

Summary Report of the
Annual meeting of the
European Evaluation Network (EVA) for Lettuce
24-25 May 2022
Salsomaggiore Terme, Italy



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The second regular project meeting of the EVA Lettuce network was held in person on 24-25 May 2022 in Salsomaggiore Terme, Italy. The agenda of the meeting is attached as [Appendix 1](#) and the list of participants as [Appendix 2](#).

1. Welcome and introduction

Massimiliano Beretta, research director of co-host ISI Sementi welcomed participants to Salsomaggiore Terme, expressing delight at finally meeting most project partners in person. The location of the meeting was chosen due to its proximity to ISI Sementi headquarters in Fidenza (PR), which the group was invited to visit at the end of the meeting. He noted the large amount of work done over the first two years of the project and was looking forward to animated discussions on next steps during the current project and a future outlook on a possible continuation of the consortium.

The EVA coordinator Sandra Goritschnig opened the meeting, welcoming also participants connecting remotely. She expressed the hope that the hybrid format of the meeting would allow all participants to benefit equally from the presentations and discussions.

After a round of introductions, S. Goritschnig provided an overview of the status of all currently active EVA networks. She reminded partners of the [website of the EVA Lettuce network](#) and the project extension until November 2023, granted by the German donor to compensate for COVID-19 related delays and difficulties.

2. Results from the EVA Lettuce project

Chair: S. Goritschnig

S. Goritschnig gave an overview of the agreed work plan for the current project, which is following a two-phased approach. During the first phase, 178 accessions of cultivated lettuce (*Lactuca sativa*) from four genebanks had been characterized in three field trials in Switzerland and Greece in 2020; these accessions were also regenerated as single seed descent (SSD) lines for phase 2. Based on lettuce typology and homogeneity of the accessions a subset of 61 accessions was selected for in-depth evaluation of SSDs in 10 field trials in multiple locations across Europe in 2021 and 2022; the first results of the trials in 2021 were presented during the meeting. In parallel, 50 wild lettuce accessions had been evaluated in duplicate lab trials for resistance to several *Bremia lactucae* strains, and several resistant accessions, which could be interesting for lettuce breeding, were identified. All multiplied SSDs and several wild lettuce accessions were genotyped using a newly developed method, Single primer enrichment technology (SPET), which yielded interesting results and could become a useful tool for the lettuce research community (see below).

Gabriella De Angelis presented a summary of the *Bremia* lab trials conducted at ISI Sementi. She explained the experimental and scoring protocol used, which follows the IBEB standard. Results were confirmed in a duplicate trial conducted by Limagrain. A related news item had been published in 2021 on the ECPGR website¹.

Tizian Zollinger, Charlotte Aichholz and Christos Vasilikiotis reported on the field trials they had conducted at their locations for phase 1 and phase 2 and presented some interesting preliminary results. The 2021 field trial at Sativa Rheinau allowed observation of *Bremia* in the field, although the identity of the strain(s) was not determined. They also observed interesting bolting phenotypes and some residual heterogeneity even in the SSDs of the accessions. Annette Haegnefelt

¹ <https://www.ecpgr.cgiar.org/resources/latest-news/news-detail/eva-lettuce-network-identifies-promising-mildew-resistant-wild-lettuce-accessions>

commented that heterogeneity in leaf phenotypes is commonly observed in Iceberg types and may be caused by transposons, making it hard to be eliminated. T. Zollinger conducted their phase 1 trials indoors while the phase 2 trials were conducted in the field. The phase 2 trials at the American Farm School in Greece were conducted in a shade house. C. Vasilikiotis observed that birds pulled out newly planted seedlings to reach worms underneath. Interestingly, both trials did not have significant bolting. With reference to scoring homogeneity, C. Vasilikiotis noted that different growth rates of accessions may affect scoring and suggested using image analysis for more exact scoring over the entire development. He also presented Multiplex Fluorescence Sensor (MFS), a non-destructive tool for measuring anthocyanin levels in the leaves, which is also used on grapes.

