Summary report of the

Annual meeting of the European Evaluation Network (EVA) for Wheat and Barley

17-18 November 2022
Freising, Germany

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The 3rd annual project meeting of the EVA Wheat and Barley network took place in person on 17-18 November 2022 in Freising, Germany, co-organized with the Bavarian Research Center for Agriculture (LfL). The agenda of the meeting is attached as Appendix 1 and the list of participants as Appendix 2.

1. Welcome and introduction

Peter Doleschel, head of the institute for crop science and plant breeding of LfL, welcomed participants to the meeting. Freising and Weihenstephan are the cradle of Bavarian plant breeding, with LfL having been funded in 1902. The institute is involved in many aspects along the life cycle of breeding activities, from breeding research to production, seed certification and quality control. In view of the European Green Deal/common agricultural policy (CAP) intentions, much work goes towards improving climate adaptation and reduction of input. LfL has lab and greenhouse facilities including an automated phenotyping platform that allows experiments above and below soil level. Apart from wheat and barley, LfL works on potato, forages, hops and other aromatic plants. The EVA coordinator Sandra Goritschnig opened the meeting, reminding participants of the expected outcomes of the meeting and highlighting the shared documents folder of the network, where partners can find all relevant templates and reference documents.

1.1 Overview of the ECPGR Evaluation Network EVA

The EVA coordinator presented a general update on the EVA project, which is currently funded by the German government through November 2023, with additional activities funded through participation of ECPGR in the H2020 project AGENT, which provides a third set of accessions for evaluation in the EVA Wheat and Barley network and runs until April 2025.

The EVA project has been promoted through presentations by the EVA coordinator and partners of EVA networks at international conferences, important venues to disseminate results and communicate our activities to the wider stakeholder community. As testimony to the success of the EVA approach, the ECPGR community is working on integrating the EVA project into the regular budget of ECPGR's next Phase XI, starting in 2024. Furthermore, other ECPGR crop Working Groups have expressed interest in establishing EVA networks for their crops.

The four other current EVA networks on Maize, Lettuce, Carrot and Pepper held their annual meetings in person in 2022, reviewing the results from trials conducted so far, planning next steps in their work plans with a large focus on data analyses, and discussed options to continue their collaborations, which were considered successful and beneficial for both private- and public-sector partners.

Overall, EVA networks conducted evaluation trials in more than 100 locations in 2020−2022. Over the last year, the EURISCO-EVA intranet has been finalized and data from more than 130 trials of EVA Wheat and Barley have already been uploaded. A hands-on session planned for the second day would demonstrate the main functionalities of the database.

Preliminary results from other networks highlighted the opportunity to access very diverse collections including also previously untapped genetic resources, and to collectively develop new tools for breeders and researchers.

The EVA Wheat and Barley network currently has 48 networks partners from 21 countries, including 25 breeding companies. Some partners have shifted their priorities in research and thus

decided to leave the consortium, while others have joined the network in the last year. The network website is available at https://www.ecpgr.cgiar.org/european-evaluation-network-eva/eva-networks/wheat-and-barley.

1.2 Review of EVA Wheat and Barley network work plan 2019–2024

The network workplan was reviewed and the time overlap between activities related to different evaluation cycles highlighted. The project funded by the German government provides for two cycles of evaluations of up to 1,500 accessions in total (multiplication of ~150–200 accessions per crop per geographic zone), including the development of the EVA-EURISCO intranet and genotyping of all evaluated accessions. The Horizon2020 project AGENT (Activated GEnebank NeTwork) contains a work package that allows for extending the current EVA Wheat and Barley network by providing funds for a third round of multiplications in 2022 for evaluations in 2023–2024.

In the first set of evaluations, 1,153 accessions of barley, soft and hard wheat were evaluated in 77 trials in 2021 and ~90 trials in 2022, with data curation and upload of the 2022 trials ongoing. With this first cycle of evaluations having been finalized in 2022, joint data analysis should now initiate to generate results of interest that can be exploited by breeders and public-sector researchers.

The second set contains 1,082 accessions, which are being evaluated over two years (2022–2023) in ~90 trials. Partners were reminded to provide their datafiles from the 2022 trials for curation and upload to the database by 20 December 2022 and notify the EVA coordinator of any failed trials.

The third set of accessions, provided by AGENT partner genebanks, has been multiplied in 2022 and all crop sets for the Southern zone as well as winter crops for the central zone have been distributed to evaluating partners. Unfortunately, the winter wheat multiplication at Nordic Seed was destroyed by snails; this set will be repeated in 2023 for distribution, delaying the evaluation by a year. Spring wheat and barley crops for the Nordic and central zone will be distributed in December 2022. Partners had reported some difficulties with seed shipments and communication, an issue particularly for the winter crops that have a short window between harvest and sowing, especially in the Nordic zone. Partners were reminded to ensure their shipping and contact information is up to date and multipliers were reminded to check deadlines given by partners as in some cases seed packages are centrally collected and distributed. Multipliers should provide tracking information on their shipments and the SMTA with all provided accessions (including check varieties), as this is an important bureaucratic aspect of seed exchange.

More than 3,800 wheat and barley accessions of sets 1 and 2 (including some accessions not yet included in evaluations) have been genotyped with high density Infinium SNP arrays and one plate could still be filled with samples. It was suggested to genotype the durum accessions from set 3 which had not yet been genotyped. The AGENT accessions in set 3 are being genotyped with DART-Seq (wheat) and Genotyping by Sequencing (barley). These data are not yet finalized but will be available to EVA partners in time for analysis of set 3 data.

2. Overview of Accessions in Evaluation

2.1 Review of the Accessions sets 1 and 2 and criteria for selection of material

The EVA Wheat and Barley network has selected ~4,500 accessions of seven crop types and adapted to three main geographic zones (Nordic, Central and Southern Europe) for evaluation in three accession sets. Two thousand one hundred nine (2,109) spring and winter barley accessions originating from 69 countries have been provided by 17 holding genebanks. Two thousand three hundred sixteen (2,316) accessions of spring and winter wheat from 63 origin countries have been provided by 16 holding genebanks. Durum wheat is only evaluated in the Southern zone – its 440 accessions from 41 origin countries have been provided by 7 holding institutes.

