Summary report
of the

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

25-26 January 2024

Fiorenzuola d'Arda, Piacenza, Italy

Contents

The 4th annual project meeting of the EVA Wheat and Barley network took place in person on 25-26 January 2024 in Fiorenzuola d'Arda, Italy, co-organized with the Genomics and Bioinformatics Centre of the Italian Council for Agricultural Research (CREA-GB). The agenda of the meeting is attached as Appendix 1 and the list of participants as Appendix 2.

1. Welcome and introduction

Luigi Cattivelli, director of CREA-GB, welcomed participants to the meeting and presented a short video showcasing the fields of the institute's experimental farm, which during the winter lie fallow. The centre in Fiorenzuola d'Arda also has an advanced genomic platform for the analysis of plant genomes, laboratories for *in vitro* culture and plant transformation, and a new phenotyping platform for physiological analyses under greenhouse conditions, which the participants were able to visit. The EVA coordinator Sandra Goritschnig opened the meeting, reminded participants of the expected outcomes of the meeting and highlighted the shared documents folder of the network where partners can find all relevant templates and reference documents. She welcomed also participants connecting online to the meeting and shared a preview of a new EVA promotional video which highlights the activities and impact of the project.

1.1 Overview of the ECPGR Evaluation Network EVA

S. Goritschnig presented a general update on the EVA project, which is funded by the German government until December 2024, extended beyond its original duration through a budget revision in 2023 which provided additional funds for data management, analysis, coordination and communication. The H2020 project AGENT provided funding for multiplication of a third set of accessions for evaluation in the EVA Wheat and Barley network and runs until April 2025.

Integration of the EVA Project into the regular work programme of ECPGR for Phase XI (2024–2028) enables continuity for network activities beyond the current duration, provided funds are identified for the networks' activities that cannot be contributed in-kind. A new EVA Legumes network has been started through the ECPGR Grant Scheme Activity ForEVA of the ECPGR Grain Legumes Working Group. This new network is being established in 2024 with the development of work plans and funding proposals, bringing together around 50 partners in seven crop groups (chickpea, common bean, fava bean, *Lathyrus*, lentil, lupine and pea) and aiming at close collaboration with ongoing Horizon projects on legumes, especially INCREASE, BELIS and Legume generation, with partners participating in multiple projects.

Ongoing involvement of ECPGR in Horizon projects AGENT and PRO-GRACE provides opportunities to participate in training activities. Several webinars are planned for promotion and training of AGENT bioinformatics tools and an in-person workshop on the evaluation of *ex situ* and *in situ* plant genetic resource (PGR) collections will be organized by PRO-GRACE in partnership with EMPHASIS-RI. More information will be provided as it becomes available.

The EVA networks have been very productive in their data collection; by the end of 2023 more than 5,000 accessions have been evaluated, the majority of which within the EVA Wheat and Barley network (~1,800 each of barley and wheat, including durum). Of the ~360 trials for which data has been uploaded to the EURISCO-EVA database more than 250 are from the Wheat and Barley network, adding up to nearly 300,000 datapoints, a complex dataset the analysis of which will likely unravel some interesting results for breeders and researchers.

Two EVA networks have published their first results in international journals. The EVA Lettuce network published the development of a new genotyping panel in *Frontiers in Plant Science*[1] and the EVA Carrot network published initial results presented at the International Horticultural Congress 2022 in *Acta Horticulturae*[2]. The EVA Maize network is also working on an article presenting the network's collections and initial results and other opportunities to disseminate project results are being discussed by all networks.

For the EVA Wheat and Barley Network, Delfina Barabaschi presented a poster at the 66th annual congress of the Italian Society of Agricultural Genetics (SIGA) and S. Goritschnig, Patrizia Vaccino and Lorenzo Maggioni participated in a Farmer's Field day[3] organized by Rete Semi Rurali in Tuscany, where they showcased the activities of ECPGR and EVA in the framework of the AGENT project.

The EVA Wheat and Barley network currently has 47 network partners from 21 countries, including 25 breeding companies. HR Smolice, a Polish breeding company is interested in joining the network and attended the meeting online. The network website is available at https://www.ecpgr.cgiar.org/eva/eva-networks/wheat-and-barley.

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

Several action points from the last meeting are still pending, including the collection of meteorological data from trial locations for analyses of environmental effects and the archiving of standard material transfer agreements (SMTAs) which should accompany all seed exchanges.

The network work plan was reviewed and the time overlap between activities related to different evaluation cycles were highlighted. In 2023, evaluations were finalized for the first two sets of accessions which had been multiplied and genotyped within the German EVA project. Most datasets for these have been collected and curated and are available in the project database, also the raw genotyping data from the SNP arrays are available there. The third set of accessions, provided within the framework of the AGENT project has been evaluated for the first year and will again be evaluated in 2024. Partners were reminded to submit their datasets ASAP so that they can be curated and made available to the network and partners involved in the analysis. Filippo Guzzon from the ECPGR Secretariat is supporting the curation and upload of data and should be contacted as needed.

