# Summary report
# of the

# Annual meeting of the European Evaluation Network (EVA) for Maize

27-28 February 2025
Bergamo, Italy

**Contents**

The EVA Maize annual project meeting took place on 27-28 February 2025 in Bergamo, Italy, co-hosted by CREA-CI. The meeting was combined with a kick-off meeting of the ECPGR Grant Scheme Activity MALANIRS, coordinated by Stephane Nicolas (INRAE). A virtual connection was established for network partners who were not able to travel to the meeting. The agenda of the meeting is attached as Appendix 1 and the list of participants as Appendix 2.

## 1. Welcome and introduction

On behalf of the local organizers, Carlotta Balconi (CREA-CI) welcomed all participants in Bergamo and at the EVA/MALANIRS meeting. The EVA Coordinator, Sandra Goritschnig, opened the meeting, welcoming in person 25 participants of the EVA network and MALANIRS project. She thanked C. Balconi and her team at CREA-CI for their help in organizing the meeting and welcomed the opportunity to kick off the MALANIRS project as an extension of the EVA Maize network.

After a round of introductions, S. Goritschnig reviewed the agenda and reminded participants of the expected outcomes of the meeting, which included a general update, a detailed discussion of the current results, and an outlook for the continuation of the network after the end of the current funding period, which will involve collaboration with MALANIRS and MineLandDiv projects.

## 2. Update on current status of the ECPGR Evaluation Network EVA

For the benefit of the new partners from the MALANIRS project, S. Goritschnig reviewed the objectives of ECPGR and its European Evaluation Network (EVA), highlighting the opportunities and benefits that the cooperation in so far six crop-specific networks has created. After several extensions and amendments, the current EVA project funded by Germany (GenR 2019-2) ends in March 2025, after a last no-cost extension was granted to allow the organization of this meeting. As mentioned previously, the coordination of the EVA networks is guaranteed to continue thanks to ECPGR support, and new initiatives such as MALANIRS provide incentives for new activities to continue the collaboration. Similarly, another Grant Scheme Activity, EuroPepLand, coordinated by the ECPGR Solanaceae Working Group, extends the collaboration of the EVA Pepper network, both in terms of new members and germplasm for evaluation. In addition, a new project, 'EVA Boost', has been granted by Germany (GenR 2024-2) to support the implementation of the EVA Legumes network and including also a preparatory action for the creation of a new network on perennial plants (berries and fruit trees)[1]. The EVA promotional video is available on the ECPGR website and YouTube[2], and participants were invited to like and promote it as well as to follow ECPGR on our new Linkedin[3] account, where we regularly post relevant information and news.

Thanks to the start of the MALANIRS project, the EVA Maize network is expanding to new partners. All new project partners were invited to join the network and sign the updated cooperation agreement, and the network now counts 24 partners in 13 countries, including 15 genebanks or research institutes and 9 breeding companies.

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[1] https://www.ecpgr.org/resources/ecpgr-publications/publication/eva-boost-2025
[2] https://youtu.be/AZ1NB6hC_ZU
[3] https://www.linkedin.com/company/ecpgr/

In terms of dissemination activities, the paper published by the EVA Maize network in the journal *Biology* in June 2024[4] has received significant interest and has been cited seven times already. A book on the characterization of Romanian maize populations was published by Danela Murariu and co-authors, and the phenotypic data included in this volume have been extracted and included in EURISCO. The work of EVA Maize has been presented at various national and international conferences, including the EUCARPIA general congress in Leipzig, Germany. Other opportunities for dissemination and communication were highlighted, and partners invited to report their local, national and international dissemination activities to the EVA coordinator for reporting to the donor.

Reviewing the action points identified in the last meeting, it was noted that most have been addressed already, and some were up for discussion at this meeting.

## 3. Results from EVA Maize project

### 3.1 Review of network workplan 2020–2024 and overview of collected trial data

Partners were reminded of the agreed work plan and outputs of the different planned activities between 2020 and 2024. With the end of the current project, all evaluations of the three sets of accessions have been finalized. In total, 612 genebank accessions from 9 holding institutes have been evaluated, as well as 360 testcross hybrids from selected accessions. EVA Maize network partners have conducted 105 evaluation trials in 25 locations across Europe.

The datasets collected include scores on 55 traits (with between 58 and 9,773 datapoints), and each accession or testcross population has between 38 to 460 or 12 to 144 data points, respectively. The evaluated traits could be grouped in plot quality (e.g. number of plants, vigour), height, flowering, morphology (kernel and ear traits), lodging, yield, biotic and abiotic stress traits. Data on height and flowering had already been analyzed for the *Biology* manuscript, and analyses of other traits data will be presented during the meeting and further coordinated. It could also be very interesting to revisit a previous proposal to compare trait expression in different environments based on common controls and collected meteorological data.

### 3.2 Review of experiments and field trials in 2024

In 2024, 19 field trials had been conducted for in-depth evaluation of per se accessions and evaluation of the testcross populations created from the third set. Some trials which had failed in 2023 due to pests or adverse weather conditions were also repeated. The reports below are complementary to those given by partners during an earlier virtual meeting.

