

ECPGR Activity Grant Scheme Proposal Form

First Call – Phase XI (2024–2028)

Activity Proposal

Activity	
Full title	MAize LAndraces traits phenomic prediction using Near InfraRed Spectra.
Acronym (or short title)	MALANIRS
Duration of Activity (in months)	36
Start date – End date	
<i>Please indicate start date not earlier than 3 months after deadline of Call</i>	September 2024 - September 2027

Applying Working Group(s)

Working Group	Indicate name and surname of Working Group Chair
1. Maize Working Group	Violeta Andjelkovic

Activity Coordinator

Activity Coordinator	
Name and Surname	Stéphane Nicolas
Working Group	Maize Working Group
Nationality	French
Current position	Researcher
Institute	INRAE
Country	France
Telephone	+33 1 69 33 23 69
Email	stephane.nicolas@inrae.fr

Activity Partners (ECPGR-funded)

Please note that each partner needs to be a member of an ECPGR Working Group to be eligible for funding. For self-funded partners please use the separate box below.

Partner ID No.	Name and Surname	Institute	Country
1	Stéphane Nicolas	Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE)	France
2	Natalija Kravic	Maize Research Institute Zemun Polje (MRIZP)	Serbia
3	Domagoj Simic	Agricultural Institute Osijek	Croatia
4	Hrvoje Šarčević	University of Zagreb	Croatia
5	Danela Murariu	Suceava Genebank (SVG)	Romania
6	Rosa Ana Malvar	Misión Biológica de Galicia - CSIC	Spain
7	Carlotta BALCONI	Council for Agricultural Research and Economics (CREA) - Research Centre for Cereal and Industrial Crops, Bergamo	Italy
8	Ana Maria Barata	Banco Português de Germoplasma Vegetal - Instituto nacional de Investigação Agrária e Veterinária (BPGV-INIAV)	Portugal

9	Beate Schierscher Viret	Agroscope	Switzerland
10	Zoran Jovović,	University of Montenegro	Montenegro
11	Najada Kadiasi	Agricultural University of Tirana	Albania
12	Pedro Mendes Moreira	Politécnico de Coimbra – Escola Superior Agrária de Coimbra (ESAC-IPC)	Portugal
13	Gönül CÖMERTPAY	Eastern Mediterranean Agricultural Research Institute (DATAEM)	Turkey
14	Sylvia Vogl	Austrian Agency for Health and Food Safety (AGES)	Austria

Self-funded partners

Partner No.	Name and Surname	Institute	Country
1	Elisabetta Frascaroli	University of Bologna	Italy
2			
3			
4			
5			
6			

Description of Activity (suggested max. 1000 words)

Please address the following aspects:

– **Background:** Explain the context behind the choice of this Activity, e.g. why this has been prioritized or selected. If this is the continuation of a preceding Activity, please indicate how and why the new Activity will build on previous results/experiences.

Maize landraces are a valuable source of genetic diversity for addressing climate change, the requirements of low input agriculture and diversification of uses as they have been selected for adaptation to local agro-climatic conditions and various human uses. Maize landraces could therefore carry favorable alleles for tolerance to abiotic or biotic stresses but also for new targeted traits linked to kernel composition. However, landraces remain underutilized in modern breeding programs and agriculture because they are poorly characterized, genetically heterogeneous and exhibit limited agronomic performance compared to elite germplasm. The development of efficient methods to characterize landraces is therefore a prerequisite to their use for broadening the genetic basis of the modern germplasm for abiotic stress tolerance, other adaptative traits and new targeted traits linked to kernel composition.

Thousands of maize landraces maintained in European Genebanks remain poorly characterized at both molecular and phenotypic levels due to lack of funding, time, and human resources. To address this issue in the ongoing ECPGR EVA Maize project (2020-2024), we already genotyped 626 landraces from eight different European genebanks using 50K Illumina SNP array (Arca et al., 2021) and evaluated 583 landraces for various agronomic traits across 22 environments (Balconi, Galaretto et al., submitted). In the SusCrop Eranet MineLandDiv project (MLD, 2023-2026), we are evaluating 300 selected

