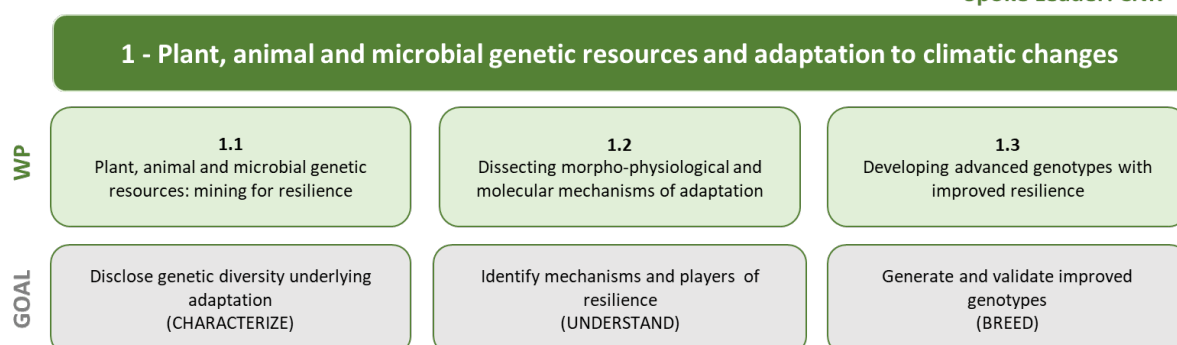


SPOKE 1

Spoke Leader: CNR



Work package number	1.1	Lead beneficiary	UNIBO
Work package title	Plant, animal and microbial genetic resources: mining for resilience		
Start month	1	End month	36

Objectives

- Disclose genetic diversity underlying adaptation
- Assess genetic variability in plant/animal/microbial gene pools at genome level, define pan-genomes in main crops/livestock and develop models of evolvability in response to climate change
- Characterize phenotypic diversity of genetic resources by phenomic and multi-omic approaches
- Build up effective networks and methodologies for germplasm storage and management
- Develop advanced methods for the analysis of complex genotypic and phenotypic data

Description of work

T 1.1.1 *Genome-wide analysis of genetic diversity, pan-genome reconstruction and modeling of evolvability in response to CC (M1-M36; task leader: UNIBO; partners involved: CNR, CREA, FEM, UNIMI, UNIBA, UNINA, UNIPD, SSSA, UCBM)*

This task will characterize the genomic makeup of plant, animal and microbial genetic resources in order to identify diversity useful to improve resilience and establish conservation units. Towards this goal, custom multispecies genotyping arrays will be developed and implemented to characterize ample collections. Genome-wide analyses, including definition of pan-genomes, will describe gene pools of selected plant crops/species and livestock, to model the response, evolvability and vulnerability to CC. Genomic data, including whole genome sequences, will be obtained from collections of plant species of known interest as tomato, durum wheat, grapevine, apple, pear, sweet orange, small fruits, maize, oak, fennel, beetroot and emerging neglected crops as teff and fonio; animals under study will be swine and lactating ruminants.

Metagenomics data from plant pathogens and mycotoxigenic fungi, as well as soil, root-associated, symbiotic and endophytic microbial communities, in agricultural and stressed environments, will be used to pursue a one-health approach, and improve plant adaptation to CC through the development of resistant/tolerant plant/microbial genotypes and combinations, and the implementation of sustainable agronomic practices.

Methods: genome characterization through whole genome sequencing, SNP discovery, resequencing, genotyping by sequencing, deep sequencing, metagenomics, comparative genomics, advanced cytogenetics.

Initial TRL: 2

Final TRL: 4

T 1.1.2 *Comparing phenotypic diversity of genetic resources through phenomic and multi-omic approaches (M1-M36; task leader: UNITUS; partners involved: CNR, UNIBO, CREA, FEM, UNIMI, UNIBA, UNIPD, SSSA, UCBM)*

Screening of plant, animal and microbial genetic resources (mutants, accessions, breeding populations, wild relatives, natural populations, introgression lines, strains) for precise dissection of intraspecies genetic-phenotypic diversity will be carried out through deep phenotyping approaches, available or developed *ad hoc* at different time and space scales. Integration of morpho-physiological traits, including root anatomy, with molecular, quality and/or nutritional/bioactive features will be carried out through models applied to multi-omics approaches and data to identify markers and superior alleles/haplotypes for CC resilience (extreme temperatures, drought, salinity) and for other stress conditions (low N, weed competition) and the basis of beneficial plant-microbe/microbe-microbe interactions. In trees, xylem anatomical traits and dendro-genomic data will be used to relate structure and functions. Plant species of interest include peach, wheat, olive, tomato, grapevine, berries, leguminous species, barley, oak, whereas animals will include swine and bovine.