Alexandre Strigens (DSP) reported on four trials in two locations in Switzerland, on per se accessions and hybrid populations. Despite late sowing dates and some lodging in one trial, the data collected were good, showing correlation between grain yield and plant height and high heritability for grain moisture traits. In their conditions, the late checks did not mature and some lines were very early, possibly indicative of inbreeding.

Natalija Kravic (MRIZP) reported on their evaluation trials, including a repeat of the 2023 per se

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[4] https://www.mdpi.com/2079-7737/13/6/454

trial, which had experienced unfavourable conditions. Early July rains caused some root lodging but overall the data collected were good.

Hrvoje Sarcevic (UZagreb) conducted evaluation of 37 maize landraces, which were grown in a lattice experimental design. The weather conditions in summer 2024 were hot to extremely hot, with above-average precipitation, most of which was due to two summer storms. Nevertheless, the trial produced useful results with a mean realized plant stand of 95%.

Pedro Mendes Moreira (ESAC) had conducted a trial on per se accessions, with the sowing of Serbian accessions delayed as they had been stuck in customs. Their trial was combined with the evaluation of Spanish and Portuguese bread flour maize accessions (Caldeirao), as part of a living lab initiative.

Amelie Le Foll (MAS) reported on their trial on hybrid populations in central France. 2024 was a difficult year weather-wise, with early cold stress and rain during grain filling, delaying the harvest.

Carlotta Balconi (CREA) had conducted a trial on set 3 hybrids. Because of heavy rains just after sowing, the crop had bad establishment and overall low yields. The data may not be useful for comparisons.

Alain Murigneux (Limagrain) summarized results from their trial on hybrid populations. They noticed lodging in around 30% of experimental plots (compared with 17% of check plots), and also a reduced yield by about 25% in the experimental population versus the checks.

Aloïs Gourrion (RAGT) noted the diversity throughout the accessions with some populations being very tall, with big and well-developed tassels, grains from every colour. Some accessions seem sensitive to lodging, but no precise notations were taken.

# 4. Data analysis

## 4.1 Results from analysis of phenotypic data of per se accessions

Rosana Malvar presented initial results from the analysis of grain yield data on per se accessions. As per trait definition, the reported grain yield (in t/ha) is adjusted to 10% grain moisture as estimated by the relevant trait. Data are available for > 500 landraces from 24 trials in 6 locations conducted over 4 years. Despite efforts to promote standardized protocols, partners did not use uniform experimental designs and these are therefore unbalanced. Using a combined statistical analysis, it was concluded that all sources of variance (landrace, trial, replicate) were significant. Because the genotype × environment (G×E) interaction was significant, the analysis was then conducted for each trial individually, and all BLUEs and standard errors were shared with partners on the project SharePoint.

Factorial regression was used to analyze G×E interactions, modelling them as functions of specific environmental and genetic covariates. The genetic covariable was the K = 9 genetic groups reported in the *Biology* paper, eliminating non-genotyped accessions and checks. Since not all climatic data were already available, the environmental variables were estimated based on mean values of check varieties for traits reported in the *Biology* paper, to characterize different trials. In the general linear model the least correlated variables were eliminated from the analysis, K7 from the genetic groups and DS (days to silking) from the traits to estimate the environmental variables. The analysis found that all germplasm groups except K2 contributed to explaining yield variability, with Dent, Portuguese and Northern Flint groups having bigger effects. Environments have a significant effect on yield, with optimal environments supporting good yields and stressful environments reducing yield.

Combining the analyses was necessary as the data between trials was unbalanced, therefore the accessions were grouped in germplasm groups and the environments by localities, which allowed the identification of the most stable genetic groups and environments. In this sites regression model, admixed accessions were grouped based on the frequency of the admixed K groups, yielding 18 genetic groups.

For future work, it is planned to use the meteorological data as environmental covariates, this is now available for all trials. An analysis of yield-related traits such as grain moisture or morphology could be considered and would be included in a manuscript in preparation. Available disease data should then also be analyzed to identify useful material for further research and breeding.

Participants questioned why the morphology traits and not the yield traits of the checks were selected to describe the environments, as this could reflect different environmental conditions. Grain yield was not selected because it would be the most important correlated trait and thus potentially skew the analysis. An alternative or addition to analyzing meteo data for use as environmental covariant could be the data on early vigor.

## 4.2 Preliminary results from testcross population trials

Cyril Bauland (INRAE) summarized the preliminary results from all testcross evaluations conducted in 2022, 2023 and 2024. Due to delays in seed shipments, the trials in 2022 and 2023 were unbalanced with regards to hybrids with the RAGT tester or the Lidea tester, respectively, while in 2024 all populations were tested in all environments. Because of the use of different testers, the trial design used complete blocks with minimal competition between testers, applying the Agricola R package with some manual adjustments. Preliminary analysis of the trials revealed some missing data, not all traits were scored in all locations, e.g. early vigour notations were not scored where the weather was not cold at emergence. Analyzing grain moisture and grain yield, the results are nicely distributed and some crosses almost reach the level of the included checks. A preliminary ANOVA detected no population effects, but some environmental and set effects. Further analyses will depend on the needs of the breeders.