During the 2020 annual meeting, the criteria for the selection of accession sets 1 and 2 were presented and summaries can be reviewed in the presentations given during the meeting[1] and the meeting report[2]. All accessions were introduced in the EVA Wheat and Barley network as SSD lines, either already available from the holding institutes or generated by the multipliers.

IPK Gatersleben (Germany) had provided SSD lines of winter wheat, spring and winter barley, which were selected from core collections created as a result of global surveys of their collections using genotyping and initial phenotyping for yellow rust. Spring wheat accessions provided by the genebank at John Innes Centre (JIC, UK) were stabilized F4 progeny of crosses between the modern variety Paragon and accessions of the Watkins collection of worldwide landraces, selected for different geographic zones based on their predicted heading dates. CREA-GB and IBBR-CNR Bari had SSDs for barley and durum wheat for the Southern zone, mostly of locally adapted landraces, which were used in set 1. Accessions for set 2 were provided by multiple genebanks, reflecting the local diversity, and SSD lines prepared by multipliers.

During three years of multiplications, multipliers Nordic seed (Nordic zone), JKI (Central zone) and CREA (CREA-GB for sets 1 and 2 and CREA-CI for set 3) have processed between 450 and 650 accessions per crop. No multiplication is foreseen for the upcoming season, as partners should discuss how to continue their collaboration and funding should be identified for a revised work plan. Accessions for wheat and barley could be made available through the AGENT network, as more than 8,000 SSD lines have not yet been included in EVA. Similarly, JIC proposed to make available accessions from the global durum wheat panel for evaluation in a future set.

2.2 Horizon2020 project AGENT

S. Goritschnig provided an overview of the Horizon2020 project Activated Genebank Networks (AGENT)[3] (www.agent-project.eu), which had been previously presented in the 2021 annual meeting. The AGENT project is interacting with the EVA Wheat and Barley network, providing materials and genotyping for a third evaluation set from precision collections created by AGENT partners that represent unique materials conserved by the genebanks. More than 6,000 wheat

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[1] https://www.ecpgr.cgiar.org/european-evaluation-network-eva/eva-networks/wheat-and-barley/eva-wheat-and-barley-project-meetings/presentations
[2]https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/EVA_Workshops/EVA_Wheat_and_Barley_May_2020/EVA_WB_project_meeting_05052020_report_FINAL_2.pdf
[3] The AGENT project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 862613.

and almost 4,000 barley accessions have been included in these precision collections, SSD lines generated, genotyped and phenotyped for flowering time, plant height and thousand kernel weight. Additional phenotyping experiments for biotic and abiotic stresses are conducted by some genebanks on their collections. In addition, historic data collected during the regeneration of accessions from the last 70 years were compiled and processed for further analysis and integration into public databases. The precision collections were selected to reflect unique locally adapted materials and to include up to one-third of international diversity. Some genebanks contributed wild relatives of wheat and barley, which were excluded from the selection of the third set for EVA. Agroscope (Switzerland) created a precision collection of *Triticum spelta*. No durum wheat collections were created within AGENT.

S. Goritschnig demonstrated the flow of data and materials between the EVA and AGENT projects. EVA receives SSD lines and genotyping information for accessions. Genotyping within AGENT has not been finalized, the practical data flow will be clarified as soon as the analysis has been completed. Access to phenotyping data from the selected lines generated by AGENT genebanks can be provided through joint partners in the two projects. Phenotyping protocols and data collection templates generated by the EVA network have been shared with AGENT and updated to their specifications. AGENT is using FAIRDOM for initial data sharing and curation and a database portal that is modelled on the EURISCO-EVA intranet for the display of accession metadata and phenotypic data. Within the AGENT project, a FIGS analysis is planned on precision collection accessions with available geographic origin data to model and predict disease resistance phenotypes.

The bread wheat and barley accessions selected from the AGENT precision collections for the different geographic zones of EVA Wheat and Barley were chosen based on the availability of GPS coordinates of their origin, matched with the EVA geographic zones, and availability of sufficient seeds from the SSD step. Selection of a third durum set depended on limited resources from AGENT, a selection provided by ICARDA and accessions from the first EVA set (provided by CREA-GB) which were not yet evaluated. As noted above, multiplied materials have been distributed to evaluators and spring crops will be distributed in December 2022.

AGENT partner Patrizia Vaccino (CREA-CI, Italy) summarized their multiplication activities for the Southern zone in 2022, supplying both EVA network partners and the farmer's network Rete Semi Rurali (RSR). Five hundred eighty-six (586) accessions received from 9 AGENT genebanks and CREA-GB were multiplied in Vercelli, harvested by hand, threshed individually and distributed to evaluators in 4 countries. Some customs issues were identified with Turkish partners and should be resolved in the coming weeks. P. Vaccino reported issues with lodging of tall landraces, as well as some *Triticum turgidum* accessions with disarticulated spikes, which made harvesting difficult. Not all accessions yielded sufficient seed for all planned trials, but seeds were sent to EVA partners for up to 150 accessions for each crop type. RSR required larger amounts of seeds for their evaluations, a second round of multiplication will therefore be conducted by them directly on the material received from CREA-CI.

Matteo Pettiti (Rete Semi Rurali, Italy) gave an overview of the involvement of RSR in the EVA network and AGENT project, performing on-farm evaluations. He introduced the farmers' network of 32 organizations working together on biodiversity management, marketing and policy issues related to organic agriculture. They work through community seedbanks, action research such as multi-actor participatory breeding, engaging the community, e.g. developing value chains and seed systems for local varieties, and policymaking at the European level, e.g. representing the

organic seed sector in consultations for the revision of the EU seed regulation. Within the context of the AGENT project, last year's accessions for on-farm evaluations were selected from the AGENT precision sets and complemented by accessions from set 1 of EVA. Selection criteria were mainly geographic origin (focus on the Mediterranean basin) and landraces collected before 1970. The prioritized selection was requested from genebanks when the required starting material was available (~100 seeds in order to produce ~500g during the first multiplication) and multiplied by partner CREA-CI. Since seed yield was not sufficient for the whole set, a second multiplication is planned for 2023 at an organic farm close to RSR before distributing to farmers. During this multiplication, a first evaluation will be performed. Before harvest, an annual farmers' field day with a specific AGENT workshop will be organized, allowing farmers to view the material before starting their own trials.