Methods: screenings *in vitro* and *in vivo*, high throughput automated phenotyping, high resolution microscopy techniques, integration of multi-omic data (phenomics/metabolomics/proteomics/volatilomics/transcriptomics), mass spectrometry.

Initial TRL: 2

Final TRL: 4

T 1.1.3 Germplasm storage and management (M1-M30; task leader: CNR; partners involved: UNIBO, CREA, FEM, UNIMI, UNIBA, UNITUS, UNINA, SSSA, ENI)

This task integrates innovative storage and management practices of germplasm collections, including development of core collections, for the valorization of genetic resources, including endangered or neglected species, herbaceous (horticultural, cereals, agro-feed stocks) and fruit trees, breeds and varieties. Conservation strategies will take advantage of tissue culture, cryopreservation, plant propagation techniques for selected crop genotypes and perennial species, and storage of beneficial fungi and bacteria. Agro-biodiversity collections will be structured and integrated with existing national and European Infrastructures. Safeguard strategies to protect animal (ovine, caprine, horses) and plant genetic resources will be implemented. Databases will be developed to incorporate information for curation and digitalization of the collections and ensure adherence to FAIR standards (FAIR - Findability, Accessibility, Interoperability, Reuse). Standardized datasets of phenotypic descriptors and molecular markers will be used.

Methods: *In situ* and *ex situ* germplasm conservation, *in vitro* and *in vivo* curation, maintenance, rejuvenation, digitalization and valorization of germplasm collections, identification and characterisation of genetic conservation units.

Initial TRL: 2

Final TRL: 4

T 1.1.4 Advanced methods for analysis/interpretation of complex data to highlight superior alleles/ haplotypes (M1-M30; task leader: UNINA; partners involved: CNR, UNIBO, UNITUS)

This task develops and implements tools for integrated analysis, meta-analysis and mining of big -omic data to dissect complex traits and discover genes/alleles/QTLs involved in the response to environmental challenges. Models and algorithms for data analysis will be developed and applied, including parametric and non-parametric machine learning techniques. High-throughput phenomic data will be matched and integrated with genomic and epigenetic variants, and geo-climatic features to shed light on biological mechanisms behind complex traits, such as innate immunity and environmental microbiome definition in response to CC. Models for efficient storage, management, interface and sharing of data will be designed in the framework of Open Science Europe.

Methods: Advanced statistical and bioinformatic tools for data management and analysis, (non)-parametric, machine learning methods.

Initial TRL: 2

Final TRL: 4

Deliverables

D1.1.1 Gene pool characterization in plant/animal/microbial species (M36)

D1.1.2 High-throughput phenotypic characterization in crop/animal/microbial species (M36)

D1.1.3 Network of genetic resource collections (M30)

D1.1.4 Bioinformatic toolbox for data integration and analysis (M30)

Milestones

M1.1.1 Identification and collection of material for genetic/genomic analysis: ≥ 30 accessions for at least 8 plant/animal/microbial species (M9)

M1.1.2 Defining a workflow/protocol for data management and analysis (including data policy): development of specific protocols/datasheets for standardized submission of different omic data (M18)

Interactions with other Spokes

The work on genome-wide analysis of genetic diversity, pan-genome reconstruction, and modeling of evolvability in response to CC will shed light on the overall genomic organization and genetic diversity of different plant, animal and microbial species of interest of most of the spokes. Genomic and comparative data, obtained using phenomic and multi-omic approaches to study genetic resources diversity, will be functional for further applications and collaborative interactions with Spoke 2 (*WP2.2 - Alternatives tools and strategies to reduce the use of synthetic pesticides and fertilizers*), Spoke 4 (*WP4.1 - Next-generation technologies for resilient traits of crop varieties and tree species*), Spoke 5 (*WP5.2 - Livestock management for improving resilience to climate change*) and Spoke 7 (*WP 7.2 - Development of multifunctional production systems*). In particular: studies focused on soil microbiota will be performed in collaboration with Spoke 2 (*WP2.2 - Alternatives tools and strategies to reduce the use of synthetic pesticides and fertilizers*); markers and different genomics tools, useful to identify phenotypic traits related to crops/trees resilience, will be developed and adopted in close cooperation with Spoke 4 (*WP4.1 - Next-generation technologies for resilient traits of crop varieties and tree species*); genetic and genomic data developed in WP1.1 will be used to screen for useful phenotypes in the collections available in Spoke 5 (*WP5.2 - Livestock management for improving resilience to climate change*); interactions with spoke 7 (*WP 7.2 Development of multifunctional production systems*) will provide molecular data on genetic resources better suited to marginal area.