2. Update from Horizon2020 project AGENT

S. Goritschnig provided an updated overview of the Horizon2020 project Activated Genebank Networks (AGENT)[4] (www.agent-project.eu), in which the EVA Wheat and Barley network is engaging as external stakeholders. AGENT provides materials and genotyping for a third evaluation set from precision collections created by AGENT partners that represent unique materials conserved by the genebanks. Combining their unique approaches, the two projects

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[1]   https://www.ecpgr.cgiar.org/eva/about-eva/latest-news/news/single-primer-enrichment-technology-a-new-genomic-resource-to-investigate-the-diversity-of-lettuce-germplasm

[2]   https://www.ecpgr.cgiar.org/resources/latest-news/news-detail/getting-to-the-roots-of-carrot-genetic-diversity

[3] https://agent-project.eu/news/cultivating-diversity-agent-wheat-and-barley-accessions-on-display-during-a-farmers-field-day-in-the-tuscan-hills

[4] The AGENT project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 862613.

work together on increasing knowledge on European germplasm, extending the range of stakeholders using PGR for food and agriculture and thus promote the use of genebank germplasm in breeding and cultivation. The EVA Wheat and Barley network has received 20% and 13% of the AGENT barley and wheat collections, respectively, for evaluation in the field. The AGENT genebanks have phenotyped their own collections for agronomic traits and biotic as well as abiotic stresses have been evaluated on subsets of the collections in some locations. In addition to phenotyping in the fields, AGENT partners also extracted data from their historical records, thus collecting data on thousands of accessions covering between two and nine decades of characterization. The data, mainly collected during regeneration cycles, allows analyses of heritability or the effect of climate change, genomic associations and predictions.

Apart from generating connected datasets in the AGENT network of genebanks, three work packages are dedicated to data management, curation and analysis, developing guidelines, templates, pipelines and other tools that enable effective information management in genebanks. The AGENT data portal developed by IPK, for example, uses the EURISCO-EVA backbone, allowing not only further exploitation of EVA project results but also providing a link and compatibility between these databases and EURISCO. A SNP-viewer, also developed by IPK, will be used to display the genotyping data from AGENT and has been updated with new features that will also be available to EVA partners in their instance of this genotyping visualization platform. The tools and pipelines developed in AGENT will be disseminated through webinars and training sessions in 2024 and further available for the wider user community through the creation of useful training materials and documentation.

Max Haupt (IPK) presented the first results from the genotyping of the AGENT collections, including examples of how the data can be used to evaluate the quality and redundancy of genebank collections and how it can drive gene discovery through association studies with new and historic phenotyping data. The AGENT collections were genotyped using reduced representation sequencing techniques, wheat by DartSeq and barley by GBS, and connected with publicly available genotyping datasets from IPK, ICARDA and other sources. Both approaches yielded a good amount of usable SNPs for diversity analyses. AGENT precision collections taken together represent a large amount of genetic diversity, while precision collections of single partners provide more focus, reflecting the selection of their collections to represent mostly unique regional material. The population structure of the collection reflects a combination of origin and biological status of the material. The material in EVA Set 3, even though only representing a small fraction of the overall collection, shows a good diversity for all crops and regions. Putative duplicate samples were identified by applying a stringent threshold for the proportion of identical markers between samples. More than 15k samples have no duplicates and have yielded hundreds of new accessions per partner to the known global diversity. Combined with associated phenotypic data, duplicates within and across genebanks provide an important resource to assess environments and bridge collections. For example, genome-wide association studies (GWAS) analysis of flowering time using historical data has identified a number of known and new associated QTLs as well as signatures of genetic differentiation that may reflect local adaptation.

It will be interesting to check the EVA-AGENT accession set for putative duplicates and to link genotyping data from EVA and AGENT for a comprehensive analysis. A large proportion of the AGENT collection can likely be mobilized for future EVA sets and selection should consider both genotyping and phenotyping data produced in AGENT as well as the biological status of the accessions, as some modern cultivars are also included.

Zakaria Kehel (ICARDA) connected online to present on Focused Identification of Germplasm Strategy (FIGS), a method to predict the performance of accessions for a specific trait based on the origin of the accession. The model uses meteorological data, data on pathogen strain distribution and phenotypic data from accessions with known origin (GPS coordinates) to predict accessions that may have evolved specific resistance to diseases. This method reduces the size of accession sets for in-depth evaluation, allowing breeders to focus on the most promising material. Results were presented on the analysis of data from the EVA project as a proof of concept. In general, combining datasets should increase the accuracy of the prediction, as long as the data is well curated and reliable. Combining data from AGENT and EVA as well as the planned integration of genomic information in the model will further improve the model, which can be applied to all traits for which enough data is available. Having access to meteorological data at daily resolution, combined with observation dates for the traits will also be beneficial. Soil moisture/precipitation and temperature were noted as the most informative environmental descriptors and could be variables used to predict certain diseases. ICARDA routinely uses this model to select material from their collections based on user requests and a Genesys tool for selection of abiotic stresses is also available. FIGS could thus be used for the selection of future accession sets for evaluation in EVA.