Breeders summarized their wishlist as wanting early hybrids that could bring new traits into their breeding programmes, that are not too badly yielding, with good diversity and disease resistance but not prone to lodging. It would be good to combine the analyses of per se evaluations and hybrids to identify the most suitable accessions for breeding. Partners were reminded that the initial selection of accessions for crosses only took the genotyping, plant height and flowering time into consideration, not lodging or biotic stress traits. Thus, a comparison of the sets and selection criteria could be interesting.

## 4.3 Results from genotyping and GWAS/Genomic prediction

Agustin Galaretto (INRAE) reported on additional analyses using the genotyping data generated in EVA for genomic predictions and association studies. Using allelic frequency predictions and admixture analyses, the EVA collection was separated into nine genetic groups with a large proportion of accessions considered admixed (< 40% assignment to a genetic group). This data had been published together with some initial phenotypic analysis of plant morphology and flowering time in the journal *Biology*.

Genomic prediction across environments used the GBLUP model on flowering and plant height, looking at the performance of each landrace in each environment. Initial analysis tested the approach in five environments and will be extended to the whole trial network. The goal is to create a tool for boosting characterization of maize landraces and a manuscript applying genomic prediction to the characterization of maize landraces is in preparation.

An important application of genomic prediction is the estimation of genetic offset as a predictor of adaptation/maladaptation, which would predict the future optimal growing conditions for a specific landrace. It would use climatic data from the trial locations to estimate genomic offset and highlight accessions that are maladapted to that trial location. This information could be added to each accession in a breeder's catalogue.

Genome-wide association studies (GWAS) were conducted using the phenotypic BLUEs estimated by R. Malvar for multiple traits and climatic variables from collection sites sourced from WorldClim2.1 (1970-2000). The model used was able to detect known genetic loci affecting flowering. Updated BLUEs will be used to do trait × trial analyses and make a list of interesting marker-trait associations. This work will also be exploited as a peer-reviewed article.

In a general discussion, partners were asked to double-check their yield data and whether the moisture correction to 10% was effected correctly, because different yields were reported in trials when the ranking of accessions by grain moisture was constant. Alain Charcosset suggested connecting all evaluations to combine GCA and per se accessions, which could improve the analysis where performance may not be the best proxy. It was also suggested to fix a threshold for yield penalty before selecting accessions for breeding based on disease resistance.

## 4.4 Development of a breeder's catalogue from the EVA Maize collection

At the last in-person meeting in Montpellier, it was suggested to develop a 'Breeder's catalogue of best performing landraces', which would combine information on the main target traits for each accession in a one-stop resource enabling breeders to select materials for further research and breeding. Similar query databases have been developed by CIMMYT (https://maizecatalog.cimmyt.org/) or Genesys. A discussion on the topic touched on possible content for the catalogue, priority traits to include, possible linkage with genetic diversity data as well as on layout and implementation. In terms of target traits, precocity was highlighted as one of the most important traits, along with biotic stress tolerance and lodging sensitivity. Breeders proposed to us BLUP of different traits for all accessions in the trials network and include also genomic prediction of each landrace in different environments. While the development of a technical solution is beyond the means of the EVA project, a simple searchable Excel template could be designed, where breeders could select landraces for prebreeding according to their own objective by combining searches for target traits. An outline for the catalogue will be prepared and presented to the network in the next virtual meeting.

# 5. MALANIRS Kick-off meeting

## 5.1 Introduction to MALANIRS

Stephane Nicolas (INRAE) presented an overview of the ECPGR Grant Scheme Activity MALANIRS, which provides an opportunity to enlarge the EVA Maize network involving new partners from the Maize Working Group as well as adding to the EVA Maize collection available for evaluation to the network. The activity was granted during the first call of Phase XI and is planned to run for three years, starting September 2024. The project builds on prior activities like 'Amaizing', 'MineLandDiv', the EVA Maize network and collaborations with CIMMYT, which have developed training sets and genotyping and analysis tools. MALANIRS plans to promote the use of landraces in breeding programmes through applying a low-cost approach for their characterization by genotyping and Near InfraRed Spectroscopy (NIRS) of kernel composition. NIRs is a low cost and non-destructive approach classically used in breeding programmes to evaluate biochemical composition (or digestibility) of seeds or silage. This biochemichal characterization is based on a model trained on a set of seed (silage) samples that were analyzed both with NIRs and with wet lab, and calibration of a NIRS equation for the relevant traits. The collected data can then be used to develop genomic and phenomic prediction pipelines to enable effective selection. A proof of concept will be developed for the use of genomic and phenomic prediction based on NIRs to characterize agronomic traits including seed composition of maize landraces from European genebanks.