Work package number	1.2	Lead beneficiary	CNR
Work package title	Dissecting morpho-physiological and molecular mechanisms of adaptation		
Start month	1	End month	36

Objectives

- Identify relevant morpho-physiological traits for organism resilience (e.g. architecture/anatomy, WUE, photosynthesis, hormones)
- Discover key loci/genes/alleles associated with organism adaptation and production
- Identify biochemical players/mechanisms involved in organism's response to environmental stresses
- Characterize plant-microorganism interactions beneficial for tolerance

Develop organism simulation models

Description of work

Task 1.2.1 *Linking phenotype and genotype: discovery of loci/genes/alleles for traits of interest (M1-M36;*

task leader: FEM; **partners involved:** CNR, UNIBO, CREA, UNIMI, UNIBA, UNINA, SIS, SSSA)

To face environmental changes, at the same time ensuring suitable quali-quantitative productions, a deep characterization of the genetic potential of national crops/breeds is fundamental. Key genetic elements controlling organism's adaptation, production and quality characteristics will be identified in relevant agriculture species (some cereals, horticultural species, fruit trees, grape, small ruminants and other animals, and *Aspergillus/Fusarium* subspecies) by integrating high-throughput genomic and phenomic data, haplotype based QTL mapping in introgression, bi- and multi-parental mapping populations, GWAS and positional cloning for gene(s) of interest, as well as forward/reverse genetics using native/artificially-induced genetic variations.

Methods: genetic, morpho-physiological and biochemical studies focused on representative plant/animal/microorganism populations will be combined with information from advanced phenomic, genomic and bioinformatic tools.

Initial TRL: 2

Final TRL: 4

Task 1.2.2 *Assessing epigenomic/transcriptomic/proteomic/metabolomic/volatilomic changes underpinning resilience/adaptation to stress conditions (M1-M30; task leader: CNR; partners involved: CREA, FEM, UNIMI, UNIBA, UNITUS, UNINA, UNIPD, SSSA, UCBM)*

Omics experiments are widely used to highlight quantitative modifications in the molecular repertoire of organisms subjected to perturbations. Thus, investigations based on the combined use of untargeted multi-omics approaches will be accomplished to assign specific representation changes in the epigenome (DNA methylation, ncRNA), transcriptome, proteome, volatilome and/or metabolome of selected genotypes of relevant agriculture species (some cereals, Solanaceae plants, fruit trees, olive, poplar, grape, and some ruminants) exposed to controlled experimental conditions mimicking those associated with real climate-environmental changes. This will allow the identification of novel molecular elements and networks essential for resilience/adaptation to stress conditions, like temperature changes, irradiation, drought, flooding, nutrient deprivation, salt stress, pathogen challenge and others.

Methods: advanced epigenomic, transcriptomic, proteomic, volatilomic and/or metabolomic technologies will be applied to body tissues/fluids/cell extracts from selected, challenged organisms; multi-omic data will be integrated by dedicated bioinformatic procedures. Data, metadata and results will be stored in dedicated structures to guarantee information for subsequent analysis in the long term.

Initial TRL: 2

Final TRL: 4

Task 1.2.3 *Biochemical mechanisms contributing to improved adaptation, production and quality traits (M1-M36; task leader: UNIMI, partners involved: CNR, UNIBO, FEM, UNIBA, UNINA, UNIPD, SSSA, UCBM)*

Specific cell structures and gene/protein families have been already identified as able of exerting a protective function with respect to abiotic/biotic stresses and/or contributing to maintained quali-quantitative production traits in these conditions. In agricultural relevant species, most of the data on these functional genes/proteins has been deduced by recognizing a sequence similarity with investigated molecules in model organisms. To fill this gap, key cellular structures, molecular players (and their structural elements), metabolic pathways, and protein interaction/modification modulatory processes punctually underpinning organism's adaptation/resistance to specific environmental stresses and/or contributing to production characteristics in agricultural relevant species will be investigated by different experimental approaches. These studies will characterize specific molecules and reactions behind the cellular processes essential for organism's resilience and adaptation to stresses, also evaluating related components/elements affecting important metabolic pathways for production. Information on key molecules in relevant agricultural species, including selected cereal and horticultural species, apple, grape, olive and some ruminants, will be translated to other related and model organisms (and vice versa). Once assigned, above mentioned molecules might possibly be the target of specific approaches focused at improving organism's adaptative and productive performances.