Bettina Bussi (Rete Semi Rurali, Italy) updated on the work of RSR in the EVA network and AGENT project, where they perform on-farm evaluations under organic conditions. Because seed yield from the multiplication in 2022 performed by CREA-CI was not sufficient for evaluation of the whole set in all locations in 2023, a second multiplication was conducted at their experimental farm in Tuscany, which was also the location of a demonstration Farmer's Field day with an AGENT workshop on 9-10 June 2023. Seeds were distributed to farmers for 2024 evaluations which are planned in nine locations, where three farms (in Tuscany, Sardinia and Sicily) will evaluate the whole set with a focus on drought stress, while other locations will grow subsets under marginal conditions and in the plains. The trial design will use a standard lattice and include local varieties as controls. Data will be collected at several time points using electronic fieldbooks. The focus of the traits will be on a comparison between locations of yield, plant height and agronomic data, as it was considered difficult to organize collection of flowering time data across many locations. Quality traits could also be interesting to evaluate, however, these analyses are not covered by the available budget. The participatory evaluations involving farmers, technicians, researchers, processors and citizens will take place at the beginning of June at the locations with the full set and partners were invited to attend these events, which will be linked with Farmer's Field Day outreach events. One partner suggested that it may be interesting to include commercial varieties in the organic trials for comparison, this may also help identify useful accessions for further breeding.

Partners discussed how the results from AGENT and EVA could be connected and how EVA could further participate in the exploitation of AGENT outputs. It was noted that the IPK wheat and barley accessions have all been previously genotyped with GBS. These data have been included in the genotyping analysis of AGENT and could help bridge the SNP array genotyping performed on the EVA accessions of Sets 1 and 2. The selection of future sets from AGENT material should take into consideration eventual duplicates and biological status of accessions in the various collections and apply FIGS approaches to select the most promising materials.

3. Review of experiments and preliminary data 2023

Partners presented preliminary results from selected trials, focusing on meteorological conditions, evaluated traits and preliminary statistics were available.

Mara Bleidere (AREI, Latvia) presented preliminary data from their trials conducted at the AREI Stende Research Centre, where spring 2023 saw drought conditions and the lack of moisture and nutrients affected the growth of the crop. This season powdery mildew was the main disease observed in all crops, which developed quickly although it started late due to the drought.

Marja Jalli (Luke, Finland) presented results from the spring barley field trials conducted at their location in Jokioinen, where they used artificial inoculation of net type net blotch and scald; spot blotch could not be evaluated. Although disease pressure was late, the data showed good distribution for both diseases and some potentially resistant accessions were identified.

Reine Koppel (METK, Estonia) reported on their trials in Estonia. She noted that despite the discussions during the last meeting, seed distribution of winter crops was still an issue, as their sowing date is very early. In their location they also had a very warm and early spring, with May and June too dry, resulting in an extreme year for spring cereals. They observed several diseases, with some showing reasonable distribution for further analysis.

Gintaras Brazauskas (LAMMC, Lithuania) presented the work of his team at the Lithuanian Research Centre for Agriculture and Forestry, showing an overview of all traits scored in the different years. In 2023, only powdery mildew and Septoria could be scored. Interestingly, they noted differences in the lodging tendency between spring wheat sets 2 and 3, where no lodging was observed in the Set 3 trial.

Esra Cakir (Çukurova University) presented initial results from their 2023 trials in Adana, Turkey, where they grew the southern zone sets 2 and 3. They observed several diseases but the scores were not well distributed for all of them. Material has been resown for replication of the evaluations in 2024.

Arzu Çelik Oğuz (Ankara University) reported on their field trials conducted in Ankara, Turkey. In 2023 they evaluated southern zone sets 1 and 2, as Set 3 had arrived late for sowing. They noted early lodging and very fast and severe disease progression in their trials. Some good resistances were observed of accessions directly adjacent to heavily diseased ones. The Set 3 will be evaluated in 2024.

4. Data analysis

F. Guzzon (ECPGR) presented an overview of the data management and available data in the EURISCO-EVA database. Partners were reminded of the content and functions of the project database and to request access by emailing the EVA coordination. Some data from evaluations in 2021 and 2022 are still not available in the database and partners will be personally contacted to inform about these missing datasets. Data for 2023 has only partially been provided and partners were reminded to provide data, especially on the repeat of Set 2 as soon as possible to allow analysis of the entire dataset. Overall, the traits scored in most trials are days to heading, plant height and lodging, with yellow rust and powdery mildew the most commonly observed diseases. Several quality and agronomic traits have been observed only in few trials and some diseases such as smut, Fusarium or barley yellow dwarf virus also have limited data available.

4.2 Results from Nordic Zone

Juho Hautsalo (Luke) presented an update on the data analysis progress for the Nordic zone data, with a focus on Set 2 as results for Set 1 had been previously presented to the network. He outlined the various steps in the analysis pipeline, highlighting some additional steps that were included for improvement. In a first descriptive analysis of the phenotypic data, he estimated skewedness and kurtosis to assess the quality and distribution of the data; high values indicate that the data are skewed and therefore not good for further analysis. Looking at the available trials for spring barley and spring wheat, most have data with good broad sense heritability and can be used for further analysis. Where available, best linear unbiased estimates (BLUEs) were calculated from all experiments to enable comparison of genotypes. Biplots were used to find contrasting and similar environments and BLUEs were used to help detect the most promising lines with good disease scoring across multiple trials.