Nine farms across Italy were identified to perform the on-farm evaluations in 2024, three conducting full trials and six evaluating partial sets, adding up to four planned evaluation sites per accession, looking at the evaluation of morphological and agronomic traits relevant to organic farming following their standard fieldbook. In addition, Prof. Stefano Benedettelli from the University of Florence will be evaluating a subset of the accessions in their experimental field.

As in the EVA network, the materials evaluated on farm are multiplied SSDs generated from landraces, which reduced the captured diversity of the landraces but allows correlation with generated genotyping data. While it may have been useful to evaluate the original landrace populations in these smaller trials, a compromise had to be found to allow the use of material from the AGENT and EVA projects. In general, during the regeneration and development of SSDs the most common type within a landrace will be enriched, so for accessions that have undergone several regeneration cycles, one line is more likely to represent the majority of the diversity in a given landrace.

3. Review of experiments and preliminary data

Partners presented preliminary results from selected trials, focusing on traits evaluated, statistics where available and discussed lessons learned and suggestions for upcoming trials.

3.1 Nordic zone

Mara Bleidere (AREI, Latvia) presented preliminary data from their trials conducted at the AREI Stende Research Centre, where there are favourable conditions for the development of different diseases. The different crop types were rotated in different fields in 2021 and 2022. Some accessions were received late and sown by hand to complement the field trial in 2022. She noted different weather conditions in 2021 and 2022, affecting the development of plants and disease pressure, and observed early lodging of accessions. Much of the tested material was susceptible to powdery mildew, other diseases were also observed. To develop a uniform methodology of field trials it was suggested to use the same number of accessions for the different sets and timely seed delivery was considered essential for trial planning.

Gintaras Brazauskas (LAMMC, Lithuania) presented the work of his team at the Lithuanian research centre for agriculture and forestry. Similar to Latvia they also experienced two different years in terms of weather, affecting the expression of some of the traits collected. Due to late seed arrival in 2020, the first-year trials were sown late, while the second-year trials were sown under optimal conditions; this is reflected in different means of agronomic traits. It was difficult to

score winter hardiness, as the last winters were rather mild. They also noted significant lodging of accessions. Disease scorings showed a low correlation between the two years, reflecting the different environmental conditions.

Charlotte Damsgård Robertsen (Sejet, Denmark) presented trials on barley and wheat conducted in two locations in Denmark. Winter barley was evaluated for different traits in the two years, again reflecting different environmental conditions, which make comparisons between the years difficult. They observed heavy lodging after rain but were satisfied with the large visual differences between barley lines. Similarly, for spring barley traits could not be repeated in both years due to low infection rates and heavy lodging. Spring wheat was mainly scored for yellow rust; however, one location in 2022 could not be scored due to a heavy attack of the barley gout fly. Given the experience reported so far, partners were encouraged to provide weather data for their experimental locations and upload them as supplemental data for their trials.

Külli Annama (Estonian Crop Research Institute) presented their experiments on spring barley, spring and winter wheat. She noted that plots were easier to evaluate than rows. In Estonia, winter wheat is typically sown no later than 15 September, but both in 2020 and 2021 seeds arrived late resulting in late sowings. The traits scored were winter hardiness, plant height and heading date in addition to several prevalent diseases. As in previous presentations they observed big differences in development between the two years. Suggestions for the future concerned a more timely seed delivery, and the question of how to proceed with materials and generated data. The winter wheat breeder noted that one of the accessions looked different from the expected landrace, which may be due to the intermediate SSD generation. It will be important to find a way to manage the SSD materials and data within EURISCO, a discussion which is ongoing in the EURISCO advisory board. Arzu Celik (Ankara University, Türkiye) inquired about some of the diseases observed, which are mainly seed-associated, asking whether any seed treatment was applied to any of the two sets or during the two years. No treatments had been applied to either seeds or fields in Estonia.

Marja Jalli (Luke, Finland) presented results from the spring barley field trials conducted at their location in Jokioinen. As in other locations, the weather conditions in 2022 deviated from the average. They performed artificial inoculation trials using a mix of fungal spores corresponding to the Finnish disease spectrum. They observed some good resistance in EVA materials for net blotch and scald but only little variation for spot blotch, for which there are also no good controls available.

3.2 Central zone

Viola Spamer (BASF) presented preliminary results from winter wheat trials conducted in France and Germany. They are also performing inoculated trials in parallel to their own material, thus internal controls ensure reliable scoring values. She noted that all accessions, including checks supplied, should be listed on the SMTA so that they can be used in trials. Due to mild winters, winter hardiness was scored effectively. Severe lodging of much of the material impacted the scoring of plant height and diseases. Diseases were scored as a percentage and translated to the EVA scoring scale with values from 1 to 9. Although it was previously agreed that in inoculated trials the scoring scales were sufficient, it may still be useful to apply percentage values which would be more fine grained to enable GWAS analysis. Average scores observed in the material were around 10−20% for resistant controls and 50−70% for EVA materials, which translates to

scores of 3–4 and 8–9, respectively. Other suggestions for improvement concerned seed distribution and SMTAs, taking into account internal shipping to different locations within an organization. Finally, it may be good to exchange information between partners on the prevalent strains used in inoculated trials.

Tanja Gerjets (GFPi) presented on behalf of partners Saatzucht Ackermann and Saatzucht Breun. The trials at Breun conducted on winter wheat, winter and spring barley included evaluation for diseases as well as agronomic traits. They reported some delay with seed distribution of EVA materials and standards, thus no standards were included in the sowing this season. They also questioned whether the seed amounts received for set 3 were adjusted by thousand kernel weight, as for multiple accessions the seeds were insufficient for two replicates. They also reported difficulties with translating the results from their recording sheets to the EVA data collection template, which was considered complicated. Comments on issues and suggested improvements were shared among German breeders.