Methods: physiological, cellular biology, biochemical and molecular biology procedures will be integrated by advanced targeted molecular approaches to achieve requested information. Specific gene mutants will be generated and tested for their morpho-physiological and biochemical characteristics.

Initial TRL: 2

Final TRL: 4

T1.2.4 *Mechanisms underlying plant/animal-microbial interactions beneficial for tolerance (M1-M30; task leader: CNR; partners involved: UNINA, UNIBO, CREA, FEM, UNIMI, UNIBA, UNITUS, UNIPD, SSSA)*

Plant interactions (above-ground/below-ground) with microorganisms have been demonstrated to contribute to tolerance to various environmental stresses and to enhance productivity. Similarly, animals are universally and persistently inhabited by microbes that are not passive players but are involved in host physiology, systemic defenses and disease resistance. This complex ecosystem of interconnected microbial communities can be influenced by several biotic and abiotic stressors, but only some of these interactions have been characterized in detail. To fill this gap, we will characterize microbial communities and will evaluate organism-microbe crosstalk/interactions in agricultural relevant species to unveil beneficial mechanisms promoting resilience and optimal quali-quantitative performances under stressful conditions. Identification of related key genes and metabolic pathways in plants, animals and microbes will also be accomplished. Beneficial modulation of some of the above-mentioned interactions by microalgae, biostimulants and/or other inorganic/organic compounds will be possibly assayed.

Methods: this activity will be accomplished on some cereals, tomato, beet, apple, grape, berry plants, pine tree, plant model organisms, beef and chicken. In plants, plot and mesocosm assays where biodiversity and ecosystem services are manipulated at realistic levels will be integrated by gene modulation/silencing, genome editing, omic-based and/or molecular docking experiments to correlate morpho-physiological data with changes in the organism's molecular repertoires. Time-course experiments with mono-, bi- and tri-microbial inocula will be eventually accomplished. In animals, related studies on intestinal bacteria and fungi will be performed.

Initial TRL: 3

Final TRL: 5

T1.2.5 Strategies and tools for genomic prediction and crop/animal simulation models (M6-M36; task leader: UNINA; partners involved: CNR, UNIBO, CREA, UNIBA, UNIPD, SIS)

Strategies for the application of genomic selection will be designed accounting for the specific genetic features of species and traits of interest. This activity will be focused on agricultural relevant species, including some cereals, legumes, tomato, strawberry and selected ruminants. Adaptive variation, genotype by environment effects on production, quality and resilience traits will be modeled for genetic prediction. Population structures and breeding practices will guide the application of predictions in genomic breeding schemes. Simulations will be used to optimize the breeding plans and leverage the use of genomic/epigenetic information in a specific production environment. Genome-base strategies for balancing selection and conservation goals are also envisaged. This activity will permit a careful evaluation of current, resilient biocapitals according to predictive-extrapolative criteria, and a putative reshaping of agroecosystems suffering climate changes through the possible introduction of selected, optimized ecotypes. Genomic and phenomic data from the previous four tasks will be used as inputs to this purpose.

Methods: Advanced statistical genetics methods for genomic selection models (including machine learning techniques). Support vector machine (SVM), random forest, neural network e deep learning approaches will be evaluated and/or compared.

Initial TRL: 3

Final TRL: 5

Deliverables

D1.2.1 Identification of loci/genes/alleles for stress adaptation, quality and yield in plant/animal/microbial species (M36)

D1.2.2 Definition of multi-omic profiles associated with resilience or adaptation in plant/animal/microbial species (M30)

D1.2.3 Identification of cellular elements and biochemical mechanisms involved in adaptation, production and quality traits in plant/animal/microbial species (M36)

D1.2.4 Characterization of beneficial microbial interactions in plant/animal species (M30)

D1.2.5 Development and implementation of genomic selection models in plant/animal species (M36)

Milestones

M.1.2.1 Genotypes segregating for loci/candidate genes for stress adaptation, quality and yield identified from at least 6 population/mutant/strain screenings (M24)

M.1.2.2 Datasets obtained from ≥ 6 plant/animal/microbial organisms exposed to different environmental conditions (≥ 3) (M20)

M.1.2.3 Estimation of genomic breeding values and structured genomic breeding plans (M30)