He outlined the proposed common approach for GWAS on all sets and across the different zones, in order to enhance confidence in marker trait associations observed repeatedly across environments. Some associations have been observed for multiple traits, which could be interesting but require further analysis. Several markers that showed association in multiple trials may be linked to known genes involved in the observed trait, for example *Ppd-H1* or *Vrn* for flowering time. Analyses were first conducted on sets 1 and 2 individually, and then on the combined sets, which for some traits increased the LOD score. Overall the data for Set 1 seemed more reliable and of better quality.

He further analyzed traits across environments using various statistical models and noted that these performed differently, suggesting that for each trait and environment, the best model needs to be assessed first to identify good associations. Matching the marker associations identified for different barley traits between the Southern and Northern Zone datasets identified some overlap.

He summarized the output available for partners, which includes the descriptive analysis of the phenotypic data, BLUEs calculated within and across environments and biplots for the comparison of environments. GWAS results have been summarized in files listing significant markers calculated using different models and graphical representations of the data as QQ and Manhattan plots. Going forward, the results should be summarized in a file with the most effective markers for each trait, indicating known genes but more importantly also putatively novel ones. In addition, accessions that have multiple interesting alleles or also rare alleles for specific traits should be highlighted for use by breeders. Finally, the results from the different zones should be compared to identify similarities and confirm findings.

4.3 Results from Southern Zone

Delfina Barabaschi updated on the analysis progress of the Southern Zone Sets 1 and 2, which used the same pipeline. Heritability calculated for different trials was generally good and allowed analysis of the datasets for multiple traits. The mixed linear model (MLM) model was applied in GWAS and two groups of significant marker-trait associations (MTAs) identified: single MTAs exceeding the Bonferroni threshold in single environments, suggesting qualitative race-specific resistance, and MTAs with lower significance observed in multiple environments, suggesting quantitative non-race-specific resistance. In general, combining the two sets in the analysis increased the significance of the association.

Results were shared with the network on SharePoint in Excel files that list MTA candidates, and define the surrounding physical region and already known candidate genes, where relevant. This output file will be updated once all phenotypic data have been analyzed and appropriate documentation will be provided for explanation of the results files.

She highlighted several accessions that were resistant to multiple pathogens and could be used in crossing schemes for further breeding as well as in in-depth evaluations with specific races under controlled conditions. For the durum set enough interesting results are available to prepare a first publication. Further work should be done to identify overlapping MTAs between traits, which could be associated with quantitative resistance.

4.4 Results from Central Zone

Albrecht Serfling (JKI) presented an update on data analysis for the Central Zone, including proposals on how to proceed with the validation of previously unknown associations. He highlighted that the SSDs for the evaluated accessions are stored at JKI and additional genotyping data is available for some that have also been included in other projects (e.g. Briwecs, Genbank 2/3 or national projects), which can provide connection for further confirmations. He highlighted the challenges associated with comparing the complex datasets generated in EVA, e.g. evaluation in different environments reduced the heritability of some traits.

He noted that some diseases such as barley yellow dwarf virus (BYDV) are difficult to score and only few datasets are available. For the barley pathogen Ramularia, which is an emerging pathogen in the Central Zone, no resistance has been identified so far, and thus the EVA dataset could be very informative, as all trials are using the same scoring protocol.

He also used different models for GWAS analysis, resulting in some differences in the associations or significance of associations. In the first step, genotyping data was used to generate a diversity structure for the whole accession set, which identified clusters associated with different origins. These data are needed to perform a kinship matrix which should be taken into account when defining different QTLs to avoid fake associations based on genetic relatedness.

For barley, some internal studies at JKI have been done on seedling resistance to leaf rust under controlled conditions, which correspond to qualitative resistance and could be compared to the data from EVA field trials, reflecting adult resistance. Some common markers were found but also new associations, which will be further investigated. For example, specific resistant material could be tested against different pathovars and used in crossing schemes to identify the genetic basis and the identified SNP could be transformed into markers for breeding. He highlighted interesting materials, which are often exotic landraces and more wild materials, and markers for different traits.

He analyzed correlations between traits, which could hint at some linkage as has been reported also for the nordic zone. For example, plant height and flowering time correlated with some diseases, which may reflect the developmental stages during which pathogens attack. This will have to be analyzed in more detail to identify false positives which could reflect the infection pressure or disease escape through early flowering. This will be an important trait in view of the climate crisis, also in helping avoid terminal drought conditions which have become increasingly common. It will be important for breeders from different zones to work together given the diversity of genetic resources available for breeding.

Similar to the Southern Zone, he identified several wheat accessions that showed multiple resistances for various pathogens. Going further, MTAs will be compared with known regions of resistance genes and quantitative trait loci (QTLs) to focus further research on novel resistances. Especially for Zymoseptoria, new fungicide-resistant isolates have been emerging and thus genetic resistance is the best approach to combat this disease. For stripe rust, results can be compared based on the race composition of the rust populations predominant in different years. Here, associations could also point to some putative unknown resistances. Accessions that show good resistance could be selected as parental lines for crossing to map the associated loci and make near-isogenic lines (NILs).

4.5 Overview of Watkins collection

Simon Orford (JIC) presented the material provided by JIC for the project which represents introgression lines of landraces in a modern cultivar background. This alternative methodology for landrace diversity selection and utilization could be useful for further work on the most promising accessions identified in the analysis of EVA field trials.