Ulrike Avenhous (W.v.Borries-Eckendorf) reported on their trials on winter wheat and winter barley. Like other partners, they had experienced delays in seed distribution. The material had lodged heavily in both years, but good variation was observed for several diseases for which natural infection was evaluated.

Alex Stride (Limagrain) seconded other partners' comments on issues with seed delivery and lodging materials. Limagrain had conducted multiple trials in France, UK and Ireland but unfavourable conditions in some locations resulted in several failed trials.

3.3 Southern zone

Arzu Celik Oguz (Ankara University) and Namuk Ergun (FCRI, Türkiye) reported on their field trials conducted in Ankara. The trial location in central Anatolia experiences cold winters and allows scoring of winter hardiness, while summers are hot and dry. All crop types were evaluated for relevant diseases and the barley set was also evaluated for agronomic traits. So far only one repetition of each set was evaluated – in the coming growing season both sets 1 and 2 will be repeated. Disease severity was generally higher in 2022, but it was unclear if it was due to the genotypes used. Winter hardiness correlated with material type (set 1 included both spring and winter barley) and origin of materials. Plant height and lodging were also positively correlated. Yield was estimated from the plots and showed variation among the material. Set 3 was not yet received but could still be sown in 2022.

Jihad Orabi (Nordic Seed) suggested combining the data for the two sets in order to gain statistical power. For barley, the differentiation between 2-row and 6-row barley should be taken into account in agronomic traits.

Rita Costa (INIAV) summarized results from their trials conducted at Elvas in central Portugal. As noted by other partners, the environmental conditions between the two years were very different, affecting the disease pressure and thus observed scores. Due to late sowing in the first year, the maturity time of wheat and barley was affected. Yellow rust and powdery mildew were the most prevalent diseases observed on wheat and barley, respectively.

3.4 Discussion on field trials

Several relevant issues had been identified by partners in their presentations and were discussed in the network. Those were related to lodging material and their effect on overall scoring, the issues with delayed seed distribution and related paperwork, and the lack of common checks which, when available, were also not always included in seed shipments.

Albrecht Serfling (JKI) commented that the difficulties with seed distribution in 2022 were partially due to the lack of dedicated personnel and the processing time needed and apologized for the inconvenience caused. At JKI, crops are harvested in late July by hand, cleaned and threshed and germination rates are analyzed before distributing seeds to more than 50 trial locations (including also for other projects), with all related logistics. He noted that some of the materials were mixes despite the SSD step, and required additional attention. The plan is to hire an assistant who would support the seed distribution efforts; this would accelerate distribution and contribute to improved communication. Overall, he noted that the data analyzed so far seemed reliable independent of the sowing date.

V. Spamer wondered whether multipliers had noted lodging within their materials. Partners argued that landraces as well as old cultivars from before the Green Revolution would grow tall and lodge. In addition, genebank material usually does not have good pedigree information associated with their passport data, which is, however, revealed through analysis of genotyping data. During multiplication, JKI is applying stem shorteners to reduce lodging, but this could affect *Fusarium* trials. However, it would be good to record information on plant height or lodging during multiplication, to inform field layouts during the evaluations.

Luigi Cattivelli (CREA-GB) noted the limits of the current project work plan, highlighted by the partner presentations. Due to the lodging common in landraces, only disease traits can be observed while adaptation, drought tolerance or quality traits are hardly evaluated. He suggested that the spring wheat accessions from JIC should not be lodging as much as landraces, as the introgression of Paragon should have cleaned up this trait. Therefore, it would be useful to look at the data on these accessions in comparison to the other materials.

Concerning the use of standard check varieties, S. Goritschnig reminded participants of previous discussions on the topic, where some partners had argued that locally adapted checks would be better to control for common diseases while others had argued that a core set of check varieties used in all trials could provide consistency over locations and years, helping to standardize the observed data and normalize for disease pressure. Partners continued to be divided on the subject, but noted that it would be beneficial to find an agreement either way. A. Serfling noted that JKI produced sufficient seeds of the standards included in the protocol and could distribute them to partners who would like to receive them, but noted that they might behave differently in different environments and be exposed to different strains of a certain pathogen. V. Spamer suggested that common EVA standards could provide consistency over different sets of accessions, as the sets are not completely overlapping in different years across different locations and geographic zones. Partners agreed to continue using their local checks as they are best adapted to the disease strains evaluated in different locations.

A. Celik noted that Ankara University could conduct disease assays under controlled conditions on seedlings of interesting material, to complement the collected field trial evaluations. N. Ergun

noted that some gaps still exist in the evaluation of the material and suggested that assessing the protein quality of durum or bread wheat could be interesting.

4. Data management

S. Goritschnig provided an overview of data management, highlighting where to access the templates and protocols, and reviewed the most important steps in completing the data collection template. She noted that two-thirds of trials from 2022 have not yet been uploaded to the EURISCO-EVA intranet and reminded partners to provide their datafiles by 20 December 2022.

Partners were reminded that the heading dates were counted as days from 1 January in order to align the different materials and zones, and because e.g. winter wheat accessions of set 1 were tested in locations in all geographic zones.

Partners continued the discussion on the scoring scales. A. Celik suggested that for disease scoring the values 1 to 9 should be used for scoring symptoms, while 0 could identify clean material. A. Serfling argued that the protocols developed at JKI used only 1−9 scales and were tried and tested by German breeders. Material not scored for a certain trait should be recorded as ND (not determined). V. Spamer added that a "0" value could confuse Excel lookup functions and was therefore not recommended as a scoring value. M. Jalli noted that using scoring scales instead of percentages produced more reliable data useful for GWAS, as the scoring always depended on the experience of the breeder. A. Serfling noted that from preliminary analysis the different environments can compensate for inaccuracy in the scoring. L. Cattivelli cautioned against changing the scoring scales at this moment, as this would produce heterogeneous data. Partners agreed to use the 1−9 scales for diseases and data curation will take this into account.