European landraces, including 150 landraces in common with EVA, across an European evaluation field/platform network using contrasted treatments for evaluating phenotyping responses of landraces to different targeted stresses (cold, nitrogen deficiency, drought, heat). In this same project, we are also developing high-throughput phenotyping for speeding up phenotypic characterization and access to new traits (Drones, Imaging, NIRs). We are also developing a pooled targeting sequencing approach to genotype 768 landraces from EVA and MLD project. Targeting sequencing offers indeed strong advantages over 50K SNP genotyping that we developed previously (Arca et al., 2021) and applied for genotyping landraces (Diaw et al., 2021, Arca et al., 2023). Targeting sequencing allows to discover new polymorphisms limiting ascertainment bias for diversity analysis, to access haplotypes and to target at high density some genomic regions of interest for abiotic/biotic stresses or grain quality (genes, quantitative trait loci for traits) at low cost. In MineLandDiv, we are also developing genomic prediction methods for characterizing traits of landraces. Genomic prediction consists in training a predictive model based on phenotyping data from field trials and genotyping data in order to predict agronomic traits for landraces based only on their genomic data (GP). A promising alternative to genomic prediction could be phenomic prediction using e.g. Near Infrared Red spectra (NIRs) from tissues (leaves, kernel...) instead of genomic data to train the model for predicting performances of unobserved individuals based on their NIRs spectrum (Rincet, Segura et al., 2018, G3). NIRs is indeed a low cost and non-destructive molecular approach that is currently available in many breeding stations and genebanks and that can be applied to large number of genebank accessions. Interestingly, NIRs can also be used to predict kernel composition providing that NIR devices have been calibrated on kernel samples representing maize diversity that have been analysed biochemically for kernel composition (NIRs equation).

In this ECPGR activity, we propose to capitalize on methodological and technology developments (Genomic prediction, Phenomic prediction, Targeting sequencing) as well as field trial and genotyping data obtained in MLD and EVA maize project to go further in genomic and phenotyping characterization of landraces by:

- 1) **Extending genomic characterization to 500 new landraces** from European genebanks that are previously genotyped neither in MLD or in EVA project by using targeting sequencing developed in MLD project. This genotyping data will be useful for performing diversity analysis but also evaluating interest in this technology for genebank management (identification of duplicates or errors in landraces multiplication). (Task 1)
- 2) **Predicting traits for these 500 landraces in contrasted environments** based on their genotyping data using a genomic prediction (GP) model trained on both genotyping data and traits measured on landraces in EVA and MLD field trials. (Task 1)

3) Evaluating use of Near Infra-Red spectra (NIRs) in place of genomic data to predict agronomic traits in contrasted environments using a phenomic prediction model trained on NIRs of kernel lots harvested and agronomic traits measured in field trials of MLD panel (Task 2)

4) Evaluating the proof-of-concept of using individual near infrared spectra (NIRs) on grains directly from genebank seed stocks to predict agronomic traits and grain composition of maize landraces by setting up a phenomic prediction model based either on NIRs spectra of kernels harvested in MLD trials for traits or NIRs of kernels for which biochemical compositions have been measured (Task 3).

– *Justification:* Explain why this Activity is justified in terms of making progress towards achieving the ECPGR priority activities and the targets of the *Plant Genetic Resources Strategy for Europe*.

This activity will support the continuation and extension to new partners of the existing EVA maize network (PGR5.1, P1), contributing to completing "a comprehensive assessment of European plant genetic resources and diversity required by users for present and future needs in food and agriculture and the corresponding gaps in the conservation system" of European maize GR. This activity will support consolidating and sustaining *ex-situ* conservation by developing methods for assessing crop diversity (PGR3.4, P2), advance creation of an exhaustive inventory of the genetic diversity conserved in national and local genebanks maintaining and characterizing maize GR (PGR3.5, P2), development of methodology to assess crop diversity required and gaps in the conservation system in cooperation with users, as a dynamic process, for current and future needs." (PGR3.4).

– *Rationale for the choice of partners:* Explain why the selected partners are the most suitable to carry out the proposed Activity and briefly describe their respective roles in the Activity.

The 15 partners involved in this project have a long history of conservation and characterization of European maize landraces accessions for breeding and pre-breeding purposes. MALANIRs involve curators of **10 national European genebanks** (INRAE, BPGV-INIAV, CREA, CSIC, SVG, MRIZP, DATAEM, University of Tirana, University of Zagreb, Agroscope), some of which are already partners in the EVA Maize network. They have therefore a strong expertise in the conservation and evaluation of maize landraces, have a good knowledge on their maize accessions, and good skills in plant breeding, field trials for evaluating/multiplying landraces and characterization of their material with Near Infra-Red spectra. This project will reinforce and maintain long-term collaboration between several partners that are involved in projects for characterizing landraces at molecular and phenotypic level in the EVA Maize Network and in the ongoing SusCrop ERANET MineLandDiv for characterization of landraces for tolerance to abiotic/biotic stresses. **MALANIRs project will also be a great opportunity to integrate in this EVA network new partners from Turkey (DATAEM), Albania (Agricultural University of Tirana),**

Montenegro (University of Montenegro), Austria (AGES) and Italy (UNIBO) that are involved in conservation and characterization of landraces.