As objectives, the MALANIRS project aims to extend genomic characterization to 500 new landraces from Europe using DNA bulk targeted sequencing approaches, to offset the ascertainment bias of the 50k SNP chips. Two relevant technologies are currently being tested within the MineLandDiv project and a pipeline will be available for use on MALANIRS samples in due course. These data will be combined with phenotypic information from EVA and MineLandDiv to develop genomic selection and prediction pipelines. MALANIRS also plans to develop and evaluate a proof of concept to use NIRs as a low-cost alternative to genotyping for predicting various agronomic traits, including grain composition. All data will be made available through public databases (EURISCO database for phenotyping and the INRAE ThaliaDB database for genotyping data) for use by farmers, breeders and genebanks. The project currently has 15 partners including ten national European genebanks, additional self-funded partners could join without budget.

As a first task for the project, the selection of 500 maize landraces in European collections for targeted sequencing should be finalized, taking into account a balanced geographical representation (also in the context of the existing MineLandDiv and EVA collections), availability of passport data including geographical origin, and existing information on interesting uses and traits.

## 5.2 Descriptions of European Maize landrace collections for MALANIRS

Presentations of Maize landrace collections and previous or ongoing research activities of MALANIRS partners are available on the project website[5].

**Albania:** Najada Kadiasi (Agricultural University of Tirana) presented the work of the Institute of

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[5] https://www.ecpgr.org/working-groups/maize/malanirs

Plant Genetic Resources (IPGR) at the Agricultural University of Tirana, Albania, which is the national institution coordinating the activities in the field of conservation and management of plant genetic resources (PGR). A recent project supported by the Crop Trust contributed to the regeneration and characterization of around 700 maize populations, which were also safety duplicated in Svalbard. Due to extensive phenotypic similarities in the collection, genotyping will be essential to help identify possible duplicates. For genotyping and NIRS analyses within MALANIRS 50 unique landrace populations were selected, which had been collected between 1976 and 2009 at elevations between 7 and 1,358 masl from all across Albania. No funding is currently available for seed multiplication, therefore the seeds provided for the project were multiplied in previous projects, and they also don't have capacity to conduct NIRS analysis in Albania.

**Austria:** Lisa Achathaler (AGES) presented on the Austrian genebank collection, which contains 58 maize accessions with associated characterization and evaluation data. While the overall production area of maize in Austria is quite large in the northern and eastern plains, only few farms still produce 'Riebelmais' varieties, which were once considered food for the poor. AGES can supply 40 accessions but has no capacity for NIRS or other evaluation activities.

**Croatia:** Domagoj Simic (Agricultural Institute Osijek) presented his institute located in northeastern Croatia, within the 'European corn belt'. The institute works on plant science and breeding of various field crops and has also developed commercial crop varieties. Its maize collection is part of the decentralized Croatian plant genetic resource (PGR) system and includes inbred lines (which have been genotyped), synthetics, OPVs and landraces. They will contribute 21 landraces and OPVs to the project, with a focus on early material, possibly originating from Italy, selected based on geographical diversity. They have experience in NIRS analysis using proximal hyperspectral handheld spectroradiometers for canopy (leaf) analysis, but not on grains.

Hrvoje Šarčević (University of Zagreb) presented their work managing the central Croatian PGR database, contributing to the collection and conservation of landraces and their maintenance, regeneration and description. U. Zagreb will provide 90 new landrace accessions from across the country for the project, including also from dry and hot climates in southern regions, where very early maturity predominates. Regenerations conducted in 2024 experienced major challenges due to drought and bird attacks, but genebank stocks will hopefully be able to compensate. They will also contribute field trials on MineLandDiv materials in 2025, but don't have their own NIRS to analyze the maize kernels. A collaboration with a local company (BC Institute Zagreb) was established and will enable them to perform NIRS assays.

**France:** S. Nicolas (INRAE) confirmed that France would contribute 40 landraces from their collection, which have already been extensively genotyped and phenotyped in previous projects.

**Italy:** Carlotta Balconi (CREA-CI) presented the work and collection of the Italian Research Centre for Cereal and Industrial Crops in Bergamo. As one of several sister institutes, Bergamo focuses on maize PGR, applying their expertise in genetics, agronomy, physiology, (bio)chemistry, NIRS and pathology to the preservation, characterization and management of maize biodiversity. Among the more than 5,000 accessions preserved in their genebank are 1,314 landraces (of which 720 are Italian), and CREA-CI contributes a diverse panel of 43 landraces to MALANIRS. Through participation in other projects, there is some overlap between collections, which may help to link the different datasets, complement and extend these existing projects. They also have expertise in NIRS, which they are happy to share with project partners.

**Montenegro:** Zoran Jovović (U of Montenegro) presented the Biotechnical Faculty of the University of Montenegro (BTF). With 87 years of existence, it is the only scientific research and higher education institution in Montenegro covering all areas of agriculture and as such acts as focal point for genetic resources. The Montenegrin plant genebank, hosted by BTF, owns a collection of 68 maize accessions collected in 2009 and 2010. MRIZP in Serbia stores another 320 accessions collected in Montenegro in the 1960s and 1970s. Local maize PGR are being maintained and cultivated by the institute in collaboration with farmers, who mainly produce them for agritourism purposes. Phenotypic data are available for all accessions and sufficient seed for the project are available for 40 accessions.