Suman Kumar (IPK Gatersleben), developer of the EURISCO-EVA intranet, provided a hands-on overview of the database for phenotypic data, demonstrating the improvements implemented over the past year. A link to the database login page is now available from the general EVA website. The EURISCO-EVA intranet is being populated with phenotypic data and already holds data from more than 130 trials within the EVA Wheat and Barley network. He showed partners how to find, search and filter experimental data and metadata, and how to upload and download datasets. Partners were reminded that each organization has one user ID that should be shared between staff involved in the project. Partners can upload data directly to the platform, using the standard data collection templates developed for this purpose. Several check steps are implemented during the upload to ensure validity of the data, e.g. comparison of allowed values for trial-ID, trait-ID and trait scores. Upload audit logs provide feedback to users and a guide was developed to assist in the use of the platform. User documents that can be downloaded from the intranet include the data collection templates and other user guides under development. Another feature is the possibility to attach supplemental data files to experiments, such as field plans, weather data or initial statistical analysis, which will not be processed and enter the database but can be used for reference. The database is not designed to hold genotyping data but will store metadata for genotyping experiments and links to data repositories. While the database does not offer functionality for statistical analysis, users can download search results and filtered data and metadata for in-depth analysis.

N. Chayut suggested that genotyping data should be made available to network partners, both as raw data and filtered datasets. Considering that the VCF files from the SNP genotyping are of a

manageable size, this can be done on SharePoint and in the EURISCO-EVA intranet. Partners agreed that Excel files of the filtered data of the different genotyping experiments should be uploaded as supplemental data files to the genotyping experiments in the database. In addition, the genotyping data should be deposited in a public repository (e.g. the EMBL-EVA), in line with FAIR principles and in time with any submitted publication.

S. Goritschnig noted the intention to implement an SNP viewer for the interactive viewing of the genotyping data, which would be available from an independent website.

It was suggested to conduct online webinars dedicated to the use of the database during the winter and to gather specific questions from partners beforehand to ensure engagement and allow partners to learn how to use the database effectively.

5. Data Analysis

Preliminary results from the analysis of genotyping and phenotyping data of sets 1 and 2 were presented for the different geographic zones. Although data from 2022 are only partially uploaded, thus far between 45 and 200 datapoints per accession have been collected for set 1. This takes into account that part of the first set of winter wheat was evaluated in all zones, thus producing more data for these accessions.

5.1 Southern zone (all crops)

Delfina Barabaschi (CREA-GB) reviewed the materials included in evaluations in the Southern zone and presented a preliminary genetic analysis of the different crops. Information on the sequence of the probes has been requested from the third-party provider, to allow mapping of the SNPs on the wheat and barley genomes. The two sets showed different levels of diversity and clustering, corresponding to the diverse origins of the materials. Within the second sets of durum wheat and barley, several near-identical lines were identified through genotyping.

Phenotyping was conducted in 13 locations across six countries in Southern Europe, with more trial sites available for durum wheat. The trials collected data for different traits producing useful data from between one and eight locations per trait in the first year. Although the disease pressure was not uniform for all locations and evaluated diseases, for some diseases the distribution of disease scores will allow these trials to be used for GWAS analyses. Several accessions were identified as consistently resistant to diseases in multiple locations.

She questioned which approach would be best to analyze the generated data, considering the year effect on the disease expression and the fact that the two sets are evaluated together only during 2022. She argued that having a larger dataset with more accessions would increase the statistical power of the analysis, but that differences in pathogen pressure and environmental conditions in the two evaluation years would complicate the analysis and interpretation. She suggested that a common pipeline should be used for the analysis of the different datasets, and that a decision should be made on which datasets to use and whether to combine datasets from different locations and years.

L. Cattivelli argued that it may be advisable to discard from the analysis the apparently redundant lines, which would likely be sister lines with large genetic similarities. On the other hand, J. Orabi argued that setting the threshold for minor allele frequency (MAF) too high would lower the

probability of identifying rare alleles for the evaluated traits, which are more interesting for breeders, as the more common alleles have likely already been identified.

5.2 Central and Nordic zone (wheat)

A. Serfling summarized the first outcomes of the data analysis from the Central and Nordic zones, highlighting also challenges for the Europe-wide evaluation of wheat and barley. The EVA network provides a significant contribution to the breeding of climate-adapted varieties by generating large datasets on European accessions from multiple locations in different geographic and climatic zones, which will allow GWAS analysis and selection of interesting breeding material to broaden the future gene pool of elite varieties. Different climatic zones have different priority traits, e.g. winter hardiness is important for breeders in Northern Europe and stem rust has so far been a problem primarily in the South. He suggested that future work could take into account abiotic stresses or emerging biotic stresses.

Preliminary analysis focused on ~330 winter wheat accessions evaluated in the central and Nordic zones, where the available data was comparable. Based on data from trials conducted at JKI, and using the molecular data, identification of accessions with resistances to multiple diseases is possible and may allow the identification of new resistance genes for breeding. Studies conducted at JKI using near-isogenic lines with different leaf rust resistance (Lr) genes indicated that only a few of the known Lr genes are effective against current strains of leaf rust. He also noted that of ~80 known Lr alleles, only ~10 have been introduced into elite varieties so far. Similarly, yellow rust resistance (Yr) genes are not widespread in cultivars as it is a fairly recent introduction to Europe; here the genetic resources may contribute to the identification of novel resistance.

For the genotyping data of wheat accessions, ~9,800 markers remained after filtering and using different approaches showing an even distribution of diversity in winter wheat accessions, a prerequisite for successful GWAS. Spring wheat accessions from JIC clustered in the analysis and may therefore not allow effective GWAS. They conducted around 50 GWAS analyses on the preliminary data on seven traits evaluated on winter wheat in different locations and identified several genome locations with significant contributions to trait expression, including regions with known causative genes as well as potential new resistance loci. Some trial locations showed differing results for the same trait, suggesting environmental effects or different pathogen strains. For traits with known lower heritability, it may be advisable to include multiple trial locations in a GWAS analysis. In order to validate field data and differentiate between new and known resistances it may be useful to test candidate accessions with pathogen strains that carry known avirulence alleles under controlled conditions. Thus, despite the issues identified by partners the collected data are well suited for the development of markers which could be developed for individual zones or partners based on the available data.