Among the activity partners, CSIC have long time expertise in evaluation of maize for tolerance to maize corn borer and cold tolerance, INRAE have long time expertise in landraces genotyping, phenotypic evaluation of landraces and are pioneering in phenomic prediction (Rincent, Segura et al., 2018, G3), CREA have a long time expertise in traditional genetic resources conservation, in evaluation of maize for nitrogen use efficiency and in NIRs evaluation of maize, MRIZP have a long time expertise in genetic resources conservation, maize breeding and field trial and NIRs evaluation. IPC-ESAC has a long-time expertise in genetic resources, phenotypic evaluation of landraces and its breeding on station and on-farm under participatory plant breeding. IPC-ESAC has been involved in breeding maize for human consumption. DATAEM have an expertise in collecting and genetic conservation of Turkish landraces but also in maize breeding in mediterranean conditions. BPGV-INIAV (<https://iniav.pt/bpgv>) have a long-time and strong expertises in the conservation and characterization of genetic resources, notably for accessions from mediterranean countries. SGV (<https://svgenebank.ro/>) have a long time expertise in conservation of cultivated species. UNIBO have a strong expertises in root phenotyping and quantitative genetics. AGES, Agricultural University of Tirana and University of Montenegro will provide their expertise on their local landraces. **MALANIRs will reinforce the building of a European field trial network for agronomic evaluation of the maize landraces as well as their genomic characterization started in EVA Maize and continued in MineLandDiv. MALANIRs will also contribute to the establishment of a network for NIRs characterization of landraces in the laboratory and in the field.**

Each genebank/institute requesting genotyping or/and NIRs in this activity will provide 50K kernels to INRAE (GQE-Le Moulon) for DNA extraction in order to perform targeting sequencing (INRAE - EPGV) and 75 kernels for individual NIRs at AGAP Institute (Montpellier, France). Each genebank will contribute to the choice of landraces and provide passport data on landraces. Partners that perform NIRs on landraces using their local device (INIAV, IPC-ESAC, MRIZP, University of Zagreb, SVG, CREA, CISC, Agroscope) will analyze a common calibration set of kernels provided by INRAE to be able to merge/combine NIR spectra measurement from different devices across the network for phenomic prediction. Partners in NIRs analysis will share their results with all network. All partners will attend videoconference meetings in order to manage project. Data/Protocols/Documents will be shared during the project across network using CGIAR EVA sharepoint (<https://cgiar.sharepoint.com/>). Cleaned data were uploaded in EURISCO-EVA for phenotypic data and ThaliaDB for genotypic data. INRAE will provide each partner access to ThaliaDB database for genotyping data of landraces evaluated in MALANIRs. INRAE will provide to partners their genomic/phenomic predictive data

analysis.

– **Methodology or Approach:** Explain how the partners will operate. Clearly explain who is expected to do what. Also explain the rationale for meeting (or not) as part of the Activity. Include a Gantt Chart, to illustrate the work breakdown structure of the project.

MALANIRs aims at (i) **using targeting sequencing for extending genomic characterization to 500 new European landraces from European Genebanks** in addition to 768 landraces that will be characterized in MineLandDiv and EVA project (Table 1) (ii) **predicting traits on these 500 new landraces in contrasted environment using genomic prediction** models trained on genomic data and traits evaluated in field trials from MineLandDiv and EVA projects (Table 1) (iii) **achieving NIRs characterization of individual kernels from 1500 landraces maintained *ex-situ* in genebanks seed stocks and kernel lots from landraces harvested in field trials** to evaluate proof-of-concept of using NIRs in place of genomic data (Phenomic prediction) for predicting agronomic traits in contrasted environment but also kernel composition (Starch, Oil, Protein content).