**Portugal:** Pedro Mendes Moreira (ESAC-IPC) presented the Coimbra Agriculture School (ESAC) in Portugal, which has been involved in regional projects promoting the use of local varieties and participatory breeding approaches for more than 40 years. An important objective of the VASO project is to motivate farmers to promote and contribute to the maize value chain. There is a trend to convert fields for organic agriculture, which can then be used to perform trials on organic research and breeding, also as part of a living labs initiative. They are thereby promoting the unique characteristics of local varieties with a DOC seal and also conduct outreach and consumer engagement through the organization of meetings and events. In the existing niche markets, farmers are able to sell their maize flour at 1 Euro/kg, and ESAC provides farmers up to 30kg/ha of improved landraces for their production. ESAC will provide five landraces to the MALANIRS project, primarily of maize used in breadmaking. The school's NIRS instrument is currently not working, but a question on how to manage different size kernels in the NIRS sample cassette was posed.

Ana Maria Barata (BPGV-INIAV) presented the Portuguese genebank in Braga, Portugal, which has been operational since 1977, starting with establishing maize collections. At the moment, 2,760 maize landraces are included in the Portuguese national collection, with the majority collected in Portugal. Seventeen accessions were included in the European Maize landraces core collection EUMLCC and already shared within the EVA network. Portugal also contributed 1,960 accessions to a Mediterranean maize collection and will provide 40 landraces to the MALANIRS project. BPGV does not have capacity or equipment for NIRS assessment but is interested in making landraces available for multilocation trials across Europe to better understand the potential and reliability of these materials and enrich their collection.

**Romania:** Danela Murariu (Suceava) provided an overview of the Suceava genebank in Romania, established in 1990 as the main custodian of the national seed collection. Their maize collection includes more than 3,500 local landraces of which so far only 161 have been safety duplicated in Svalbard. Around 1,300 accessions have been morphologically or biochemically analyzed before 2003. Most of the local landraces were collected in mountainous regions across Romania, while in the lowlands, the landraces have been largely replaced or contaminated by modern varieties, resulting in genetic erosion over the past decades. A recently established *in situ* database aims at supporting and improving on-farm conservation. NIRS analysis of genebank material could be done through collaboration with the University of Suceava, which has a new 9500 NIR apparatus. Unfortunately, no material can be provided to the MALANIRS project for genotyping and other experiments, because the necessary quantities are not available in genebank stocks and there is no funding for regeneration of accessions.

**Serbia:** Natalija Kravic (MRIZP) presented the ongoing work and existing maize collection of the Maize Research Institute Zemun Polje, Serbia. The institute has one of the largest maize collections in Europe, including more than 2,217 local landraces from the area of former Yugoslavia, of which they will provide 150 (from Serbia, Bosnia and Herzegovina, North Macedonia and Slovenia), covering some origin countries not present in the project. They will also conduct field trials under drought conditions but are limited in contributing to NIRS analysis as their instrument cannot analyze dark maize kernels.

**Spain:** Pedro Revilla (MBG-CSIC) presented the work and collection of the Mision Biologica de Galicia in Pontevedra, Spain. As part of the Spanish National Research Council (CSIC), the MBG specializes in plant genetics, crop improvement and sustainable agricultural practices. Its research aims to enhance food security, biodiversity conservation and environmental sustainability. The institute is involved in a number of national and international projects, collaborating also with the private sector. The MBG maize collection includes 241 populations, of which 100 are currently being analyzed by NIRS, following an improved calibration protocol for coloured grain. MBG aims to provide 40 new landraces to MALANIRS, having already included a large number of Spanish accessions in the EVA collection.

**Switzerland:** Beate Schierscher-Viret (Agroscope) presented her institute, which combines research, policy advice, enforcement, knowledge exchange and technology transfer in all areas of agriculture and food production. Maize has a long history in Switzerland, having been mentioned for the first time in 1571 and being used in traditional food products such as polenta, ribel and cornbread, albeit with small production areas. The Swiss maize collection includes 209 local varieties, which had been collected since the 1940s and are locally adapted to mountainous and valley conditions. Several thesis projects have characterized the genetic diversity of Swiss landraces. Switzerland already contributed a significant part of their collection, including landraces and inbred lines in the EVA network and are now providing 40 new landraces from southern and eastern Switzerland, collected mostly in the 1940s.

**Turkiye**: Gönül Cömertpay (DATAEM) informed on the activities and collections at the Eastern Mediterranean Agricultural Research Institute in Adana, Turkiye, which is part of the General Directorate of Agricultural Research and Policies (TAGEM). The Turkish genebank contains nearly 2,090 maize landraces, collected between 1974 and 1981; however, permission for use in international collaborations is currently regulated. The DATAEM institute in Adana has 500ha research and production area and conducts maize breeding programmes, using also SSR markers for genotyping. Other projects work on maize epiphytic flora and biotic stresses. DATAEM included 32 Turkish landraces from their collection in MALANIRS and MineLandDiv, and are also conducting field trials and NIRS analyses.