He summarized the main challenges and issues to be resolved at JKI. As multipliers, they would work on expediting seed distribution and improve communication with evaluators, especially around deadlines for shipping. A preselection of the materials for evaluation could facilitate trial planning by partners and reduce lost data due to lodging. Standards could be distributed to all partners if needed. Streamlining of seed distribution should enable partners to contribute more evaluation locations and compilation of data in provided templates should be made as easy as possible.

M. Jalli questioned how to make the analysis more efficient and identify traits for which more data may be required to improve significance. A. Serfling noted that yield was difficult to evaluate based on the agreed trial design. *Zymoseptoria* resistance may be an interesting trait to evaluate more in the future, as fungicide resistance has been observed for some strains.

N. Chayot commented on the effect of population structure in the Watkins-lines on GWAS. He suggested that, since most of the diversity observed would be contributed from the landraces and not the Paragon parent, the association should still be possible, even if perhaps with a wider peak.

J. Orabi reminded partners of several online tools to monitor distribution of pathogen races, for example the rust-tracker provided by CIMMYT[4].

U. Avenhaus suggested that in addition to location-specific analyses, breeders would also be interested in regional summaries as the breeding is done for a bigger region. She also reiterated their interest in identifying rare alleles, and suggested to use a lower threshold for MAF.

5.3 Brainstorming and discussion on data analysis

L. Cattivelli introduced the topic of data analysis. From the results seen so far, there are some potentials but also limitations of the data to make them useful for breeders and researchers. A first limitation is that we are currently dealing with two sets of accessions, with corresponding datasets that are partially overlapping across zones, but are mostly separate. This means that independent data sets can be analyzed separately for the different zones and also use different pipelines, as appropriate. Secondly, the main priority for breeders and thus the data most meaningful for analysis are on disease traits. Thirdly, not all trials will have produced equally robust data for all diseases, necessitating data analysis steps for each trait to select the data with the highest potential of success for GWAS. In order to identify rare resistance alleles, manual selection of interesting accessions may be preferable to reducing the MAF in the filtering step. It will be necessary to select accessions with good performance and high resistance in multiple locations and potentially proceed with biparental mapping, if the consortium agrees to include such pre-breeding activities within EVA.

Discussions on data analysis approaches were started with a brainstorming exercise where each partner was asked to respond to an online survey with the following questions: 1) What are your main objectives to get from the data (research/breeding)? 2) Which specific questions would we like to answer? 3) How should we analyze the data (methods/approach)? 4) What could you contribute to the analysis? and 5) What would you consider a minimum embargo period for data before publication?

Within each geographic zone, a partner should be identified who would take the responsibility to coordinate data analysis and select the appropriate pipeline. As agreed previously, JKI and CREA-GB will lead data analysis in the Central and Southern zones, respectively. M. Jalli agreed that Luke could take responsibility for analysis of Nordic zone data, provided there would be some financial support. Juho Hautsalo will be in charge, with a detailed description of the required work to be defined based on the material and available data. A. Serfling suggested that the zones should be more connected in their analysis in order not to duplicate work.

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[4] https://rusttracker.cimmyt.org/

Much of the resistance observed in the EVA accessions may probably already be known and used in breeding, and it was unclear how many novel alleles we may be able to identify. As shown, GWAS analysis and potential markers could be provided to breeders already from the datasets analyzed so far. N. Chayut noted that we should consider the possibility that novel allelic variation could be identified on previously known resistance loci. It was recommended to prioritize analysis on the datasets from 2022, when both sets 1 and 2 were evaluated together, as this would increase the statistical significance. It was agreed that all analyses should use the same GWAS pipeline and all results should be available for the whole consortium.

6. Outlook

6.1 Dissemination and exploitation of results

From discussions so far, it was clear that the desired output of the EVA project focuses on disease resistance. Actual outputs of the project could be the multilocation field trials which help identify materials with the better or best performance in multiple locations. This preliminary work would require several follow-ups, which could be considered for a work plan for EVA Wheat and Barley 2.0. The best-performing accessions could form the basis for future work, for example through additional testing against known pathogen strains under controlled conditions and could be used to generate crosses or near-isogenic lines for further genetic analysis or pre-breeding.

L. Cattivelli suggested that for future collaboration, it may be worthwhile to consider an approach similar to that used by JIC for the Watkins collection and to select a panel of interesting material, cross them with a modern variety and develop lines that would allow evaluation of genomic regions instead of genotypes. This would require several years of preparatory work but could result in better trials and evaluations, facilitate analysis, and allow testing of many more characteristics from the landraces. Thus, an EVA 2.0 project could work on preparing the lines that could then be evaluated in the field in an EVA 3.0 project. It should be noted that such a preparatory phase would require 3–4 years to prepare several generations of crosses, and would ideally include genotyping of the created lines.

J. Orabi agreed with the assessment and mentioned a project in barley, where Klaus Pillen and colleagues identified the 25 best accessions of *Hordeum spontaneum* and crossed them to elite material, creating NAM populations that provided material for multiple research projects. He suggested the consortium could consider selecting 20–30 most promising accessions from the first step of evaluations (i.e. the first two accession sets) and develop such populations which could be used in future work.

N. Chayut suggested that the work done in EVA on the Watkins collection could serve as a pilot for the suggested possible EVA 2.0 approach. Possible questions to be answered by the data could be whether the flowering time prediction matched the evaluations, and how the lodging expressed in the paragon crosses. A possible publication could describe the workflow for the description of landraces using this approach; how many lines per accession would be optimal for such a description or the minimum number required to assess the landrace genome; whether the backcross is a limiting factor for GWAS on disease resistances. Thus, this analysis may show whether the proposed approach could work and inform the project planning for EVA 2.0. A. Serfling noted that in the genotyping data, the Watkins lines should cluster separately from the other spring wheat accessions.

