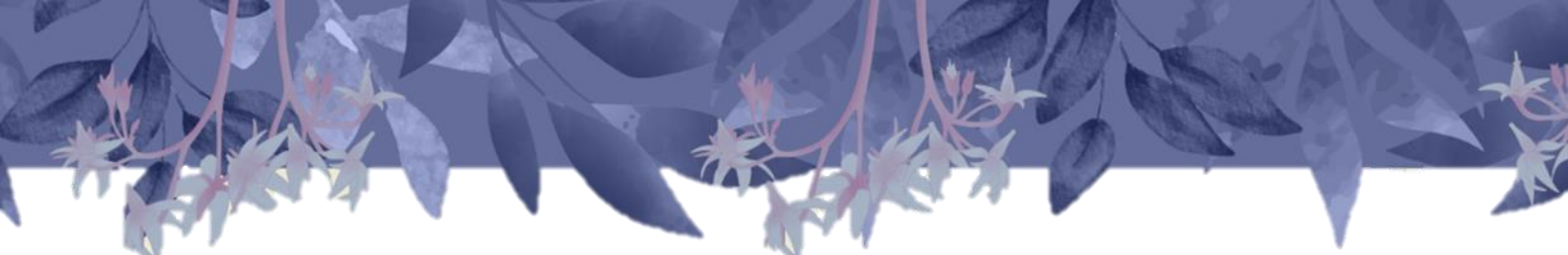
WP4- Proof of concept: pollinator selected F1 varieties, *lead partner: Aquilino Sánchez* (UNISEM)

Partner Jose Ángel Martín (CSIC-CEBAS) presented Task 4.1, about phenotyping and selection of tomato parent lines based on breeder and pollinators' preferences and Task 4.2, regarding the generation of F1 hybrids:

-Partner Jose Ángel explained that inter-sector differences registered this season at the greenhouse will be solved in the next trial. The low repeatability difficulted the obtention of statistical significance in the comparative analysis. The proposal is to reduce the number of lines to be assayed in the next trial but increasing replicates (meaning the location of four antennas per genotype and sector) and to increase climate change sectors (four) while reducing the control ones (two).

-Regarding isogenic lines from WP3 to generate F1 hybrids (by crosses with UNISEM elite and breeding lines), four lines -instead of two- selected by the pollinator and/or breeder are being considered. Elite and breeding lines from UNISEM will be reduced to two and eight, respectively, making a total of 56 F1 hybrids generated by UNISEM over next season. Hybrid seeds will be ready by next June. Both WP3 selected lines and UNISEM lines will be grown and evaluated for pollination and agrophysiological parameters next season at the WP2 platform.

-From the hybrids generated, 14 will be phenotyped at the WP2 platform. The project coordinator suggested to use same tomato kind lines from UNISEM lines (for instance cherry-type) to reduce variability in number of flowers and flower size. These hybrids are expected to be pre-commercial lines to the market.

-WP3 leader José Jiménez suggested to include the WP3 parental line cv. Moneymaker in the crosses to be made by UNISEM, which is accepted by WP4 leader, partner Aquilino.

WP5 – Project Management, Dissemination, and Communication, *lead partner: Francisco Pérez-Alfocea* (CSIC-CEBAS)

Partner Maialen Ormazabal (CSIC-CEBAS) reviewed the Task 5.4 about dissemination and communication activities:

-Regarding publications: CEBAS team in collaboration with partner Antonio (CSIC-CAR) published one scientific article which is among the top recommended in Research Gate during September and another one is under review.

-Project partners attended several symposia and fairs to showcase the Darkwin project, including the Science Week at Murcia, thanks to which the number of Instagram followers considerably increased, and more than 2,000 Instagram accounts were reached. On the other hand, the project got more than 1,000 followers on LinkedIn and top accessions to the website are from United States followed by Spain and China.