The germplasm research unit at JIC was presented as a one-stop-shop, where the genebank not only provides material worldwide but is also involved in providing relevant background information through a public search interface, actively collaborating with research partners in pre-breeding activities and accepting improved material back into their collection.

The Watkins collection from the 1920s was presented to the network previously and was safety duplicated in Svalbard in 2021. It was highlighted that the collection showed a large range of diversity for phenotypes. However, due to difficulties in management (lodging, diseases) this valuable resource is not easy to work with for breeders, who had been looking at the stabilized landraces but were discouraged by the difficulties in management. Therefore a bi-parental crossing strategy where stabilized landraces were genotyped and crossed to the elite cultivar Paragon to establish mapping populations and NILs was employed to introgress as much diversity of the landrace panel into a stable background.

The process of achieving a workable EVA panel involved several selection steps which decreased diversity but at the same time increased genotypic/phenotypic stability and usability. Of the historical landrace set those that were successfully stabilized and genotyped informed the selection of a core set of 119 landraces capturing most of the entire diversity, which was used for production of bi-parental populations, containing 50% of each parent. For the EVA project, 40 populations from across the world were selected of which 3–5 accessions were chosen for each zone, based on their flowering time data to be most suitable for that zone. Thus, the approach reduced the complexity of the collection on offer while still aiming to provide as much diversity as possible to the user.

Looking at the wider context, EVA is tapping into the UK initiative delivering sustainable wheat (DSW), evaluating biparental populations of landraces to make them available for breeding of elite material. This initiative also creates links between public research and the private sector, with several breeding companies involved both in EVA and DSW. The collaboration between the sectors is an important give and take where JIC provides material and information and breeding companies contribute trial sites and the necessary feedback on breeding priorities. Through this project, backcross populations are being developed that are presented back to breeders twice a

year for their feedback and selection. It should be noted, however, that these represent much less diversity than what has been provided to EVA.

To assess the usefulness of this alternative methodology, it will be necessary to analyze the data and see whether the flowering time prediction worked, whether the material was easier to analyze as compared to pure landraces and whether the diversity was useful to identify resistances or whether the reduced diversity lowered the germplasm usefulness. It was suggested that mixing bi-parental lines could give improved phenotypes, as it combines interesting traits from various backgrounds, especially under organic conditions.

4.6 Discussion

Considering the presented findings, it is clear that the multilocation evaluations have identified several novel resistance sources and associations, which can be provided to partners as a deliverable. However, not all evaluation data have been analyzed yet and a comparison of the results between the different zones and with existing literature still needs to be completed.

Going forward, the first priority is therefore to finalize the curation and analyses for the datasets from the different zones and partners were urged to provide any missing data ASAP. Generally, quality-checking and cleaning of the datasets is very important, due to the complexity of the data. In terms of quality checking of datasets, heritability was considered a good measure of quality and a threshold for usable datasets should be defined. A global comparison of the results and shared analysis should allow to categorize markers into promising loci and possible new QTLs, which could then be checked in all available accessions for genomic predictions. It will be interesting to assess whether differences observed between sets are related to germplasm or location effects. A. Serfling noted that combined analysis improved the logarigthm of odds (LOD) only in some traits and may have to be assessed on a case-by-case basis. It was suggested to use different statistics that are more robust to analyze data that are not normally distributed. In general, BLUEs are used to normalize the data, but for some traits data are available only for a subset of accessions and for these the analyses would have to be adjusted individually. Further discussions are needed to see how these can be improved.

It was noted that GWAS may not be the best approach to identify rare alleles, which may not pass filtering criteria. Similarly, MTAs that only appear in single or few environments should need further scrutiny to eliminate false positives, for example by comparing score distributions with other trials and confirming the identity of the pathogen strain. Promising accessions should then be used in crosses for genetic analysis and the creation of introgression populations. Also, accessions that show resistance to multiple pathogens will be very interesting to include in further research to identify the underlying genetics.

Correlations between QTLs for flowering and various diseases show the direct impact of plant development on the disease, especially for pathogens infecting during flowering where early flowering varieties may be able to escape infection and thus appear resistant. Here, overlap between putative resistance QTLs and known flowering loci should be taken with a grain of salt. Some diseases are very dependent on weather and environment so differences in developmental stages between wheat and barley during the same time of pathogen pressure could explain differences in correlations observed for those two crops. Caution should be taken in the interpretation of correlations as the coefficient also depends on the number of accessions in the

dataset. This highlights the challenge of a big multi-location evaluation programme such as EVA and the complexity of the datasets which sometimes compare different sized accession sets.

Analysis approaches could address various questions. Data analysis of the different regions should continue as now, to identify (novel) sources of (multiple) resistance that can be inserted into breeding programmes. It may be difficult to use the EVA materials directly in breeding programmes, being too far from modern elite varieties and an approach like that done for the Watkins lines should be considered to create pre-breeding materials. The analysis of general adaptation of landraces across different latitudes from south to north as indicated by the correlations between resistance and flowering time could be very interesting but also more complex. A good way to further exploit the data could be to prepare a publication describing the collection for each crop and climatic zone and to describe the novel sources of resistance.