L. Cattivelli suggested that the consortium consider jointly publishing an overview paper of the activities of the EVA Wheat and Barley network, describing the material and diversity analysis from the genotyping data as well as a general summary of the phenotyping activities, without going into detail on marker-trait associations. This would benefit both the breeders and researchers within the network.

Another question that could be addressed in a publication is how genebank genetic diversity is used in modern breeding, describing the best way to extract information from datasets useful for existing breeding programmes, such as that generated in EVA. This could focus on the genetic resources, not going too much into detail and covering for example data on flowering time and a selected disease.

In order to allow timely publication of the results, the question of the embargo period was discussed again, and partners agreed that publishing and breeding interests were not mutually exclusive. Companies are not opposed to publishing but wish to be involved in the process. They suggested publishing on a topic relevant to breeders, rather than focusing only on diversity studies and requested to keep partners' breeding and pre-breeding activities in mind when planning the publishing and before making all data fully public.

For breeders, a strong association between a marker and phenotype could be sufficient output, but this alone may not be enough for publication. J. Orabi noted that any publication as suggested could provide the authors with a base to do additional research on a certain genotype or trait, identifying a genetic basis or molecular mechanism. It would depend also on how the data are prepared, whether a study broadly covered data from all trials of a certain zone and producing a list of lines or loci of interest or if it should be more specific on a certain trait and locus.

T. Gerjets suggested that a data analysis strategy should be developed together with a publication strategy and that should be done jointly by scientists and breeders, coming up with concrete ideas for publication. A task force was established to address disease analysis questions and develop a publication strategy which would incentivize the contributions of the public sector partners. The task force is composed of T. Gerjets (chair), S. Goritschnig (coordinator), D. Barabaschi, A. Serfling, Juho Hautsalo (Luke), N. Chayut, J. Orabi and G. Brazauskas and will meet in early 2023 for a first review of the data.

6.2 Discussion on long-term planning for EVA Wheat and Barley

As noted during the project overview, the current project funding through the German project covers 2023 and, through the AGENT project, support for EVA Wheat and Barley was extended until early 2025. In principle, EVA networks should continue their collaborations as self-sustained networks, keeping in mind the required budget for funded activities such as multiplications, genotyping and data analysis.

Lorenzo Maggioni (ECPGR) informed on the proposal to include funds for the EVA project into the regular ECPGR budget of the next Phase XI, noting that this would be negotiated during the upcoming ECPGR Steering Committee meeting in June 2023. Any funds would be used to support the existing five networks and possibly others under development. It would therefore be important to get some commitment from the networks and it was suggested to prepare a perspective document that could outline planned activities, e.g. multiplications or population

development for the future work plan, and would constitute an expression of interest of the EVA Wheat and Barley network to continue their collaboration.

Breeders were in general supportive of the opportunity to access and evaluate genebank accessions within this unique international public–private network and would support future collaboration. However, before commitments for future activities could be considered, the issues identified in the meeting would need to be resolved and initial results show the benefit of the joint work. Public partners agreed that this project is good and useful but reiterated that for their continued contribution, publications are a prerequisite to secure funding.

Partners noted a need to complete data analysis of at least set 1 and years 2021 and 2022 to review the results and inform proposals for future work. Thus, an actual decision on future collaborations will only be possible in a year from now.

Since this year no multiplication activities have been included in the work plan, there will be no additional accession set 4 for distribution in 2023. However, as noted the Nordic zone will still multiply the set 3 winter wheat and JKI also has some additional lines of spring and winter types that are still in the multiplication pipeline. Furthermore, additional SSD lines could likely be provided from the AGENT project for soft wheat and barley, as well as from JIC for the global durum panel. Material from both sources could be preselected to reduce the problems experienced with lodging. T. Gerjets suggested that a lower number of accessions included in each set could reduce the costs and efforts associated with multiplication and distribution.

6.3 Next steps and closing of the meeting

Priorities for the coming months were outlined and relevant action points are collected in Appendix 3. Distribution of spring crops should be coordinated before the end of December 2022 to ensure partners receive seed shipments in time. All missing datasets should be collected and curation finalized by end of the year so that effective analyses could be started on the entire data. Similarly, the genotyping data should be made available to partners as soon as possible. The task force should meet in early January to develop a strategy and plan for data analysis and publications. Webinars on the use of the EURISCO-EVA intranet for data management should be planned for early 2023, and partners invited to attend open training events organized within the AGENT project.

A virtual meeting in spring 2023 will be planned during which initial results would be disseminated to partners and the strategy and possible future work plan presented and discussed. During the spring, partners would also need to decide whether a new set 4 should be proposed for multiplications in 2024 and funds identified for that. Another in-person project meeting would best be planned during the winter months, as breeders across Europe have more downtime in winter; a possible date would be January 2024 and CREA-CI Vercelli suggested co-hosting the meeting.

S. Goritschnig thanked participants for their active participation in the discussions and partners at LfL for their assistance in co-hosting the meeting and closed the meeting.

