

Brussels, 12 May 2023

COST 067/23

# DECISION

Subject: Memorandum of Understanding for the implementation of the COST Action "Reproductive Enhancement of CROP resilience to extreme climate" (RECROP) CA22157

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action Reproductive Enhancement of CROP resilience to extreme climate approved by the Committee of Senior Officials through written procedure on 12 May 2023.





# MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

# COST Action CA22157 REPRODUCTIVE ENHANCEMENT OF CROP RESILIENCE TO EXTREME CLIMATE (RECROP)

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to understand the basis of the sensitivity of crop reproduction under abiotic stresses. The Action will identify the weakest links and create a roadmap for crop improvement in a sustainable manner using genetic and agronomic approaches, such as the application of biostimulants. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.



# OVERVIEW

#### Summary

Climate change is a threat for food security as extreme weather phenomena will reduce the yield of all major crops. Grain and fruit crops which consist the core of human diet are particularly vulnerable due to the sensitivity of sexual reproduction process to abiotic stresses. Consequently, there is an urgent need to generate elite varieties with enhanced reproductive stress resilience. RECROP (Reproductive Enhancement of CROP resilience to extreme climate) is a team of agronomists, physiologists, geneticists, biologists, bioinformaticians and researchers from the field of Machine Learning from public organizations and private sector which will use holistic approaches to understand the grounds of crop sensitivity and design solutions for yield stimulation in the era of climate change. RECROP aims to: (1) Identify the genetic, molecular, and physiological makeup of the sensitivity of crop reproduction, (2) Create a roadmap for the generation of resilient crops, and (3) Provide guidelines of exogenous treatments to increase resilience in a sustainable manner and push the limits of the genetically inherited stress tolerance. The aims will be fulfilled by four Working Groups (WGs) which in addition to research discussions will organize training schools, workshops, conferences, and dissemination activities. RECROP will actively support Early Stage Career researchers through training and networking and support interactions with Near Neighbouring and partner COST countries. RECROP members will be actively involved in building communication channels with Policy Makers to provide scientific advice and support them in scientific-based context of future policies on biotechnology, technology and agriculture sectors.

Areas of Expertise Relevant for the Action	Keywords
• Agriculture, Forestry, and Fisheries: Agriculture related to	Crop improvement
crop production, soil biology and cultivation, applied plant	abiotic stress
biology, crop protection	reproduction
Agricultural biotechnology: Genetic engineering, transgenic	• yield
organisms, recombinant proteins, biosensors for agricultural	tolerance
biotechnology, animal biotechnology	
Agricultural biotechnology: Databases, data mining, data	
curation, computational modelling	
Biological sciences: Genomics, comparative genomics,	
functional genomics	
Biological sciences: Plant biology, Botany	

# **Specific Objectives**

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

#### Research Coordination

• Define standards for experimental setup, data collection, analysis and result interpretation, including both wet lab and in silico methodologies and determination of the basis for the integration of interdisciplinary approaches from small to large scale on different levels, and further to Big Data analysis and modelling.

• Coordinate the collection of state-of-the-art data on response and resilience of plant reproduction to high temperatures, drought and flooding as major stress factors in the era of climate change and build up-to-date models regarding response description and prioritize specific genes/pathways as targets for crop improvement.

• Coordinate experiments across different laboratories for crop model validation and hypothesis testing and organise benchmarking of pipelines and workflows across different research groups with various expertise.

• Define standards and requirements for integration of interdisciplinary approaches on different scales (from single cell to tissue/organ, and from single molecule to global –omics) to build models for stress response.

• Bring together expertise, share of knowledge on experimental design and execution, data collection,



analysis and result interpretation in a multidisciplinary scale, share infrastructure and instrumentation, datasets and molecular and genetic tools to expand the accessibility of partners to a wider range of resources.

• Provide input to stakeholders for the current status and future projections of yield for important crops, guidelines as solutions to improve thermotolerance and yield to assist constructing a roadmap for strategic collaborations and future investments.