Pasquale Tripodi presented an initial analysis of the genotyping using SPET. SPET allows decomplexing genomes with a focus on specific regions and has been successfully used in different crops. The new lettuce SPET panel developed in collaboration with IGA Tech is based on SNPs identified in a genome resequencing study². The generated dataset identified ~2 million polymorphisms, of which more than 81,000 SNPs with density of one SNP each 28kbp passed the quality filtering, and can be used for phylogenetic and population analyses. The fact that SPET uses a sequencing approach for the genotyping allowed observing heterozygosity in sequenced accessions. Preliminary population analysis clearly separates the wild lettuce accessions and allowed the grouping of accessions into six sub-populations based on taxonomy and typology. Excluding the wild accessions prior to principal component analysis as well as considering available multi-crop passport descriptors (MCPD) data may allow refining of the genetic groupings. The next analysis steps include assessing different filtering criteria and refining statistical analysis approaches. Combining genotypic and phenotypic data will require reliable data for accessions from both approaches, thus any missing data may need to be complemented through additional genotyping or field trials. Because of the collaboration with IGA Tech, the panel is in principle publicly available, but the EVA lettuce network will benefit from priority pricing also in the future.

3. Data management

Chair: S. Goritschnig

S. Goritschnig presented the standardized data collection template, which had been developed in parallel with the EURISCO-EVA intranet, to be used by all EVA networks for direct upload of phenotypic data into the database. Several iterations of the template had been circulated at various times, but the current version should be considered the final one compatible with the automatic uploader. Stephan Weise, calling in remotely, presented the upload process and responded to questions from the audience. During a hands-on session, partners provided feedback on the portal, which will be communicated to the EVA developer for implementation. Accession metadata in the database can also include pictures, and partners agreed to include photos of lettuce accessions, depicting representative field plots, cut heads, and top and bottom cuts. Partners were reminded that phase 2 trials were evaluating SSD lines of the original accessions used in phase 1 and should be recorded using the assigned EVA-IDs for the accessions.

S. Goritschnig reminded partners that the EURISCO-EVA intranet was designed to only hold raw phenotypic data, although an extension for SNP viewing may be feasible. Genotypic data and

² <https://doi.org/10.1038/s41588-021-00831-0>

analysis results will be shared via project-specific platforms and ultimately deposited in public repositories.

4. Data analysis

Chair: M. Beretta

Following up on the presentation of preliminary results, participants engaged in brainstorming and discussions about suitable data analysis approaches. The goal is to assess the environmental effects and heritability of the different traits evaluated and potentially link them with genotyping data in a genome-wide association studies (GWAS) analysis. Partners were reminded that most selected plant material was suitable for open field and that head formation consequentially may not be easy to score in greenhouses.

Damien Peltier agreed that Limagrain would conduct an initial statistical analysis on the phenotypic data from the different trials, which would be the first step in assessing data quality and comparability of the different datasets. A. Haegnefelt noted that it could be interesting to correlate day length and bolting tendencies, also days to maturity would likely be affected by the environment. I. Kalfas suggested considering environmental data in statistical analysis by downloading weather data from European public databases of each trial location. Parameters such as relative humidity of air and soil, soil and air temperature, degree days, day length or light intensity could be gathered for each location and help make the data from different trials comparable. Furthermore, it was noted that day-degree data could be the right parameter to consider a correlation with bolting. G. Briand also suggested normalizing bolting data in comparison to reference varieties, to account for differences in development in different locations and seasons.

A priority would be now to upload trial data to the EURISCO-EVA intranet. C. Vasilikiotis noted that outliers present in a dataset could be discarded to simplify the statistical analysis.

Based on the quality assessment of individual trials, the network will decide on how to proceed with the data analysis, considering whether data can be combined or additional trials should be planned.

The current datasets are likely not suitable for GWAS. However, a marker-associated analysis may be feasible.

5. Outlook – EVA Lettuce 2.0

Chair: M. Beretta

5.1 Dissemination and exploitation of results

S. Goritschnig informed participants about several opportunities to present the EVA networks to different stakeholders, having participated in international (online) conferences and workshops. Such outreach is an important aspect of the project, providing information to potential new partners and donors, and also reaching other stakeholders and the general public.

Partners agreed that publication of the new lettuce SPET panel together with some population genetics data from the EVA accessions in a scientific paper was a priority, as it is a useful tool for the lettuce research community and represents a significant output of the EVA lettuce network. Other phenotypic data, especially disease-resistance results, should remain embargoed and not

be used for publication immediately, also considering that publication of those would likely require additional data generation.