## 5.3 Work planning of MALANIRS activities

After presentation of the diverse collections which will be included in MALANIRS, the group discussed the work planning and next steps.

Within Task 1 (Targeting sequencing of 500 new landraces from different genebanks for diversity analysis and genomic prediction), the final selection of the accessions is a priority as seeds need to be provided to INRAE for processing by March at the latest due to the upcoming retirement of the trained lab technician. It was again highlighted that the collection should be geographically balanced and complement the existing EVA collection, and all selected landraces should have associated passport data and be shared with SMTA. In a next iteration, the whole EVA collection could also be included in the targeted sequencing effort, to enrich the SNP dataset. The plant material list will be finalized to include information on whether material has been included in other projects (e.g. EVA, MLD, Dromamed), and thus enable a bridging of the different accessions and datasets. It was clarified that ideally, all accessions included in the targeted sequencing task should also be analyzed by NIRS, including the existing EVA collection. However, genotyping will be prioritized over NIRS in cases where not enough seeds of interesting materials are available. DNA extracted for the sequencing task will be stored for eventual follow-up experiments.

Patricia Favreau (INRAE) explained the targeted sequencing approach, which uses microhaplotypes (markers with at least two SNPs within a 300 bp window) to differentiate accessions. As in previous approaches, the DNA is extracted from 25 plants per accession to maximize the population effect. Currently, two platforms (NEB next direct and Allegro SPET v2) are being tested in proof-of-concept studies on 110 targeted SNP regions in known QTLs for abiotic stresses and flowering time. In this first experiment, different biological materials (landraces, inbred lines, F1 hybrids and control pools with genotypes mixed in different proportions) were included to develop the analysis pipeline for estimating haplotype frequency. The Allegro SPET approach seems the most promising, also in terms of economy, bioinformatic support and read length, and for the MALANIRS project the approach will be applied to 5,000 target SNPs on the selected 500 landrace samples.

**Task 2** (NIRs of kernels harvested in field trials to train phenomic prediction model for traits and grain composition) is planned to be conducted centrally on kernels harvested in three field trials conducted within MLD. MALANIRS has also planned two field trials in Zemun Polje (on the full MLD set) and Zagreb (on a subset) in 2025, using materials regenerated in MLD, which will also be harvested and analyzed with NIRS. Other additional field trials should be conducted by EVA partners in the following years, for available landraces. EVA will share the experimental and phenotyping protocol also for regeneration.

No funding is available for the development of a new NIRS equation through combination with wet lab experiments, and therefore, labs with calibrated instruments will perform this task. The phenomic predictions calculated in this task could then be confirmed by field trials within the EVA network. Similarly, correlations between field trial results and NIRS could be used to predict other traits, not only grain composition, but appropriate traits will need to be selected, in alignment with those evaluated in EVA and focusing on priority traits for future evaluations. A protocol for seed processing for NIRS after harvest will be developed and shared with partners.

In **Task 3** (Proof-of-concept of phenomic prediction of traits and grain composition of landraces based on individual NIRs of kernels from genebank seed stocks), the NIRS approach is planned to be applied to phenomic predictions of genebank material stocks. For this task, genebanks should provide 250 kernels sampled from kernel lots of genotyped landraces (from EVA, MLD or MALANIRS) to INRAE for centralized NIRS analysis. Partners who have NIRS in their institutes should use these for the analyses. SUCEAVA, ESAC, CSIC, CREA and DATAEM confirmed their ability to conduct NIRS analyses and UZagreb may be able to collaborate with the BC institute on this task. Some standardized samples (including control hybrids) could then also be shared between labs to conduct a ring test to validate the approach using different NIRS instruments and confirm the NIRS equations. As with genotyping, the data collected within MALANIRS will complement datasets from MineLandDiv and EVA to enable analysis of a larger collection.

**Task 4** (Feeding and sharing data using EURISCO database for phenotyping and ThaliaDB database for genotyping) concerns data management and analysis tasks and will make use of existing infrastructure such as the EURISCO-EVA intranet and associated templates for collecting and sharing raw data. All EVA partners will get login credentials to the ThaliaDB where the genotyping data is available and visualized.

# 6. Integration of EVA Maize and MALANIRS activities

No specific regeneration activities have been budgeted in MALANIRS and materials used in field trials will be sourced from previous regenerations in MineLandDiv and EVA. Future regeneration activities should be considered and prioritized for high-potential materials as identified by genomic and phenomic predictions.

Priority traits for future field trials were discussed (see flipchart). It was proposed to adjust the definition of yield traits for better standardization, to use yield per plot in combination with Thousand-kernel-weight to measure the number of kernels per plant. Flowering traits, architecture traits (plant and ear height), grain yield, TKW and lodging were considered important. As previously commented, kernel colour and type should be scored on regenerated material to avoid cross-pollination effects. Other morphological traits would not need to be scored in multiple locations, reducing the workload of evaluators. Breeders commented that priority traits also depend on the intended use of landraces, either in breeding or directly (e.g. for food in high-value markets such as polenta, cornflakes, bread). Kernel quality traits such as vitrosity, starch and protein content are traits important in the varieties used in breadmaking; these analyses require specific equipment and are more costly. Milling yield (flour fraction) and test weight could also be interesting to evaluate for food maize. NIRS spectra collected in MALANIRS could be reused by developing and applying calibrations for new traits and associated predictions.