• Coordinate discussions with private enterprises on short and long-term requirements of the market regarding resilient germplasm and support of crop improvement, map short- and long-term R&D priorities and the strategic collaborations that need to be built.

• Disseminate up-to-date models, future guidelines for improvement of crop resilience, standardise methods and pipelines as well as the principles, prospective and workflow for interdisciplinary approaches on exploring and improving stress resilience of plant reproduction and yield.

• Inform the public on the relevance of stress resilience for food production, current threats and strategies to tackle the problem.

#### Capacity Building

• Promote the exchange of knowledge through a Pan-European network of scientists from universities, research institutes, SMEs, farmer and consumer associations to tackle the problem of the effect of global warming on crop yield, public awareness and strategic solutions.

• Engage scientists from different research disciplines (biologists, geneticists, biochemists, mathematicians, bioinformaticians, big-data scientists, botanists, agronomists, engineers) to develop holistic approaches to tackle the description of stress response and develop complex and robust solutions to enhance yield in the era of global warming.

• Create a central hub for a network of scientists aiming to understand crop thermotolerance and offer solutions for crop improvement via enhancement of the resilience of plant reproduction through innovative approaches.

• Foster a group of early stage researchers and group leaders, particularly female researchers and with respect to diversity, equity and inclusion.

• Promote the interactions between public research bodies and private sector particularly SMEs from different European regions with different climate types and predictions on global warming effects, to define prerequisites and priorities towards the development of resilient germplasm.



# **TECHNICAL ANNEX**

# **1. S&T EXCELLENCE**

# 1.1. SOUNDNESS OF THE CHALLENGE

# 1.1.1. DESCRIPTION OF THE STATE OF THE ART

The core of human diet is based on plant products. The increase in crop yield and quality of the produce has been an ongoing challenge for hundreds of years. In the last decades, advances in biology, genetics and agricultural practices have been the drivers for a steep increase in yields of many crops. Production has been boosted by breeding of elite varieties, the development of hybrids but also the application of fertilizers, and the technological progress in farming. Along with these, biotechnological approaches and genetic engineering revolutionize molecular plant breeding and speed up the development of high yielding crop varieties to push food production even further.

Due to the increasing human population and the current geopolitical situation there is currently an even stronger demand for higher yields to prevent poverty and malnourishment. It is estimated that food production needs to be increased by 70% by 2050 to feed a population of approximately 9.8 billion people (www.fao.org). One of the goals of the United Nations (UN) '2030 Agenda for Sustainable Development' is 'End hunger, achieve food security and improved nutrition and promote sustainable agriculture' (sdgs.un.org/2030agenda). Despite intense efforts, an increase in production is hampered by two main factors: the limited availability of arable land and the negative effects of climate change. Current climate models predict a global rise of annual average temperature of 0.9 to 2.0 °C till 2050 according to intermediate emission scenario RCP4.5 of IPCC (www.ipcc.ch/report/ar6/wg3/). For every 1°C increase in growing-season minimum temperature a decline in grain yield of 10% has been reported for rice and 5% for wheat [1,2]. Every single day with air temperature rise above 30°C results in a 1% decline in maize yields [3]. In addition to the long term effects of mild temperature elevations, extreme weather phenomena such as heat waves and prolonged drought periods or even heavy precipitation causing floods are expected to become more intense and frequent, causing devastating effects for agriculture and food production (www.ipcc.ch/report/ar6/wg3/).