-The project workshop celebrated on June attracted 13 companies and four of them are still in contact with project partners for further collaboration.
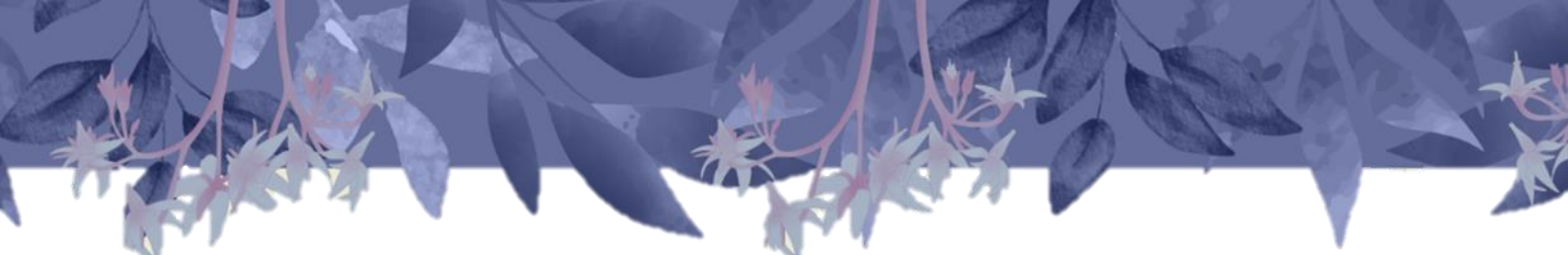
-The project was disseminated through TV and the EU Deepsync platform. Also, a Youtube channel has been launched and the CEBAS team started to collaborate with a TikTok influencer with more than 360,000 followers.

-Finally, collaborations between project partners continue as partner Juan José Guerrero (CSIC-CEBAS) is currently working on his PhD stay at MPIMP under supervision of the WP3 partner Alisdair Fernie and Juan actively collaborates with project partner Guillaume.

-Partner Maialen recalled the need to help the project network growing and gave some recommendations to do it.

Finally, the project coordinator reminded the Project and Management administration tasks (included in Task 5.1; 5.2 and 5.3) to the rest of the partners:

-The RP2 (month 13-24) is a precondition to receive the payment and has to be submitted 60 days after the end of each RP. It consists in two parts: Part A which is automatically generated by the system and part B which is a narrative part uploaded by the coordinator.

-The coordinator asked for partners´ input by January 17, as the Technical Report (part B) draft needs to be submitted two weeks before RP2 meeting (January 22).

-If RP2 approved, they payment will be received after 90 days from RP2 end, if rejected the clock will start over again.

-All beneficiaries will receive a notification and must contribute to the Technical Part for part B, complete their own Financial Statement, sign it by PFSIGN and submit it to the coordinator.

-The coordinator showed the RP2 Agenda which is focused on WP2, WP3 and also WP4.

-The coordinator asked the partners for innovations to get them included in the EU Comission Innovation Radar, to which the project already applied with several innovations.

-Also the coordinator recalled the compromise to complete the roadmap to the market.

-The review of the project will have place with the new PO, Lazaros Perakis, assisted by the already known experts Elena Alexandrovna Fesenko, Fred Stoddard and Maurizio Trovato.

-About the next Interim Payment, 100% of costs implemented until December 31 will be reimbursed within the limit of 90% pre-financing.

The next Annual Meeting (5th) of the project is scheduled for Spring 2025, partners need to decide if it will be held online or presential.

All partners are invited to organize the meeting in case they were nterested.

The meeting closed at 14:00h.
Screenshot
End of the 4th Annual Meeting

ANNEX 1

Attendees 3rd Annual Meeting 06/06/2024	Partner
1. Antonio Jiménez	CAR-CSIC
2. Francisco Pérez Alfocea	CEBAS-CSIC
3. Ángela Sánchez	CEBAS-CSIC
4. José A. Martin	CEBAS-CSIC
5. Maialen Ormazabal	CEBAS-CSIC
6. Juan José Guerrero	CEBAS-CSIC
7. Cristina Martínez-Andújar	CEBAS-CSIC
8. José María Jiménez	CBGP-CSIC
9. Félix Martínez Rivas	CBGP-CSIC
10. Ignacio Bartomeus	EBD-CSIC
11. Francisco de Paula Molina Fuentes	EBD-CSIC
12. Aquilino Sánchez	UNIGENIA
13. Juan Pardo	NOVAGRIC
14. Guillaume Decros	MPIMP
15. Tristán Duminil	DORIANE