

Summary Report of the Annual meeting of the European Evaluation Network (EVA) for Lettuce

**31 August – 1 September 2023
Wageningen, the Netherlands**



Table of Contents

1. Welcome and Introduction	3
2. Results from EVA Lettuce project	3
3. Outlook – EVA Lettuce 2.0	5
4. Action points	7
Appendix 1. Meeting agenda.....	9
Appendix 2. List of participants	10

The third regular project meeting of the EVA Lettuce network was held in person on 31 August- 1 September in Wageningen, the Netherlands. The agenda of the meeting is attached as [Appendix 1](#) and the list of participants as [Appendix 2](#).

1. Welcome and introduction

Theo van Hintum, head of the Centre for Genetic Resources of the Netherlands (CGN), welcomed the EVA Lettuce meeting participants in Wageningen. CGN is part of Wageningen University and Research (WUR) and as such well located at the intersection of conservation, research and use. CGN manages animal, plant and forest genetic resources, with a strong focus on horticultural crops in their collection. Funding is secured by the Dutch government, and the CGN budget has recently doubled, highlighting the importance of genetic resources in government planning. With the increased budget CGN is hiring new staff and planning to move to a new, larger building. He emphasized the interest of CGN in participating in initiatives such as the EVA networks, which connect the genebanks and their collections with breeders and end users.

Rob van Treuren, genebank curator at CGN, also welcomed participants and provided an overview of the activities at CGN. The genebank is actively collaborating with the private sector to finance collecting missions and also to regenerate accessions, as there is not sufficient capacity for this at WUR/CGN. The lettuce collection at CGN is growing via collecting missions and also has been well studied through projects involving industry, such as LettuceKnow.

The EVA coordinator Sandra Goritschnig opened the meeting, welcoming also the participants connecting remotely. She noted that several network partners had also attended the EUCARPIA Leafy Vegetables conference in Utrecht, where project results from EVA Lettuce had been disseminated and some contacts made with potential new partners for the network. She highlighted the expected outcomes of the meeting, which included a discussion on the current results, a detailed work plan for the project until December 2024 and a proposal for continuation of the network after the end of the current funding period.

2. Results from EVA Lettuce project

During a general project update, S. Goritschnig informed participants of a project and budget extension granted by the donor at the end of 2022, which allows the current project to run until December 2024 and provides additional funds to support data analysis and communication activities and to implement a single-nucleotide polymorphism (SNP) viewer to display genotypic data. In 2024, ECPGR is also starting a new 5-year phase (Phase XI), and the EVA networks have been firmly integrated into the ECPGR work programme for the next five years, with guaranteed funding for coordination until 2026. The EVA networks are also growing, an EVA Legumes network is currently under development through the grant scheme activity "[Fostering the need of implementation of the ECPGR European Evaluation Network \(EVA\) on Grain legumes](#)" ([ForEVA](#)), of the ECPGR Grain Legumes Working Group under the leadership of Creola Brezeanu. This project has 25 project partners (mainly genebank curators and researchers), has attracted more than 25 stakeholders (private and public sector breeders and researchers) and will develop a work plan and budget for the future EVA Legumes network during a meeting in Bucharest in October 2023.

Some personnel changes in EVA Lettuce partners Gautier Semences, ISI Sementi, NordGen and Zollinger were announced and will be reflected in the project database. Massimiliano Beretta, scientific lead of the network and instrumental in developing the work plan of the network had to reduce his involvement in the project due to a change in his role at ISI Sementi, and all network partners appreciated his dedication and impact on the project.

A major output and dissemination activity of the EVA Lettuce network is the recent article describing the '[Development and application of Single Primer Enrichment Technology \(SPET\) SNP assay for population genomics analysis and candidate gene discovery in lettuce](#)', published in *Frontiers in Plant Science*, one of the most impactful journals for plant research¹. This work was also presented at the EUCARPIA Leafy Vegetables conference² through an oral presentation by S. Goritschnig and a poster presented by Damien Peltier and has received a lot of interest and positive feedback. Pasquale Tripodi, the lead author of the article and mainly responsible for the analysis of the described data, also presented this work at the 66th Annual Congress of the Italian Society for Agricultural Genetics (SIGA) in Bari, Italy³. This work was further promoted on the ECPGR and EVA websites⁴, on social media⁵ and through Eurekalert⁶, targeting international media outlets, enhancing the impact of the EVA Lettuce project globally.