Since the field trials would be open pollinated, different options for field design were suggested to limit the xenotic effect on kernel and quality traits. For example, four rows could be planted with only the innermost harvested and the outermost, acting as a barrier to cross-pollination. In any case, it was considered to continue with a two-row design with two replicates and inclusion of relevant commercial hybrid checks.

The experimental and trait scoring protocols will be updated as relevant to reflect the modifications to the experimental design.

## 6.1 Discussion on continuation of EVA Maize network activities

EVA partners provided opinions on their future collaboration within the network. RAGT confirmed their support for the network, although they would perhaps focus more on the per se accessions to add diversity to their breeding germplasm and not continue testcross evaluations. DSP would be interested in doing field trials to confirm genomic and phenomic predictions. For this, it was suggested to select contrasting groups for specific traits and to choose the landraces for trials based on priority traits. A. Charcosset and P. Mendes-Moreira suggested that the EVA network could connect better with other actors in the value chain and direct use of maize landraces.

INRAE has available seed stocks of the ~300 test cross populations, which could be evaluated for priority traits in joint experiments of the three sets in 2026, with results combined with the per se accessions and previous testcross trials. KWS could perhaps conduct nursery trials on biotic stresses for promising accessions and related testcrosses. DSP also expressed interest in conducting a follow-up trial on the testcrosses.

V. Andjelkovic noted that the analysis of biotic stress data should be important to select priority materials for further work. Breeders in general are looking for landraces with acceptable yield, minimal lodging and promising resistance traits, keeping in mind also the changing environment.

R. Malvar reminded that BLUEs for most traits and trials are already available, and the file will be finalized with any missing data. These would then be useful for the generation of the breeders' catalogue and to inform future work.

V. Andjelkovic reminded participants of an upcoming symposium on the occasion of MRIZP's 80th anniversary in the first week of October 2025. Several project partners have been invited to attend this symposium and a satellite meeting for MALANIRS and EVA could be organized during that week.

## 7. Wrap up of meeting

S. Goritschnig summarized the main results of the discussions during the meeting and actions to be taken by participants (see action list in Appendix 3). She thanked all partners, including those connecting online, for their active participation and especially local host CREA-CI for their assistance with logistics.

## Appendix 1. Meeting agenda

### Venue: Hotel Cappello d'Oro, Bergamo, Italy

| **DAY 26 FEBRUARY 2025** | | |
|---|---|---|
| 13:00 – 14:00 | Registration | |
| **14:00 – 14:30** | **Welcome and introductory session** | |
| 14:00 – 14:10 | Welcome by local host and ECPGR | *C. Balconi*<br>*S. Goritschnig* |
| 14.10 – 14:30 | Update on current status of the ECPGR Evaluation Network EVA | *S. Goritschnig* |
| **14:30 – 16:00** | **Results from EVA Maize project** | **CHAIR: S. GORITSCHNIG** |
| 14.30 – 14:45 | Review of network workplan 2019-2024 and overview of collected trial data | *S. Goritschnig* |
| 14:45 – 15:30 | Review of experiments and field trials in 2024 (5 min each) | *A. Strigens (DSP)*<br>*N. Kravic (MRIZP)*<br>*C. Balconi (CREA)*<br>*H. Sarcevic (UZagreb)*<br>*P. Mendes Moreira (ESAC)*<br>*A. Le Foll (MAS, online)*<br>*A. Murigneux (Limagrain, online)*<br>*A. Gourrion (RAGT, online)* |
| **15.30 – 16.00** | **TEA/COFFEE BREAK** | |
| | **Data Analysis** | **CHAIR: A. CHARCOSSET** |
| 16.00 – 16:20 | Results from analysis of phenotypic data of per se accessions | *R. Malvar* |
| 16:20 – 16:40 | Preliminary results from testcross population trials | *C. Bauland* |
| 16:40 – 17:00 | Results from genotyping and GWAS/Genomic prediction | *A. Galaretto/S. Nicolas* |
| 17:00 – 17:10 | Development of a breeder's catalogue from the EVA Maize collection | *S. Goritschnig* |
| 17:10 – 18:00 | Discussion and Dissemination planning | *All* |
| **20.00** | **SOCIAL DINNER** | |