Interactions with other Spokes

Information generated regarding epigenomic/transcriptomic/proteomic/metabolomic/volatilomic changes underpinning resilience/adaptation to stress conditions will provide tools and biochemical markers useful for the identification of plants and animals better adapted to specific stress conditions and resilient to climate changes. Application of these findings will take advantage of the collaboration with several spokes, including Spoke 2 (WP2.2 - *Alternatives tools and strategies to reduce the use of synthetic pesticides and fertilizers*), Spoke 4 (WP4.1 - *Next-generation technologies for resilient traits of crop varieties and tree species*) and Spoke 5 (WP5.2 - *Livestock management for improving resilience to climate change*). Investigation of the role of plant microbiota in the modulation of plant growth and defense will be carried out in collaboration with Spoke 2 (WP2.2 - *Alternatives*

tools and strategies to reduce the use of synthetic pesticides and fertilizers), also studying natural antagonists of pests and pathogens.

Work package number	1.3	Lead beneficiary	CREA
Work package title	Developing advanced genotypes with improved resilience		
Start month	1	End month	36

Objectives

- Develop efficient selection and morpho-physiological phenotyping strategies for the production of novel genotypes better suited to cope with climate changes
- Develop and implement novel genetic improvement strategies (New Genomic Techniques, NGTs) in species of agricultural and forest interest
- Assess the performance of varieties/breeds microbial strains in different environments and cultivation systems
- Develop new tools to facilitate genotype identity assessment and traceability of agricultural products

Description of work

T1.3.1 Improved genotypes / varieties / breeds / microbial strains using genomic and phenotypic information (M1-M36; task leader: CREA, partners involved: CNR, ENI, FEM, SIS, UCBM, UNIBA, UNIBO, UNIMI, UNITUS)

Genomic based breeding and marker assisted selection will be used to develop novel plant and animal genotypes with improved characteristics. Depending on the species of interest (which will include major seed crops such as wheat, rice, barley, soybean as well as grapes, apple and pear, olive and citrus trees, and horticultural crops, such as tomato, bean, and blueberry, and forage plants, such as alfalfa; cattle and pigs), the work will focus on different traits, which will include yield stability, tolerance to abiotic and biotic stresses, resource use efficiency and quality. New genomic indexes will be developed in support of animal breeding, and selection protocols will be developed to remove deleterious recessive alleles. Genetic and phenotypic information will also be used to improve microbial strains with beneficial effects. These activities will make extensive use of phenotyping and genotyping tools, and will be performed at different scales, ranging from controlled environment tests to field/farm conditions, in order to evaluate the agronomic, nutritional and technological characteristics of the new genetic materials and to assess their commercialization potential.

Methods: genotyping, phenotyping, marker-assisted selection, genomic selection.

Initial TRL: 3

Final TRL: 6

T1.3.2 Development and implementation of advanced phenotyping approaches for selection (M1-M36; task leader: SSSA; partners involved: CNR, UCBM, UNIBA, UNIBO, UNIPD, SIS)

Phenotyping tools are central to the study of the response to changing environmental conditions and to the selection of superior genotypes. This requires the implementation and use of a large range of different platforms, and of standardized protocols for data acquisition, management and distribution. The activities will focus on the development and implementation of phenotyping approaches that can be useful for the selection of novel genotypes in plant, livestock and microbial species. Response to specific stresses will be monitored in order to identify indicators of resilience and adaptability. While some phenotyping tools will operate in laboratory conditions, others will be implemented in controlled environment conditions. In addition, field/on-farm phenotyping, including *precision livestock farming*, will be functional to the development of new breeding strategies and will allow to accelerate the screening of genotypes during the whole selection process, also integrating classical and high-throughput phenotyping approaches. The activities will be focusses on major crops such as wheat (bread and durum) and barley.

Methods: imaging, data analysis, microbial high-throughput phenomics, controlled environment phenotyping, field phenotyping, on-farm phenotyping.