Table 1: Summary of landraces and samples analyzed in EVA, MineLandDiv and MALANIRs project

Task Description	ECPGR EVA Maize (2020-2024)	MineLandDiv (2023-2026)	MALANIRs (2023- 2027)
# Unique Landraces accessions	626	300*	896
Task 1: Targeting sequencing of landraces for genomic prediction	No	Yes	Yes
Development of targeting sequencing methods	No	Yes	No
#Unique Landraces for targeting sequencing	0	768	500
Origin of DNA samples studied by targeting sequencing	476	300*	492
Field trials evaluation used for training genomic prediction model	Yes	Yes	Yes
#Unique Landraces evaluated in field trials	626	300	400
#Environments (Location x Treatment x Year)	22	14	2
Task 2: NIRs of kernels harvested in field trial	No	Yes	Yes
Development of NIRs methods for phenomic prediction	No	Yes	No
Field trials evaluation used for training/validating	No	Yes	Yes

phenomic prediction model			
#Landraces evaluated in field trials	0	300	350
#Trials (Location x Year)	0	5	6
#Environments (Location x Treatment x Year)	0	10	6
#NIRs samples	0	2460	1440
Task 3: Phenomic prediction of traits and grain composition based on individual NIRs of kernels from genebank seed stocks	No	No	Yes
Development of NIRs individual kernel evaluation	No	No	Yes
#NIRs Samples	0	0	2105
#Origin of landraces samples analyzed	476*	300	724
Task 4: Feeding EURISCO Database	Yes	Yes	Yes
Task 5: Face-to-Face Meetings and training	Yes	Yes	Yes
#Meetings	1	2	2
#Persons	5	5	5

*** 150 landraces were shared between EVA and MineLandDiv panel**

For NIRs characterization, we will set up two approaches: (i) **NIRs characterization of kernel lots harvested in field trials** using either robotized or manual devices of each partner (Table 1), (ii) **NIRs characterization of individual kernels (50 per landrace)** from genebank stocks which will allow to assess average but also within landrace variability. To combine NIR spectra from different devices and approaches (local and AGAP devices, individual kernel vs kernel lot), we will test the interest of using a common calibration set of kernels on different devices.

We split MALANIRs in five tasks corresponding to: Targeting sequencing of 500 new landraces for performing diversity analysis and genomic prediction (Task 1); NIRs of kernels harvested in the field trial to train phenomic prediction model for traits and grain composition (Task 2); Phenomic prediction of traits and grain composition of landraces based on individual NIRs of kernels from genebank seed stocks (Task 3), Feeding databases with phenotypic (Eurisco database) and genotypic data (ThaliaDB: <https://thalia.moulon.inra.fr/>) (Task 4), organization of meeting and training for phenomic prediction (Task 5).

Task 1: Targeting sequencing of 500 new landraces from different genebanks for diversity analysis and genomic prediction

We will genotype 500 new landraces from European genebanks using a pooling targeting

sequencing approach that we are developing in the MLD project. This ECPGR activity will complement targeting sequencing of 768 European landraces already planned in MLD project including landraces from EVA and MLD panel (Table 1). For selecting these 500 landraces among 856 proposed by 13 partners (Table 2), **we will prioritize landraces that originated from geographical area and/or genebanks that have been not well covered in previous projects (Albania, Montenegro, Turkey, Austria), that have good passport data and geolocalization, that could be interesting for stress tolerance and/or for grain composition to fit animal/human consumption.** The 13 partners will send 50 seeds to INRAE for DNA extraction, for performing pooling targeting sequencing and retrieving genotyping from targeting sequencing. INRAE will share with all partners genotyping data from targeting sequencing analysis. Genotyping data will be used for performing diversity analysis in combination with EVA/MLD data but also for testing ability for identifying duplicated accessions within/between genebanks. Genotyping data will also be used by INRAE to predict agronomic traits of these 500 landraces based on models calibrated on the EVA and MLD datasets. The genomic prediction model will be trained using phenotypic and genomic data already obtained in the EVA Maize project with the evaluation of agronomic traits of 583 landraces across 37 environments as well as in MLD, where 300 landraces will be evaluated in 14 environments. MALANIRs will also take advantage of methodological development for genomic prediction for landraces and targeting sequencing achieved in MLD project.