The output for breeding companies' partners should be marker-trait associations but also genebank material that harbours valuable traits and could be included in breeding programmes. Before providing a definitive list of promising accessions and MTAs to the breeders, the results should be manually curated to check for the presence of known loci and to compare the results between the climatic regions. Breeders emphasized their priority to obtain markers for new resistance traits over the material. Since working with landraces used in the project is very difficult, it is unlikely that they will be commercialized immediately and a process of introgression into elite material is necessary to ensure the quality and yield of the final variety.

Whether or not joint efforts for pre-breeding could be a task within the EVA network would depend on the interests of partners, which will need to be assessed through a survey. Furthermore, before starting extensive crossing activities and population development it may be wise to confirm resistance of promising accessions against known strains of the pathogen under controlled conditions. Thus, for further analysis of novel traits identified, more research and validation is necessary that is in essence beyond the scope of the EVA project and should be pursued by partners in follow-up projects. This could be particularly interesting for multi-resistant accessions or for the analysis of overlap between phenological and resistance traits.

5. Outlook

5.1 Dissemination and exploitation of results

From discussions so far it was clear that the main outputs of the EVA project to breeders are marker-trait associations for novel resistance loci as well as promising materials with validated resistance phenotypes, ideally against multiple pathogens or pathogen strains. The preliminary results will be made available to network partners on SharePoint, with the caveat that not all data have been analyzed yet and so these are not finalized. The data analysis task force will meet to discuss further steps and consolidate the output for partners.

Having finalized the genotyping from all zones and all crops, the SNP Viewer will also be implemented in collaboration with IPK to provide a visual presentation of the diversity of the EVA accessions.

Follow-up research will be needed as there is a clear gap between the identification of a landrace with a novel resistance and the actual use of the underlying resistance gene in a breeding programme. Backcrosses and NIL populations need to be developed to clean up the background and identify and validate the resistance gene. This work requires substantial funding and takes

several years, thus is beyond the current EVA project. The output from EVA, however, could feed into such further projects which would first develop the material and populations for the detailed analysis of the resistance locus and its use in breeding. Speed breeding technology could speed up this process, but requires more external funding. The genebanks in the EVA network could be involved in the population development for their own material, provided this is feasible from a capacity and economic standpoint. A breeders' toolkit similar to what was presented for the UK DSW initiative could thus be developed by EVA and European genebanks for further research and breeding.

In terms of dissemination of the results beyond the network, publications should be planned. Detailed analysis of novel resistance loci will require additional research, but some preparatory studies could already be considered for publication, and outlines should be prepared and shared with the network to identify contributors to the manuscripts.

One possibility could be a general position paper, outlining the strategy of the EVA project and participatory phenotyping, highlighting the novelty of connecting public and private partners from different geographic zones to work together on identifying resources to breed for climate adaptation through participatory phenotyping. This could include descriptions of the global diversity of the collections for each crop and climatic zone used in EVA and phenology data could also be included, e.g. to describe adaptation through flowering time in different locations. Another interesting approach could be to compare results from analysis of durum and soft wheat from all zones, as some of the resistance loci against fungal diseases reported in the literature are the same and could be applicable.

Another study, already suggested in a previous meeting, could compare the results from the analysis of landraces versus the Watkins lines in order to assess the benefit of employing a strategy with evaluating biparental lines, incorporating also experience from the DSW initiative. This study could focus on phenology data which are available from most trials, describe how the diversity in flowering time is captured by the different approaches, and should be led by JIC (Noam Chayut). It could also exploit the genotyping data, assessing the diversity of the EVA lines compared with the original landrace. Another paper which could be very interesting could be on the use of genebank genetic diversity in modern breeding, describing also how the EVA database could be used to extract relevant information.

Another suggestion was the evaluation of subsets of accessions in targeted environments to study genotype-by-environment interactions (GxE) effects on important agronomic traits in different zones based on common genotypes (e.g. the IPK winter wheat set that was evaluated across Europe as part of Set 1). A description of specific genotypes that could be useful in different areas could also be interesting to publish.

5.2 Proposals for EVA Wheat and Barley 2.0 – continuation of network activities

In terms of work planning for 2024, the evaluations of all sets will be finalized. Some partners already communicated about postponements of trials, and feedback will now be collected from all evaluators to finalize trial planning for 2024. Similarly, the curation of all available evaluation datasets should be finalized to enable the completion of the data analysis task and further dissemination and exploitation as discussed.

An important aspect for further discussion is also in which way data should eventually be incorporated into EURISCO, since for the most part the evaluated material (SSD lines) are not

yet included in the database, only the parental material. In addition, the selection of datasets for upload should be discussed, whether BLUEs or raw data are more appropriate and how statistical analysis of data quality should be considered. The deposition of data has to be approved by the National Focal Point (NFP) of each holding institute and this discussion could also include the EURISCO advisory board. Importantly, conservation of SSD lines generated during the EVA project should be secured by the holding institutes. A survey will be conducted among genebanks to establish next steps in the conservation of these valuable resources.