M. Beretta informed participants of a planned conference, the 10th EUCARPIA meeting of Leafy Vegetables, which will be held in the Netherlands in 2023. This meeting could be a good opportunity to present EVA Lettuce results (e.g. the SPET panel) and potentially also plan a next network meeting in conjunction.

Other public outreach activities included an article about the meeting on the Italian website of *Fresh Plaza*, the global trade media platform for the fresh produce industry³, as well as an article on the EVA Lettuce network in the Italian horticultural newsletter *Fresh Cut News*⁴.

5.2 Discussion of potential continuation of the EVA Lettuce network

Meeting participants engaged in brainstorming discussions on potential common goals for a continuation of the network. All partners agreed that their collaboration so far has been very fruitful, not only generating large amounts of phenotypic and genotypic data but also providing a platform for the exchange of knowledge and expertise.

Rob van Treuren reminded partners of the initial intention to perform GWAS, which was abandoned because of the reduced sample size, but could be considered in a follow-up project. P. Tripodi estimated that data from around 150 accessions would be needed to perform a meaningful GWAS analysis. Partners suggested that, with a focus on limited traits with high heritability, a larger number of accessions could be managed at various locations.

Partners from private companies noted that they would not be interested in collaborating in very competitive activities, such as generating pre-breeding material for *Bremia* resistance. However, they agreed to continue collaborating on the evaluation of relevant traits such as research of new *Bremia*, bolting and tip burn resistance. Investigating insect resistance under controlled conditions on a new panel of lettuce accessions was also considered an interesting area to study.

Partners were also interested in exploring qualitative traits such as bitterness, taste and texture, which could be evaluated using tasting panels or metabolomics, however, with associated additional costs.

S. Goritschnig provided an overview of the funding situation. In the current project, Germany funded the coordination of the networks, the development of the EURISCO-EVA intranet, as well as network-specific activities. For the EVA Lettuce network, genotyping and project meetings are covered by project funds. Partners contribute in kind to regenerations, evaluations and data analysis. If partners continue their in-kind contributions, funds for a follow-up project would need to be identified for coordination, genotyping and meetings, and any other experiments that may require funding. A closer collaboration with the ECPGR Leafy Vegetables Working Group (WG) may facilitate identifying additional partners and financial resources.

In the EURISCO database, more than 10,000 cultivated and wild lettuce accessions are documented but only a fraction of these have associated C&E data. These may be available for future evaluations; thus it was considered a useful approach to get more members of the Leafy Vegetables WG involved in the project and increase the number of participating genebanks.

R. van Treuren presented an overview of the Dutch collection at CGN, which holds over 2,500 lettuce accessions, including a substantial collection of wild lettuces as well as modern varieties. Collecting missions to Jordan and Uzbekistan have identified additional material which is being

³ <https://www.freshplaza.it/article/9431948/la-lattuga-al-centro-di-un-incontro-internazionale/>

⁴ <https://www.freshcutnews.it/2022/06/03/isi-sementi-eva-lettuce-costruiamo-la-lattuga-perfetta/>

added to the collection over the coming years. A special lettuce collection of 470 SSD lines, most of which have been resequenced, is currently being used in the research project '[LettuceKnow, Science-Based Improvement of Salad](#)' for phenotypic and transcriptomics analyses. He also noted that their *L. serriola* collection is currently being screened for *Bremia* resistance (BI32-BI35, US9) in a project with private partners.

E. Vasileva presented the lettuce collection of the genebank at IPGR Sadovo in Bulgaria. Their collection of Bulgarian and worldwide accessions mainly of *L. sativa* include cultivars and breeding lines. Limited morphological phenotyping data has been collected but is not available in electronic form. A large variety of wild lettuce accessions grow *in situ* in Bulgaria, but collection missions have not been undertaken.

6. Conclusion and next steps

Based on the discussions during the meeting, the following agreements, action points and open questions were defined:

6.1 Current activities

- Genotyping data analysis is a priority, focusing on cultivated lettuce accessions and preparing a publication of SPET panel in collaboration with IGA Tech. Activity led by P. Tripodi.
- Final field trials of Phase 2 will be concluded by end of summer 2022. Priority is to upload all trial data into EURISCO-EVA using the correct format.
- Phenotypic data analysis of the field trials will initiate with statistical analysis to assess the quality of data of individual trials, conducted by Limagrain (led by D. Peltier). Based on this analysis, a decision on how to combine data and whether additional trials should be planned will be taken.
- All phenotypic data remains under the embargo period of three years from when it is first entered into EURISCO-EVA.
- Data analysis would focus on marker-associated analysis, as the panel is not large enough for GWAS.