Table 2: Contribution of each partner and origin of landraces and to MALANIRs

Research Institute / Genebanks	Genebank curators	ECPGR Funding for activities	Countries	Task 1 : #Landraces proposed for targeting sequencing and Individual NIRs	Task 2 : Field trial	Task 2 : NIRs on kernel from harvest	Task 3 : #NIRs samples on genebank
INRAE	Yes	Yes	France	40	MLD	600	1500*
MRIZP	Yes	Yes	Serbia	150	ECPGR	200	
University of Zagreb	Yes	Yes	Croatia	90	ECPGR	100	
Agricultural institute Osijek	No	Travel	Croatia	21			
SGV	Yes	Yes	Romania	40	ECPGR	100	75
CSIC	Yes	Yes	Spain	40	MLD	200	100

CREA	Yes	Yes	Italy	50	MLD	100	50
INIAV/BPGV	Yes	Yes	Portugal	40			40
Agroscope	Yes	No	Switzerland	40			40
University of Montenegro	Yes	Travel	Montenegro	100			100
University of Tirana	Yes	Travel	Albania	200			200
ESAC-IPC	No	Yes	Portugal	5	ECPGR	100	
DATAEM	Yes	Travel	Turkey	40	ECPGR	40	
AGES	Yes	Travel	Austria	40			
UNIBO	No	No	Italy				
TOTAL				896		1440	2105

***NIRs sample (kernel) will be provided by all genebanks**

Task 2: NIRs of kernels harvested in field trials to train phenomic prediction model for traits and grain composition

We will carry out NIRs characterization of grains harvested in eight field trials including three from the MLD project panel as well as in five new field trials in MALANIRs on a subset of the MLD landraces panel and the new local landraces (Table 2) for evaluating accuracy of phenomic prediction of agronomic traits and grain composition (oil, starch, protein) of landraces in contrasted environments and the transferability of this phenomic prediction across environments and devices. To achieve these objectives, MALANIRs will take in charge:

- **NIRs characterization of kernel lots harvested in 3 MLD field trials (Bergamo, CREA, Italy ; Saint Martin de Hinx, INRAE, France ; Pontevedra, CSIC, Spain).** In this way, we will benefit from agronomic trait evaluation of these landraces in contrasted environments and treatments (drought, nitrogen deficiency, cold) obtained in the MLD project to train phenomic prediction models with NIRs characterization from MALANIRs. MALANIRs will complete NIRs characterization of kernels harvested in the MLD field trial network since NIRs characterization of MLD panel is planned for the whole panel only in one field trial (Montpellier, INRAE, France). These NIRs data in combination with agronomic traits will allow to evaluate accuracy of phenomic prediction across different environments and NIRs devices.
- **NIRs characterization and agronomic evaluation of a subset of landraces MLD panel in two additional field trials in 2025** by two partners that are not involved in MLD project (MRIZP, University of Zagreb). These field trials will thus extend the European field trial network in which the MLD panel is evaluated. Genebanks of origins for landraces from MLD panel will provide seeds for these new trials.

- **NIRs characterization (ECPGR funding) and evaluation for agronomic traits (Own funding) of new local landraces in 3 additional local trials (SVG, ESAC-IPC, DATAEM).** In addition to extend the EVA/MineLandDiv Maize evaluation network these local trials will be useful for evaluating transferability of the phenomic prediction model using local devices for new landraces in new environments.

NIRs characterization of kernels harvested in field will be made either by robotized devices from experimental unit of Diascope (France, Montpellier) or by manual local NIRs devices from genebank/trial stations. NIRs and agronomic trait for landraces from the MLD panel will be combined to train different phenomic prediction models. We will evaluate their accuracy for predicting agronomic traits of new landraces in same environment (cross-validation approach), of same/new landraces in other environments, of combining NIRs from different devices for predicting new landraces in new environments. In addition, grain composition of landraces will be inferred based on NIRs using a model training on samples that have been analyzed biochemically to obtain kernel composition (so called NIRs equation). If available on local devices, NIRs equation will also be used to obtain kernel composition for landraces (Starch, Oil, Protein content). In the end, we will also compare genomic and phenomic prediction accuracy. For evaluating agronomic traits in new trials, we will use standardized evaluation protocols for landraces developed in EVA and MLD. Tolerance to abiotic stresses will be evaluated by using classical stress index based on relative differences of agronomic performance (yield, biomass) between normal and treated trials at same location but also the response of each genotype across different environments (location x year x treatments) to climatic/soil variables (environmental classification).