As noted during the project overview, the EVA network has become a priority initiative within the ECPGR work programme for Phase XI (2024–2028) and thus a continuation of the EVA Wheat and Barley network beyond the current project funding as a self-sustained network based on in-kind contributions is possible, keeping in mind that funding for activities such as multiplications, genotyping and data analysis would need to be identified. One possibility to secure funding is to apply to the ECPGR Grant Scheme activity, which can support projects involving the relevant Working Groups and addressing the ECPGR priority areas.

The work plan for the current approach should be reviewed to optimize activities in line with available capacities. Aspects to be considered are the ideal size of accession sets and the extent of annual overlap for trials to provide linkage between years, keeping in mind that in practice evaluation of two overlapping accession sets including multiplication and analysis will require a five-year project duration. The timing of multiplications and evaluations to resolve issues around logistics of seed distribution should be reviewed, and of course also the selection of new crop sets for multiplication from AGENT precision collections (wheat, barley) or the Global durum panel (JIC), taking into account existing phenotypic data (PH, flowering time), genotyping and FIGS in the selection. Pre-breeding approaches could be included in the EVA activities, if partners are interested and funding can be secured.

Partners were invited to explore funding opportunities that could support some research activities within the EVA network or further exploit EVA results. For example, the EVA network could contribute to stakeholder engagement of larger European projects as is being done in AGENT, but other funding opportunities are also possible to fund follow-up research or development of populations that then could feed back into EVA Wheat and Barley in the future. For example, EVA Maize is continuing their collaboration within the SusCrop ERANet project MineLandDiv. The GreenERAHub (https://www.greenerahub.eu/) or PRIMA-Med (https://prima-med.org/) regularly open calls for projects on agricultural and breeding topics and could be used to support research in the EVA Wheat and Barley network. Going forward it will be important to identify the capacity for in-kind contributions of network partners, the minimum funding required for activities as well as the ambitious approaches that could be implemented with adequate project funding.

An initial round of feedback was collected from partners present at the meeting. Partners from Turkey are interested in working with soil-borne and cryptic pathogens, especially in barley where loss of resistance has recently been observed. They could conduct validation of pathogen tests in the lab, provided there is financial support, and have also developed some crosses between CWR accessions and cultivated barley which could be interesting for the network. Greece also has interesting material to share and Ioannis Mylonas offered to contribute to the analysis of phenotypic data. Direct deployment of promising accessions could be interesting especially for organic farming and in marginal areas. Rete Semi Rurali highlighted the demand for organic varieties based on the EU Farm2Fork strategy which requires an increase in organic farming. Including farmers and end-users directly would be beneficial for this but requires support from

researchers and the possibility of scoring more qualitative traits. L. Cattivelli emphasized that the priority now should be to finalize the current project and finish the exploitation of results. The approach to follow in future will depend on the stakeholders and could perhaps be informed by the proposed strategy paper that could serve as a basis for future activities. M. Bleidere noted that the multilocation trials clearly produced valuable information for breeders. Most partners were supportive of continuing the current approach and some expressed interest in evaluating also quality traits, however, this would require additional funding. Members of the data analysis task force agreed to work closely together to compare their results and provide the output to the network in a suitable form. A meeting of the task force will be scheduled to discuss this in detail. E. Mazzucotelli argued not to reduce the size of future accession sets as they need a minimum size for association analyses. Septoria and Fusarium should be tested in artificial inoculations, which may require funding. In the future it may be useful to focus on specific diseases that are evaluated in targeted environments with more reliable conditions, thus increasing the quality of the datasets and reducing the efforts in phenotyping. In general, she noted that we should demonstrate that the approach followed is good for breeders. S. Orford suggested thinking about how material is delivered to breeders, the output should be reduced to the most useful lines, considering that the landraces still have a lot of genetic background that needs to be cleaned up. Breeders were in general happy with the results and the approach, although some improvements could be made. Future accession sets should be selected based on adaptation and data from other projects, logistics and paperwork should be improved and data quality should be increased. Harmonization of trial design and inclusion of official checks in all locations could facilitate comparison of trials and data analysis. Sharing of results, both markers and promising accessions should be done in a systematic way. Multipliers have kept seed stocks of the SSDs, which can be multiplied for distribution of most promising accessions for further research and breeding. Some budget should be allocated to this activity.

A survey will be conducted to collect feedback from all partners about their priorities and capacities for a continuation of the EVA wheat and Barley network, to facilitate planning of activities of an EVA 2.0 and development of project proposals for additional funding.

5.3 Next steps and closing of the meeting

Priorities for the coming months were outlined and relevant action points are collected in Appendix 3. Collection of missing data and finalizing the last round of evaluation trials are the main priorities. Data analysis will continue with a meeting of the task force and harmonization of approaches and outputs and comparison of results. Sharing of results will also be centralized and communicated to partners. Publication planning will be followed up with the involvement of the whole network. Surveys will be conducted about the status of conservation and data management for the SSD lines generated and also about the priorities and capacities for the continuation of EVA Wheat and Barley. Based on these, a proposal for EVA Wheat and Barley 2.0 will be presented to the network towards the end of 2024. At the same time, partners are encouraged to develop project proposals for suitable calls. It is not clear whether another in-person meeting will be necessary and possible, a virtual meeting will be convened in due course to update the network on developments.