Initial TRL: 4

Final TRL: 6

T1.3.3 Development and implementation of novel biotechnological approaches, including cisgenesis and genome editing, for accelerated precision breeding (M1-M36; task leader: CNR; partners involved: CREA, FEM, SIS, UNIBA, UNIBO, UNIMI, UNINA, UNIPD, UNITUS)

Plant and animal breeding is a long process and, for instance, it can take up to more than ten years to develop a new plant variety. There is therefore an urgent need to accelerate the breeding process in order to rapidly respond to the challenges imposed by climate change, sustainability goals and evolving market conditions. Recently introduced new genomic techniques (NGTs), together with speed breeding have a great and still largely unexploited potential to accelerate the breeding process and contribute to the development of an efficient and sustainable agricultural system. This will require continuous technological innovation and specific efforts to adapt the new techniques to the individual characteristics of each plant/animal/microbial species. This task will deal with the implementation of available technologies, such as cisgenesis and genome editing, in plant/animal/microbial species (including major seed crops such as wheat, maize and rice, as well as other species with a relevant role in the national landscape such as citrus, apple, pear, grape and tomato, and farm animals such as pigs) with the development of innovative breeding tools and protocols able to accelerate the breeding process, thus contributing to the release of new varieties/breeds/microbial strains able to sustain innovation in the farming sector. The task will deal with different species including cereals, legumes, fruit trees, grapes, and horticultural/industrial plants such as tomato.

Methods: genome editing, cisgenesis, tissue culture, speed breeding.

Initial TRL: 2

Final TRL: 4

T1.3.4 Testing new genotypes for performance in multiple environments and cultivation systems (M6-M36; task leader: UNIPD; partners involved: CNR, CREA, ENI, FEM, SIS, UNIBA, UNIMI, UNINA)

The aim of this task will be the validation of already available and newly developed genetic materials in different environmental and cultivation conditions, including marginal lands and low-input conditions, in order to provide farmers with a valuable toolbox to overcome in a cost-effective way abiotic stress and climate change phenomena, still preserving overall productivity and quality of primary production. The work will include the assessment of Genotype x Environment interactions through multiple environment trials, the comparison of different cultivation/farming systems, and controlled environment tests. The tests will involve different species and will mainly be addressed to compare genotypes from major seed and protein crops (brads wheat, durum wheat, soybean), oil (energy) crops, horticultural crops (tomato and berries), grapevine, apple, forage legumes, olive trees, other fruit trees, and poplar.

Methods: evaluation of yield, quality and expression of new agronomic and genetic traits in different farming conditions, statistical analysis, controlled environment experiments.

Initial TRL: 6

Final TRL: 7

T1.3.5 Development of innovative tools for genotype identity assessment and intellectual property protection (M12-M36; task leader: UNIBA; partners involved: CNR, UNIMI, UNIPD)

The availability of tools to assess genotype identity is central for distinctness, uniformity, stability (DUS) testing, and for the protection of intellectual property rights. Genetic methods (e.g. KASP Markers, SSRs, GBS), phenotyping methods, and analytical (e.g. mass spectrometry, near infrared spectroscopy, nuclear magnetic resonance) protocols will be developed and validated to facilitate genotype identification and assessment of the characteristics of the different genetic materials in a cost-effective way.

Methods: single nucleotide polymorphism (SNP) fingerprint, simple sequence repeat (SSR) markers, mass spectrometry, nuclear magnetic resonance, phenotyping, digital code (QR code).

Initial TRL: 5

Final TRL: 7

Deliverables

D1.3.1 Improved genotypes /varieties / breeds / microbial strains (M36)

D1.3.2 Precision phenotyping and speed-breeding applied to plant/animal/microbial species (M36)

D1.3.3 Novel breeding protocols applied in plant/animal/microbial species (M36)

D1.3.4 Data from multi-environment testing and identification of superior genotypes (M36)

D1.3.5 Development of novel tools (markers, protocols) for IP protection (M36)

Milestones

M.1.3.1 Validation of mutants/genotypes in at least 3 traits of interest per 3 plant/animal/microbial species and selection of mutated progeny (M24)

M.1.3.2 Definition of the experimental strategy for field-testing of novel genotypes: protocols for testing in at least 4 different locations/conditions (M24)

Interactions with other Spokes

Newly improved genotypes such as plant varieties, animal breeds and microbial strains will be further tested for specific traits at different levels (laboratory, phytotron, greenhouse, field, farm) in collaboration with Spoke 2 (*WP2.2 - Improved genetic materials to reduce the use of agrochemicals*) to further characterize the genetic materials with respect to response to pests and pathogens, with Spoke 4 (*WP4.2 - Smart-climate and resilient agriculture and forestry: from sustainable products to the bioeconomy*) to assess their farm-level performance, with Spoke 5 (*WP5.2 - Livestock management for improving resilience to climate change*), where genetic materials and data made available by this WP will be instrumental to phenotype collection for testing resistant and resilient animals, and with Spoke 7 (*WP7.2 Development of multifunctional production systems*), to identify genetic materials best suited to marginal areas.