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

To achieve this goal, we will measure individual NIRs of 50 kernels each from 1500 landraces originated directly from genebanks seed stocks by using either automated NIRs devices from AGAP Institute at Montpellier (France, 1500 samples) or local NIR devices available in the genebank/institute (7 partners, 605 samples, Table 2). These NIRs will be first used to infer grain composition (oil, starch, protein) based on "NIRs equation" available on each device obtained by calibrating a phenomic prediction model on maize kernels that have been measured by NIRs and biochemically analysed to obtain grain composition in oil, proteins, starch content. This action will be done by each partner on their own funding since this calibration curve is specific for each device. In addition, NIR spectra of kernels from genebank will be combined with NIRs spectra from kernels harvested in field trials to predict traits of landraces maintained ex-situ in genebanks using phenomic prediction. Interestingly, phenomic prediction based on NIRs of 50 individual kernels for each landrace will allow to assess phenotypic variability within landraces which could be useful to evaluate plasticity and resilience of landraces to stresses. Since NIRs capture a part of Genotype by Environment interaction, phenomic prediction will be useful for evaluating tolerance to abiotic stresses. In order to be able to combine different NIR spectra from different devices, a calibration set of same kernels will be defined and shared. 13 genebanks partners will send 75 kernels from 1500 selected landraces to AGAP institute for performing NIRs on automated devices. These 1500 selected landraces comprised 776 landraces from

EVA and MineLandDiv panel, 500 new landraces selected for targeting sequencing. The 224 remaining NIRs samples will be used to analyze kernels harvested in different MineLandDiv field trials on a common set of genotype (checks) in order to combine NIRs between different local devices for phenomic prediction. In addition, 7 genebank will analyze NIRs on individual kernels of 605 local landraces originated from their genebank with their local NIRs device (Table 2). NIRs on individual grain based on their local device and centralized devices will be compared.

Task 4: Feeding and sharing data using Eurisco database for phenotyping and ThaliaDB database for genotyping

We will share data while ensuring good data/accession traceability and connection with previous data obtained in EVA maize project. The EURISCO Database will be fed with phenotyping and NIRs characterization data obtained in this activity in order to share the characterization of European landraces with maize working group and stakeholders of maize genetic resources.

Task 5: Face-to-face meetings in person and training

We will organize two "in-person" project meetings (2025, 2027) as satellite meetings of in-person meetings of the MLD project (2023-2026) and the EVA maize network, taking advantage of available funding to bring together people from these three projects (Table 2). The joint meetings will help to promote, discuss and disseminate the methods and results of MALANIRs to the maize working group members and stakeholders (private companies) through the EVA maize network. In particular, these meetings will also be used as an opportunity to train Maize Working Group scientists in the use of tools developed for the characterization of their maize genetic resources (diversity analysis, duplicate identification using genomic data, phenomic/genomic prediction). The two face-to-face meetings will be dedicated to discussion and training on (i) "NIRs measurement of landraces in genebank for phenomic prediction and kernel composition" (ii) "Diversity analysis of landraces using pooling targeting sequencing approach". Specific videoconferencing meetings for MALANIRs project will also be organized to manage the project and share results. In the ECPGR activity, priority for travel funding will be given to partners who are not involved in MLD and who have contributed to the NIRs/field experiment activity by providing material or performing experiment.

Table 3: Gantt Chart for MALANIRs project

Task Description		2024	2025				2026				2027	
		Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
T1	Targeting sequencing of 500 landraces		■	■								
	Genomic prediction for 500 landraces				■	■	■			■		
T2	Field trial			■	■			■	■			

	NIRs of kernels harvested in field trial																		
	Phenomic prediction in field																		
T3	NIRs of kernels from seed stock in genebank																		
	Phenomic prediction in genebank																		
T4	Feeding and sharing data																		
T5	Meeting in person / videoconference																		

– *Description of genetic material:* If your Activity is focusing on genetic material, please describe in detail, as far as possible, who is providing this genetic material, its status and the number of accessions under investigation (for example: *This Activity aims at molecularly analysing / safety-duplicating / evaluating / collecting XY accessions (listed) of “Genus species”, provided by genebank Z/ farmers in country W /to be collected in country P..., etc.*).

This activity will genotype 500 new maize (*Zea mays*) landraces maintained ex-situ using targeting sequencing approach out of 856 proposed by 13 genebanks/institute from 11 countries (Table 2). For selecting these 500 landraces among 856 proposed by 13 partners (Table 2), we will prioritize landraces that originated from geographical area and/or genebanks that have been not well covered in previous projects (Albania, Montenegro, Turkey, Austria), that have good passport data and geolocalization, that could be interesting for stress tolerance and/or for grain composition to fit animal/human consumption.