| **DAY 27 FEBRUARY 2025** | | |
|---|---|---|
| **8:30 – 10:30** | **MALANIRS intro and Collections** | **CHAIR: V. ANDJELKOVIC** |
| 8:30 – 8:45 | Introduction to MALANIRS | *S. Nicolas* |
| 8:45 – 10:30 | Descriptions of European Maize landrace collections for MALANIRS<br>(~7-8 minutes per partner) | • *S. Nicolas (INRAE)*<br>• *N. Kravic (MRIZP)*<br>• *D. Simic (A.I. Osijek)*<br>• *H. Šarčević (U Zagreb)*<br>• *D. Murariu (SVG)*<br>• *R. Malvar (MBG-CSIC)*<br>• *C. Balconi (CREA-CI)*<br>• *B. Schierscher-Viret (Agroscope)*<br>• *Z. Jovović (U of Montenegro)*<br>• *N. Kadiasi (Agricultural University of Tirana, online)*<br>• *P. Mendes Moreira (ESAC-IPC)*<br>• *G Cömertpay (DATAEM)*<br>• *L. Achathaler (AGES, online)*<br>• *A. Barata (BPGV-INIAV, online)* |
| **10:30 – 11:00** | **TEA/COFFEE BREAK** | |
| | **MALANIRS – work planning** | **CHAIR: S. NICOLAS** |
| 11:00 – 13:00 | Work planning of MALANIRS activities<br>- genotyping<br>- NIRS<br>- phenotyping/field trials<br>- data management<br>- data analyis | *All* |
| **13:00 – 14:00** | **LUNCH** | |
| 14:00 – 15:30 | Integration of EVA Maize and Malanirs activities<br>- Regeneration activities<br>- Material for field trials<br>- Planning of additional field trials<br>- Management of EVA and Malanirs collection<br>- timeline | *All* |
| **15:30 – 16:00** | **TEA/COFFEE BREAK** | |
| | **Discussions (continued)** | **CHAIR: S. NICOLAS** |
| 16.00 – 17:30 | Discussion on continuation of EVA Maize network activities<br>Discussion on work planning for MALANIRS<br>Any other open questions | *All* |
| **17:30** | **END OF MEETING** | |

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# Appendix 3: Action list

| # | Activity | Action | Responsible | Due date |
|---|---|---|---|---|
| 1 | General | Finalize updated cooperation agreement with signatures from Coimbra, INRAE, Albania, Montenegro, Bologna | S. Goritschnig | 31/05/2025 |
| 2 | General | Report dissemination activities within EVA Maize network | all partners | 30/06/2025 |
| 3 | Data management | Upload meteo files to EURISCO-EVA as metadata for individual trials | S. Goritschnig | 31/05/2025 |
| 4 | work planning | Centralize trial design in future, possibly using pipeline developed in AGENT | S. Goritschnig | 31/01/2026 |
| 5 | Data management | Check for grain moisture correction in reported yield data. | all evaluators, S. Goritschnig to coordinate | 31/05/2025 |
| 6 | Dissemination | Consider how to report genetic offset in a breeders' catalogue. | A. Gallaretto | 31/05/2025 |
| 7 | Dissemination | Develop breeders' catalogue based on input during meeting | S. Nicolas and S. Goritschnig | 30/06/2025 |
| 8 | Dissemination | Collect feedback on a draft breeders catalogue from partners | S. Goritschnig | 31/07/2025 |
| 9 | Data management | Collect Metadata for MineLandDiv and MALANIRS collections and experiments for inclusion in EURISCO-EVA | S. Goritschnig | 30/06/2025 |
| 10 | Data management | Collect and curate available phenotypic data from previous projects for inclusion in EURISCO (e.g. Switzerland, Croatia) | S. Goritschnig | 31/05/2025 |
| 11 | Data management | Update geographic location metadata on EVA accessions in EURISCO-EVA and implement map of landrace locations | S. Goritschnig | 30/06/2025 |
| 12 | Data management | Compare accession lists of EVA, MineLandDiv, Dromamed and MALANIRS to flag overlaps | S. Nicolas and S. Goritschnig | 30/06/2025 |
| 13 | Work planning | Update and share the regeneration, experimental and phenotyping protocol from EVA Maize with MALANIRS | S. Goritschnig | 31/05/2025 |
| 14 | Work planning | Develop protocol for seed processing after harvets for NIRS | S. Goritschnig | 31/05/2025 |
| 15 | Work planning | Select priority traits for which phenomic prediction by NIRS could be developed | S. Nicolas and S. Goritschnig | 30/06/2025 |

| # | Activity | Action | Responsible | Due date |
|---|---|---|---|---|
| **16** | Work planning | Plan Ring Test to compare NIRS equations between participating laboratories | S. Nicolas | 30/06/2025 |
| **17** | Data management | Provide access to Thalia DB for EVA partners | S. Nicolas and S. Goritschnig | 30/06/2025 |
| **18** | Work planning | develop protocols for priority traits on kernel quality | S. Goritschnig | 30/06/2025 |
| **19** | Work planning | Get info on available testcross seed stocks. | C. Bauland | 30/06/2025 |
| **20** | Work planning | Analysis of accessions used in testcrosses relative to overall genetic diversity of EVA | C. Bauland | 30/06/2025 |
| **21** | Work planning | Plan testcross trials in 2026 on available material. | C. Bauland and S. Goritschnig | 30/09/2025 |
| **22** | Dissemination | develop a plan for connecting EVA Maize with other actors in the maize value chain | P. Mendes-Moreira | 30/09/2025 |
| **23** | Meeting | Plan possible EVA Maize/Malanirs meeting in Belgrade | V. Andjelkovic and S. Goritschnig | 10/10/2025 |