6.2 EVA Lettuce 2.0

- GWAS on some traits (to be defined) could be done on all sequenced accessions, depending on the final quality of genotyping data and perhaps discarding some accessions with low-quality genotyping data.
- New traits of interest for taste and quality should be considered, also the need for new partners to contribute to their evaluation.
- Wild lettuce accessions which can be introgressed into cultivated lettuce may be interesting for testing for *Bremia* resistance. *L. virosa* may also be useful to screen for insect resistance. Partners will review their collections or the EURISCO database to identify additional accessions that could be used in the project.

6.3 Dissemination

- Focus on the publication of the lettuce SPET genotyping panel, which should be timed to also be presented at the 10th EUCARPIA meeting of Leafy Vegetables in 2023.

6.4 Plant materials

- The SSD lines generated during regeneration in the EVA project are currently stored by Limagrain but could be sent back to the genebanks that provided the original material. R. van Treuren indicated that CGN could take the lead in storing the whole collection, with a minimum of 100 seeds per SSD line needed for safety backup.

Appendix 1. Meeting agenda

Venue: Hotel Villa Fiorita, Salsomaggiore Terme

TUESDAY 24 MAY		
08.45 – 09.00	Registration	
	Welcome and introductory session	CHAIR: M. BERETTA
09.00 – 09.10	Welcome by ISI Sementi, ECPGR	<i>M. Beretta, S. Goritschnig</i>
09.10 – 09.20	Introduction of participants	<i>All</i>
09.20 – 09.30	Overview of the current status of the ECPGR Evaluation Network EVA	<i>S. Goritschnig</i>
	Results from EVA Lettuce project	CHAIR: S. GORITSCHNIG
09.30 – 09.40	Review of network work plan 2019-2023	<i>S. Goritschnig</i>
09.40 – 09:50	Lab tests on wild lettuce for <i>Bremia</i> resistance	<i>G. De Angelis</i>
09.50 – 10.20	Field trials – Phase 1 (2020/2021) – Phase 2; past and ongoing	<i>T. Zollinger, C. Aichholz, C. Vasilikiotis</i>
10.20 – 10.40	Preliminary results of Lettuce genotyping	<i>P. Tripodi</i>
10.40 – 11:00	TEA/COFFEE BREAK	
11:00 – 11:45	Discussion	<i>All</i>
	Data management	CHAIR: S. GORITSCHNIG
11.45 – 12:00	Review of data collection templates	<i>S. Goritschnig</i>
12:00 – 13.00	Hands-on session with EURISCO-EVA intranet	<i>S. Goritschnig</i>
13:00 – 14.30	LUNCH	
	Data analysis	CHAIR: M. BERETTA
14.30 – 15.45	Brainstorming and discussion on data analysis – possible questions, approaches, volunteers	<i>All</i>
15.45 – 16.00	TEA/COFFEE BREAK	
16.00 – 17.30	Planning of data analysis work pipeline for 2022/23	<i>All</i>
20.00	SOCIAL DINNER	

WEDNESDAY 25 MAY		
	Outlook – EVA Lettuce 2.0	CHAIR: M. BERETTA
09.00 – 09.45	Dissemination and exploitation of results	<i>S. Goritschnig</i>
09.45 – 10.30	Proposals for EVA Lettuce 2.0 – continuation of network activities Lettuce accessions in European genebanks	<i>M. Beretta, S. Goritschnig R. van Treuren, E. Vasileva</i>
10.30 – 11.00	TEA/COFFEE BREAK	
11.00 – 12.00	Discussion on continuation of network activities after end of current project (Nov. 2023)	<i>All</i>
12:00 – 12.30	Wrap-up of meeting	<i>S. Goritschnig</i>
12.30 – 14.00	LUNCH	
14:30 – 16:30	Field trip to ISI Sementi	<i>M. Beretta</i>
16:30	End of meeting	

Appendix 2. List of participants

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