In addition to genotyping, this activity will generate NIRs data for ~1500 genebank seed stock accessions which will be used in phenomic prediction models for field trial performance and grain composition traits. To train these models NIRs data will also be generated on 300 accessions which originate from the MLD project and will be phenotyped in field trials, also within MALANIRs (Table 2).

– *Expected impact.* Clearly specify the expected impact from this Activity for the respective ECPGR priority activity, compared to the current state of progress of the respective *Plant Genetic Resources Strategy for Europe* target. Explain how the impact will be obtained.

MALANIRs (Task 1, 2, 3, 5) will promote sustainable use of PGR by consolidating and extending the EVA maize network in order to increasingly characterize and evaluate under standard conditions as well as genotype maize genetic resources (Priority P1 in PGR 5.1). MALANIRs will help to maintain existing collaboration between partners but also extending to new maize genebanks and research institutes (Priority P1 of PGR 5.1 for ECPGR 2024). Task 4 of MALANIRs will compile and transfer existing/new characterization and evaluation data for maize landraces by feeding EURISCO Database with phenotypic data from MineLandDiv project as well new and existing NIRs characterization of kernel composition for landraces from activity partners (Priority P1 of PGR 4.3 for ECGR 2024). In the end, MALANIRs will contribute valuable grain quality data that can be exploited to breed improved landraces both for farmers and other producers (Priority 1 of PGR 2.1.)

Task 1, 2, 3 of MALANIRs will help to consolidate and sustain ex-situ conservation of maize landraces by developing and setting up methods/tools for characterizing at low cost and large scale promising diversity of maize landraces for tolerance to abiotic stress, new traits that are required for addressing climatic change and low-input agriculture challenges. Task 1, 2, 3 of MALANIRs will contribute for maize landraces to achieve “a comprehensive assessment of European plant genetic resources and diversity required by users for present and future needs in food and agriculture and the corresponding gaps in the conservation system has been completed and is regularly updated” (Priority P2 of PGR 3.4).

MALANIRs will also contribute to providing “an exhaustive inventory of the genetic diversity conserved in all national or local genebanks maintaining and characterizing maize GR” (Priority P2 of PGR 4.5).

MALANIRs will be helpful for genebank management by developing methods based on NIR and/or genotyping for identifying duplicate which will increase efficiency in genebank management and reduce financial resources for collection maintenance. MALANIRs will then support countries to identify and include material into AEGIS, including regeneration and safety-duplication. (Priority P1 of PGR 3.3 for ECPGR 2024)

– *Links with other non-ECPGR projects or individuals:* If applicable, clearly explain the objectives of the linked projects and the reasons for complementarity with the ECPGR Activity.

This activity will benefit from the SusCrop EraNet project MineLandDiv (2023-2026, PI S. Nicolas) that aims at mining allelic diversity for tolerance to abiotic/biotic stresses by combining genomic prediction, genome wide association studies, detection of selective footprints for pre-breeding approaches. In MALANIRs, we aim at extending genomic characterization to 500 new landraces from new genebanks not involved in EVA maize and MineLandDiv (T1) and setting up phenomic prediction of traits in European genebank based on low cost and non-destructive molecular characterization of kernels by NIRs (T2 and T3). MALANIRs will be focused on NIRs characterization of landraces in genebanks and field for implementing phenomic prediction in genebanks while MineLandDiv is more

focused on deep genomic characterization and genomic prediction of landraces for tolerance to abiotic stress. However, these three projects (EVA maize, MineLandDiv, ECPGR activity) are highly complementary in their objectives/data generated while sharing genetic material, data, methods to feed each other.