S. Goritschnig reminded partners of the agreed work plan for the current project, taking into account the project extension until December 2024, which will allow the network to complete the current data collection and strengthen the association analyses made possible with the SPET genotyping data.

P. Tripodi reviewed the genotyping analysis and highlighted some aspects that were not included in the publication and could inform future work of the EVA Lettuce network. One of the main advantages of SPET is that through the probe design, the genotyping can focus on genes and also identify SNPs in gene clusters, thus the SPET panel covers gene regions to 96%, compared with SNP arrays that have a lower number of SNPs associated with genes (e.g. pepper SNP array 45%) or other reduced representation library approaches that provide only random coverage of the genome (e.g. genotyping-by-sequencing (GBS)). This is especially important when performing genome-wide association studies (GWAS) as it increases the likelihood of identifying markers directly associated with genes, as described in the paper. In general, the collection represents a good diversity panel, again important for GWAS, although the provenance and typology are perhaps sometimes misleading, especially for breeding material. He noted that together with the phenotypic data and photographs taken of the accessions, the typology of some lines in the EVA collection could be corrected and should be communicated to the responsible genebanks to review the passport data. The typology of research material could be associated with one of the parents; using the SPET data might enable the identification of shared fragments

¹ Tripodi P, Beretta M, Peltier D, Kalfas I, Vasilikiotis C, Laidet A, Briand G, Aichholz C, Zollinger T, Treuren Rv, Scaglione D and Goritschnig S (2023) Development and application of Single Primer Enrichment Technology (SPET) SNP assay for population genomics analysis and candidate gene discovery in lettuce. *Front. Plant Sci.* 14:1252777. doi: [10.3389/fpls.2023.1252777](https://doi.org/10.3389/fpls.2023.1252777)

² <https://www.uu.nl/en/research/eucarpia-leafy-vegetable-conference-28-31-august-2023-in-utrecht-the-netherlands>

³ <https://sigannualcongress.it/>

⁴ <https://www.ecpgr.cgiar.org/resources/latest-news/news-detail/single-primer-enrichment-technology-a-new-genomic-resource-to-investigate-the-diversity-of-lettuce-germplasm>

⁵ <https://twitter.com/ECPGR/status/1698604069818958182>

⁶ <https://www.eurekalert.org/news-releases/1000089>

associated with certain lettuce types. The traits which had been included in the SPET paper (seed colour, leaf colour and bolting time) had shown high heritability, which could compensate for some missing data, as only one trial had evaluated all accessions simultaneously. For the GWAS analyses, the trials were analyzed separately to account for missing data, the quality of the phenotypic data was considered most crucial and also applying several statistical models to the GWAS was deemed important to identify robust associations. In order to make the best use of the current dataset, he suggested analyzing other traits collected by the network on the current EVA lettuce collection and also moving forward, additional accessions from other collections could be genotyped and combined with the current dataset.

D. Peltier highlighted the importance of ensuring the reusability of the generated SNP data and interoperability with future datasets. One of the advantages of SPET is that target regions are consistently recovered, thus allowing the same regions to be sequenced in new samples. The newly discovered SNPs in the dataset are closely linked to the target SNPs; it should therefore be possible to identify also these in new experiments. He also suggested investigating how well the SPET probes cover conserved gene clusters, such as the major resistance loci.

Ilias Kalfas suggested investigating whether the genotyping could identify genetic signatures or genomic regions specific to the different lettuce typologies, which could be responsible for some typical characteristics within the different types or different behaviour depending on the growing season. Franco Donati noted that the main outcome of the current study is that butterhead types could be clearly separated from the others based on SPET genotyping.