Appendix 1: Meeting Agenda

Venue: LfL, Freising, Germany

THURSDAY 17 NOVEMBER 2022		
13:30 – 14.00	Registration	
	Welcome and introductory session	CHAIR: LORENZ HARTL
14.00 – 14.10	Welcome by local host, ECPGR	*Peter Doleschel (LFL)* *S. Goritschnig*
14.10 – 14.20	Overview of the current status of the ECPGR Evaluation Network EVA	*S. Goritschnig*
14.20 – 14:30	Review of EVA Wheat and Barley network workplan 2019-2024	*S. Goritschnig*
	Overview of Accessions in Evaluation	CHAIR: LORENZ HARTL
14:30 – 15:00	Review of the Accessions sets 1 and 2 and criteria for selection of material. – Multiplication activities – potential materials for future evaluations	*S. Goritschnig*
15:00 – 15:30	Horizon2020 project AGENT - AGENT Precision collections - Interaction between AGENT and EVA WB (access to data and material) - AGENT multiplication - Rete Semi Rurali – Farmer's network evaluation	*S. Goritschnig* *P. Vaccino (CREA-CI)* *M. Pattiti (RSR, online)*
15.30 – 16:00	**TEA/COFFEE BREAK**	
	Review of experiments and preliminary data	CHAIR: LORENZ HARTL
16:00 – 16.30	Northern Zone – selected trials presentations - Traits evaluated - Preliminary results from trials - Lessons learned and suggestions for upcoming trials	*M Bleidere (AREI)* *G. Brazauskas (LAMMC)* *C. Damsgård Robertsen (Sejet)* *K. Annama (ECRI)* *M. Jalli (LUKE)*
16:30 – 17:00	Central Zone – Selected trials presentations - Traits evaluated - Preliminary results from trials - Lessons learned and suggestions for upcoming trials	*V. Spamer (BASF)* *T. Gerjets (GFPi, online)* *U. Avenhaus (WvB-E)* *A. Stride (Limagrain)*
17:00 – 17:30	Southern Zone – selected trials presentations - Traits evaluated - Preliminary results from trials - Lessons learned and suggestions for upcoming trials	*A. Celik, (Ankara U)* *N. Ergun (FCRI)* *R. Costa (INIAV)*

17:30 – 18:00	Discussion on field trials - Scoring protocols - Check varieties - Data collection and reporting - Timelines	*All*
19.00	**SOCIAL DINNER**	

FRIDAY 18 NOVEMBER		
	Data management	**CHAIR: S. GORITSCHNIG**
08:30 – 08:45	Overview of data management Review of data collection templates	*S. Goritschnig*
08:45 – 09:30	Hands-on session with EURISCO-EVA intranet	*S. Kumar (online)*
	Data Analysis	**CHAIR: L. CATTIVELLI**
09:30 – 10:15	Preliminary results from analysis of genotyping and phenotyping data for Set 1 – Southern zone (all crops) – Central and Nordic zone (wheat)	*D. Barabaschi* *A. Serfling*
10.15 – 10.45	**TEA/COFFEE BREAK**	
10:45 – 11:00	Data analyis approaches in EVA networks	*S. Goritschnig*
11:00 – 11.45	Brainstorming and discussion on data analysis – questions to be adressed, approaches, possible contributors	*All*
11.45 – 12.30	Planning of data analysis work for 2023-24	
12.30 – 13.30	**LUNCH**	
	Outlook	**CHAIR: L. CATTIVELLI**
13.30 – 14.00	Dissemination and exploitation of results	*S.Goritschnig*
14.00 – 14.30	Discussion on Long term planning for EVA Wheat and Barley - Project's funding coverage - Options for continuation of network after end of current project (Nov. 2023) and AGENT extension (April 2025)	*All*
14.30 – 15.00	**TEA/COFFEE break**	
15:00 – 16.30	Final discussions and wrap-up of meeting	*S. Goritschnig/All*
16:30	*End of meeting*	

Appendix 2: Participants list

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Appendix 3: Action list 2022/23

#	Section	Task	Responsible	Due date
1	1.2	Provide data from evaluation trials 2022, using data collection templates	All evaluators	20.12.2022
2	1.2	Finalize spring crop distribution lists for 3rd set of evaluations	S. Goritschnig	15.12.2022
3	1.2	Check genotyping of durum lines and sequence set 3 if necessary	S. Goritschnig/P. Vaccino	31.01.2023
4	2.2	Discuss genotyping data access from AGENT during GA	S. Goritschnig	31.05.2023
5	3.1	Provide weather data for experimental locations and upload to database as supplemental files	All evaluators, S. Goritschnig to coordinate	28.02.2023
6	3.2	Collect information on prevalent strains used in inoculated trials/encountered in field	Evaluators, S. Goritschnig to coordinate	28.02.2023
7	3.4	Include pre-selection criteria during multiplication (plant height, lodging) in experimental protocol	S. Goritschnig	28.02.2023
8	3.4	Compare lodging data from different material types (Watkins vs other)	N. Chayut	28.02.2023
9	3.4	Include 'request standards' field in seed distribution matrices	S. Goritschnig	28.02.2023
10	4	Check for consistency in curated data (heading date, winter hardiness, '0' score in disease traits)	S. Goritschnig	31.01.2023
11	4	Archive copies of SMTAs used for seed exchange	All senders and recipients of seeds	31.05.2023
12	4	Make raw genotyping data available on SharePoint	S. Goritschnig to coordinate	31.12.2022
13	4	Update genotyping metadata and upload datafiles to EURISCO-EVA database	S. Goritschnig	31.01.2023
14	4	Coordinate deposition of genotyping data in public repository	S. Goritschnig	31.12.2023
15	4	Organize webinars for use of EURISCO-EVA intranet and gather specific questions from partners in advance	S. Goritschnig	31.02.2023
16	5.1	Identify and flag genetically near-identical accessions from the Southern zone sets	D. Barabaschi	28.02.2023
17	5.2	Share publications on JKI studies using NIL lines with Lr and Yr genes	A. Serfling	31.01.2023
18	5.2	Align analysis methods and pipelines for genotyping data and GWAS	Task force	31.01.2023
19	5.3	Discuss terms and work description for Nordic zone data analysis with Luke	S. Goritschnig, J. Hautsalo	31.01.2023
20	6.1	Provide input on data analysis priorities and publication ideas to task force on data analysis through survey	All partners	31.12.2023
21	6.1	Organize virtual meeting of task force on data analysis	S. Goritschnig	15.12.2023
22	6.1	Task force to prepare publication and data analysis strategy for discussion with network	Task force	28.02.2023

#	Section	Task	Responsible	Due date
23	6.2	Task force to prepare a perspective document for possible future activities (EVA Wheat and Barley 2.0)	Task force	28.02.2023
24	6.3	Virtual meeting to review data analysis strategy, perspective documents and preliminary results.	All partners	31.03.2023
25	6.3	Consider possible multiplication of set 4 in 2024, identify materials	All partners	31.03.2023
26	6.3	Organize in-person annual meeting, in collaboration with CREA-CI Vercelli	S. Goritschnig, P. Vaccino	31.01.2024