MALANIRs will benefit from field trial and agronomic evaluation achieved in MLD project to perform NIRs characterization of kernel harvested from these MineLandDiv trials allowing combination of the datasets for training a predictive model of agronomic traits in contrasted environments. Reciprocally, this activity will benefit MineLandDiv by extending phenotypic characterization of landraces to other traits, notably kernel composition. This activity and MineLandDiv could also benefit mutually by allowing the comparison of prediction accuracy based on genomic data and NIRs but also combining the two types of data. The ECPGR activity will also take advantage of methodology development for targeting sequencing in MineLandDiv project to characterize genetic diversity of large collection of new landraces maintained in genebanks and use them for genomic prediction. Reciprocally, MineLandDiv project and EVA maize will benefit from the extension of the genomic characterization to new landraces from genebanks in countries/region that are not well covered in these two projects such as Albania, Austria, Turkey and Montenegro and that could then feed back into the EVA Maize trial network. MineLandDiv could also benefit from new trials for new landraces in ECPGR activity to evaluate these landraces in more environments and therefore identifying new genomic regions involved in abiotic stresses. Reciprocally, ECPGR activity will benefit from genomic prediction model calibrated for different traits in MineLandDiv and EVA project to predict traits of these new landraces genotyped by targeting sequencing. EVA Maize network will benefit from ECPGR activity by achieving targeting sequencing for landraces evaluated in EVA maize network but also by integrating new members in the network.

Expected products and related ECPGR Priority activity

List concrete products and results that are obtained by the Activity and the corresponding number(s) of the ECPGR Outcome(s) and/or Output(s) and/or Activities to which each product/result will contribute.

Expected products/results	Corresponding contribution to <i>Plant Genetic Resources Strategy for Europe</i> target
<p>1 High density genotyping of 500 landraces using targeting sequencing in the continuation of EVA maize project by completing remaining landraces genotyped with 50K in EVA maize panel (200) in addition to new landraces from different genebanks (300)</p> <p>Genomic prediction of new landraces based on model train on field trial and genomic data from MineLandDiv targeting sequencing</p>	<p>PGR 5.1 “Collections of PGR in Europe are increasingly characterized and evaluated under standard conditions, as well as genotyped with suitable sets of molecular markers”</p> <p>PGR 3.4 “A comprehensive assessment of European plant genetic resources and diversity required by users for present and future needs in food and agriculture and the corresponding gaps in the conservation system has been completed and is regularly updated”</p> <p>PGR 4.5 “An exhaustive inventory of the genetic diversity conserved in all national or local genebanks maintaining and characterizing maize GR”</p> <p>PGR 3.3 “identify and include material into AEGIS, including regeneration and safety-duplication. (P1 of ECPGR 2024)</p> <p>PGR 5.1 Collections of PGR in Europe are increasingly characterized and evaluated under standard conditions, as well as genotyped with suitable sets of molecular</p>
<p>2 NIRs characterization of 1,500 landraces on kernel from genebanks including those that are genotyped by targeting sequencing.</p>	<p>markers (P1 continuation of EVA network)</p> <p>PGR 3.3 “Identify and include material into AEGIS, including regeneration and safety-duplication. (P1 of ECPGR 2024)</p> <p>PGR 3.4 “A comprehensive assessment of European plant genetic resources and diversity required by users for present and future needs in food and agriculture and the corresponding gaps in the</p>

- conservation system has been completed and is regularly updated”
- NIRs characterization of kernel lot from 300 landraces harvested in 4 field trial
- 3 Phenomic prediction of trait for 1500 landraces from genebanks based on NIRs
- Feeding and sharing data using Eurisco database for phenotyping and using ThaliaDB database for genotyping
- 4
- 5 Trainings and meetings
- PGR 5.1 Collections of PGR in Europe are increasingly characterized and evaluated under standard conditions, as well as genotyped with suitable sets of molecular markers (P1 continuation of EVA network)
- PGR 5.2 Data and accessions in the public domain, including those with relevant agronomic and quality traits identified at molecular level, are available to users through open centralized information systems, including Crop Portals.
- PGR 5.1 Collections of PGR in Europe are increasingly characterized and evaluated under standard conditions, as well as genotyped with suitable sets of molecular markers (P1 continuation of EVA network)

Workplan for the proposed period of the Activity

Brief description of meetings and/or main actions of the Activity.

Type of Action <i>(indicate if "meeting" or "other action")</i>	
1	Task1: Targeting sequencing of 500 new landraces from different genebanks for diversity analysis and genomic prediction (Other action)
2	Task 2: NIR of kernels harvested in the field trial to train phenomic prediction model for traits and grain composition (other action)
3	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 (other action)
4	Task 4: Feeding and sharing phenotyping data with Eurisco database and genotyping data with ThaliaDB database (Other action)
5	Task 5: Face-to-face meetings in person and training (Meeting)

Additional remarks

Indicate any additional remark(s) that is/are important for the evaluation/implementation of the proposed Activity

Remarks:

Please send the completed form together with the budget table to the Chair of the submitting Working Group for submission of the Activity proposal.