D. Peltier presented his work on statistical analysis of phenotypic data collected during trials from 2020 to 2022. The data can be categorized based on accessions (species, typologies), traits (agronomic, morphological, stresses), scoring scales (continuous, nominal), and trials (locations, time series, replicates), all of which are important when considering the appropriate statistical method to analyze the data and improve association analyses. Specifically, it will be important to decide on how to process trait scores of replicates or time series during an experiment, whether these should be averaged or not. Also, not all traits have been observed in all trials or on all accessions; it would be good to discuss if missing data should be complemented through additional trials. Observations follow different distributions, depending on the trait, with continuous variables showing normal distributions. Detailed analysis of traits per trial will be done to determine whether enough data is available for association studies, and then ANOVA analyses will be done to look for gene–environment (GxE) effects due to environmental differences in trial locations before running GWAS. One approach to normalize across locations could be to transform data against controls and use relative values. P. Tripodi noted that even traits that may not look very diverse in the collection could yield strong associations, e.g. leaf colouring was only observed in few accessions but had a clear presence/absence occurrence. This analysis should also help define traits that are useful and easy to score for future trials. Furthermore, participants noted that accessions scored sensitive to *Bremia* in any field trial, should be considered susceptible for further analysis, especially given that the *Bremia* races present in the field are usually unknown.

P. Tripodi proposed to publish the results of GWAS and GxE analyses on agronomic traits from the current collection, taking advantage of the existing genotyping data.

3. Outlook – EVA Lettuce 2.0

S. Goritschnig reminded participants that the EVA project has become an integral part of the ECPGR work programme for the upcoming Phase XI (2024—2028), securing funding support for coordination and data management. Funding for network activities (e.g. genotyping, evaluations) would need to be secured from external sources for a new project or provided by partners through in-kind contributions as before. During the EUCARPIA Leafy Vegetables conference, several participants expressed interest in EVA Lettuce, wishing to become partners in the network. These collaborations would provide access to other existing lettuce collections in the UK (Vegetable Genetic Improvement Network VEGIN⁷) or Spain (Vegetable Germplasm Bank of Zaragoza), for which some characterization and evaluation (C&E) data have already been collected.

Rebecca Stevens presented the INRAE lettuce collection, which has been partially characterized through collaborations with French breeders also involved in regeneration activities. A paper describing the INRAE vegetable collection and its use in genetics and breeding has recently been published⁸. She noted that the paper lists the full collection, part of which is reserved for the collaboration with breeders and only the national collection with accessions from France and of national interest is freely available and documented in EURISCO. Currently, work is ongoing to curate the C&E data for upload into the INRAE database Florilege and subsequently connect to EURISCO.

Partners decided to divide the discussions on work planning into short-term activities that could still be done within the current project to complement existing data, and topics that could be part of a future project EVA Lettuce 2.0 and brought to potential new partners for discussion.

In 2024, project partners could plan field trials with the existing materials to either complement the current dataset, fill missing datapoints or add datapoints or traits that could be interesting for GWAS analysis. The current single-seed descent lines (SSD) subset used in Phase 2 could also be extended based on genotyping data, to collect additional data on those accessions. I. Kalfas suggested that it would be good to include in field trials traits that have a real impact on marketability and are most useful for farmers. These priority traits could then also be tested on the next accession set in EVA Lettuce 2.0. Breeders noted that bolting time, tip burn sensitivity, anthocyanin in leaves and heart formation are very important to be scored in the field. New traits of interest could be the shape of the bottom (important for machine harvesting), bottom cut (particularly in butterhead), leaf count/weight of the head or the percentage of useful parts of the head (weight of leaves without core or stem), both directly related to yield.

Partners were asked to confirm their capacity for additional trials in 2024 and provide detailed descriptions of the proposed new traits. Seed stocks of the accessions should also be checked and communicated. D. Peltier agreed to send ~100 seeds each of all SSD lines to CGN for safekeeping.

Some partners suggested doing *Bremia* lab trials with newly emergent strains (up to BL 40) using the available *L. serriola* accessions as well as additional ones from partner genebanks. Given that the SPET genotyping also worked well on *L. serriola* this data could be used for GWAS in that population. The current pricing for the SPET assay will need to be confirmed with IGATech, but some of the funds in the current EVA project budget earmarked for lettuce data analysis could potentially be used for this activity.