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

Appendix 1: Meeting Agenda

Venue: Magazzino Gelmetti, Fiorenzuola d'Arda, Italy

THURSDAY 25 JANUARY 2024		
08:30 – 09.00	Registration	
	Welcome and introductory session	CHAIR: LUIGI CATTIVELLI
09.00 – 09.10	Welcome by local host, ECPGR	*L. Cattivelli* *S. Goritschnig*
09.10 – 09.20	Overview of the current status of the ECPGR Evaluation Network EVA	*S. Goritschnig*
09.20 – 09:30	Review of EVA Wheat and Barley network workplan 2019-2024	*S. Goritschnig*
	Update from Horizon2020 project AGENT	CHAIR: S. GORITSCHNIG
09:30 – 10:45	Update from Horizon2020 project AGENT - progress in AGENT (genotyping, phenotyping) - FIGS prediction for phenotypic traits - Training opportunities within AGENT (data curation, data management, bioinformatics pipelines) - Rete Semi Rurali – Farmer's network evaluation - discussion on opportunities for exploitation of AGENT results by EVA WB	*M. Haupt* *Z. Kehel (online)* *S. Goritschnig* *B. Bussi* *All*
10.45 – 11:15	TEA/COFFEE BREAK	
	Review of experiments and preliminary data 2023	CHAIR: F. GUZZON
11:15 – 12.45	Comments and highlights from evaluators of 2023 trials - Traits evaluated - Preliminary results from trials - Lessons learned and suggestions for upcoming trials	*M. Bleidere* *R. Koppel* *M Jalli* *G.Brazauskas* *E. Cakir* *A. Celik*
12:45 - 13:45	LUNCH	
	Data Analysis	CHAIR: LUIGI CATTIVELLI
13:45 – 14:00	Overview of data management and review of available data	*F. Guzzon*
14:00 – 14:30	Update on data analysis of genotyping and phenotyping data for Sets 1 and 2 - Nordic zone	*J. Hautsalo*
14:30 – 15:00	Update on data analysis of genotyping and phenotyping data for Sets 1 and 2 - Southern Zone	*D. Barabaschi*

	Data Analysis ctd	CHAIR: LUIGI CATTIVELLI
15:00 - 15:30	Update on data analysis of genotyping and phenotyping data for Sets 1 and 2 – Central zone	A. Serfling (online)
15:30 - 16:00	**TEA/COFFEE BREAK**	
16:00 – 16:20	Preliminary analysis of data on Watkins collection lines evalutated in EVA	S. Orford
16:20 – 17:00	Discussion on data analysis – additional analyses, questions to be adressed, approaches, possible contributors	All
17:00 – 18:30	Visit to CREA-GB facilities	A.Tondelli, E.Mazzucotelli
20:00	**SOCIAL DINNER**	

Venue: Magazzino Gelmetti, Fiorenzuola d'Arda, Italy

FRIDAY 26 JANUARY 2024		
	Outlook – EVA Wheat and Barley 2.0	CHAIR: SANDRA GORITSCHNIG
08:30 – 09:00	Dissemination and exploitation of results Planning for publications	S.Goritschnig
09:00 – 10:00	Proposals for EVA Wheat and Barley 2.0 – continuation of network activities - Work planning for 2024 - Long term planning for EVA WB: Options for continuation of network after 2024	All
10:00 – 10:30	**TEA/COFFEE BREAK**	
10:30 – 12:30	Development of work plan for EVA Wheat and Barley 2.0	All
12:30 – 13:00	Final discussions and wrap-up	All
13:00	End of meeting	

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Appendix 3: Action list 2024

#	Task	Responsible	Due date
1	Provide data from previous evaluation trials, using data collection templates	All evaluators	29.02.2024
2	Curate data and finalize upload of all missing data for Set 1 and Set 2	F. Guzzon	29.02.2024
3	Task Force meeting to coordinate analysis activities and harmonize output	DA TF	29.02.2024
4	Provide feedback on SSD management in survey	Genebanks	29.02.2024
5	Provide feedback in survey on priorities and capacities for EVA WB 2.0	All network partners	31.03.2024
6	Provide weather data for experimental locations and upload to database as supplemental files	All evaluators, F.Guzzon to coordinate	31.03.2024
7	Collect information on prevalent strains used in inoculated trials/encountered in field	Evaluators, S. Goritschnig to coordinate	29.02.2024
8	Make project output (MTAs and list of promising accessions) available on SharePoint, including relevant documentation	DA TF, S. Goritschnig to coordinate	31.03.2024
9	Plan activities to make promising accessions available to partners for breeding and further research	S. Goritschnig	30.06.2024
10	Archive copies of SMTAs used for seed exchange	All senders and recipients of seeds	31.05.2024
11	Implement SNP viewer for wheat and barley genotyping data of Set 1 and 2	F. Guzzon, DA-TF and IPK	31.03.2024
12	Share publications on JKI studies using NIL lines with Lr and Yr genes	A. Serfling	31.03.2024
13	Develop publication plan, identifying topics and outlines, leads and contributors	S. Goritschnig to coordinate	30.06.2024
14	Use FIGS on AGENT accessions to select new set for EVA	S. Goritschnig to coordinate	30.06.2024
15	Virtual meeting to update network on developments	All partners	31.07.2024