⁷ <https://warwick.ac.uk/fac/sci/lifesci/research/vegin>

⁸ <https://doi.org/10.3390/plants11030347>

As concerns further planning for EVA Lettuce 2.0, partners agreed that it would be most interesting to expand to other collections and involve academic partners who might be able and interested in evaluating complex traits in laboratory settings, which could be complemented with the multilocation field trials conducted by EVA company partners. This could also be done on the current EVA collection, adding to the existing datasets. Traits of interest would be biotic stresses (e.g. Tomato spotted wilt virus (TSWV), Bremia, Fusarium), abiotic stresses (e.g. salinity, tip burn) metabolites and nutritional quality (e.g. vitamins, phenolic compounds). Complex traits such as root architecture shelf life are also of interest, but may require additional partners with the relevant expertise.

Focus should be on initial screening of collections with the possibility of generating recombinant inbred populations to be discussed within the network in the future.

In terms of financing a future EVA Lettuce 2.0 project, partners would explore possibilities from national funding agencies but also through EU funds. Proposals for future work will depend also on the possible input from new partners which should be assessed and then discussed by the enlarged consortium in 2024. The next virtual meeting to plan activities in 2024 will be held in November 2023.

After the meeting, participants visited CGN and the newly established NPEC facilities at Wageningen University and Research.

4. Action points

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

	Action	Responsible	Due date
1	Schedule calls with new staff of EVA Lettuce partners to inform and involve them in project planning	S. Goritschnig	31.10.2023
2	Confirm capacity for Field trials on SSDs in 2024	S. Goritschnig/ all partners	31.10.2023
3	Check seed stocks of SSD and availability for additional trials	D. Peltier	31. 10.2023
4	Send 100 seeds of genotyped SSDs to CGN for safe-keeping	D. Peltier/R.v. Treuren	31.10.2023
5	Develop trait descriptions for new traits proposed for field trials	I. Kalfas/F. Donati/S. Goritschnig	31.10.2023
6	Contact potential new partners for EVA Lettuce 2.0	S. Goritschnig	31.10.2023
7	Check with IGATech pricing for SPET for EVA Lettuce	S. Goritschnig	31.10.2023
8	Investigate availability of wild lettuce accessions in partner genebanks	S. Goritschnig	31.10.2023

9	Next virtual meeting	All	30.11.2023
10	Explore national funding options for EVA Lettuce 2.0	All	31.12.2023
11	Upload photos of lettuce accessions to EURISCO-EVA database	F. Guzzon	31.12.2023
	Collect revised typology and share info with responsible genebank curators	S. Goritschnig	31.12.2023

Appendix 1. Meeting agenda

Venue: WUR, Wageningen; Impulse building, [Innovation room](#)

THURSDAY 31 AUGUST		
14.00 – 14.30	Registration and coffee	
	Welcome and introductory session	
14.30 – 14.45	Welcome by CGN, ECPGR	<i>R. v. Treuren S. Goritschnig</i>
14:45 – 15:00	Overview of the current status of the ECPGR Evaluation Network EVA	<i>S. Goritschnig</i>
	Results from EVA Lettuce project	
15:00 – 15:15	Review of network work plan 2019–2024	<i>S. Goritschnig</i>
15:40 – 16:00	Genotyping analysis and discussion of SPET paper	<i>P. Tripodi</i>
15:15 – 15:40	Statistical analysis of phenotypic data	<i>D. Peltier</i>
16:00 – 16:30	Discussion	<i>All</i>
16.30 – 17.30	Transfer to Hotel by bus	<i>All</i>
19.00	SOCIAL DINNER Restaurant Magnolia , Rhenen	

FRIDAY 01 SEPTEMBER		
08.00 – 09.00	Transfer from Hotel to WUR by bus	
	Outlook – EVA Lettuce 2.0	
09.00 – 09:30	Proposals for EVA Lettuce 2.0 – continuation of network activities	<i>S. Goritschnig</i>
09:30 – 10.30	Development of work plan for EVA Lettuce network 2.0	<i>All</i>
10.30 – 11.00	TEA/COFFEE BREAK	
11.00 – 12.00	Development of work plan for EVA Lettuce network 2.0	<i>All</i>
12:00 – 12.30	Wrap-up of meeting	<i>S. Goritschnig</i>
12.30 – 14.00	LUNCH	
14:30 – 16:30	Visit to WUR facilities	<i>R. van Treuren</i>
16:30	End of meeting and Transfer to Hotel/train by bus	

Appendix 2. List of participants

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