The majority of a plant-based diet is comprised of fruits and seeds, the products of successful sexual reproduction. The majority of these crops are cultivated in open fields, and are therefore vulnerable to adverse environmental conditions such as elevated temperatures that lead to failures in fertilization or abortion of seeds and fruits [4]. Increased temperatures can cause early or late flowering, asynchrony in male and female reproductive development, a shorter the period of stigma receptivity, as well as defects in male and female gametes that lead to sterility. In addition, prolonged warm conditions or heat waves can cause defects in parental tissues such as carpels and stamens, abnormalities in ovaries, and even trigger ovule and flower abortion. In insect-pollinated crops, heat-dependent disruption of key secondary metabolic pathways can lead to a major reduction of flower volatile and nectar production, thereby reducing pollinator attraction and pollination efficiency. High temperatures during the period of seed filling can also accelerate senescence, reduce overall seed mass and yield due to limitations in photosynthates. All these effects are exacerbated when heat conditions co-occur with other abiotic stresses, like drought (two stresses that typically coincide).

In general, the effects of high temperatures alone or in combination with other stresses on sexual reproduction processes at the physiological level have been described in many crops. Studies focusing on the molecular and biochemical aspects of stress responses have been mainly focusing on pollen, as the male gametophyte has been proposed to be the weakest link of sexual reproduction in plants and the exposure to higher temperatures during meiosis can lead to developmental arrest and failure to produce mature pollen grains [5]. However, the physiological and molecular basis for the response of different reproductive stages and tissues to a single stress and combinations of stresses remains unknown, hindering the development of resilient crops. Furthermore, while many studies have shown that the physiological status of the vegetative organs such as leaves and roots under stress conditions have an impact on the resilience of plant reproduction, the ground of this relation is not well understood.

Understanding the basis of resilience or sensitivity requires a multilayer approach that produces information ranging from molecular to physiological responses, across a population of individuals from COST Association AISBL

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various genetic backgrounds. Such information allows the identification of key regulatory hubs for stress responses and resilience. While such information exists to some extent for vegetative tissues, knowledge is scarce when it comes to the effects of abiotic stresses on reproduction and the reproductive tissues [6]. Modern technologies allow the global analysis of DNA, RNA, protein and products of primary and secondary metabolism in great depth and in a multidimensional fashion (e.g. modified molecules, structural dynamics, macromolecular complexes, etc.). State-of-the-art powerful imaging techniques decipher structural changes at the cellular, tissue and organ levels. Despite the fact that all this information is interconnected, current approaches consider only few levels of regulation, mostly due to the costs of analysis and lack of expertise, thereby providing only limited information and missing the overall view. Instead, integrative approaches that utilise multiple levels of information provide deeper insights into the biological system. The latter requires the employment of machine learning (ML) based predictive algorithms in order to describe and understand complex biological systems, discover key pathways and biomarkers across different conditions and genetic backgrounds [7]. RECROP (Reproductive Enhancement of CROPs to extreme climate) COST is a multidisciplinary consortium bringing together experts from different and complementary fields that share knowledge and expertise in order to design strategies to tackle the problem of crop resilience in the era of climate change in Europe and worldwide. RECROP focuses on heat, drought and flooding as abiotic stress with the most devastating effects for agriculture.

# 1.1.2. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Plant reproduction is very sensitive to high temperatures and therefore global warming is considered as a major driver of yield reduction. The most recent models on the effect of global warming predict significant losses in average productivity ranging from 6 to 24% for crops like maize, soybean and rice till the end of the century. In addition, the co-occurrence of other stresses such as drought during critical developmental periods can further intensify yield losses. The impacts of adverse climate can be mitigated by the use of better-adapted and resilient varieties. The task to create stress resilient varieties and hybrids of crops by improving reproductive performance under stressful environments is one of the greatest challenges for plant biologists, as *it has to be done on a global scale, within an unprecedented short time and in parallel for many crops.* In addition, innovative agronomical tools can increase the genetically predetermined stress resilience capacity. Biostimulants acting as stress priming agents can increase the capacity of crops to minimize crops losses [8].

Albeit many research groups focus on exploring stress response and tolerance of reproductive tissues, there is only little progress on understanding the basis of the sensitivity and the development of resilient crops. One reason for this, is that the very nature of resilience is a major obstacle as in many cases it has been shown that there is a trade-off between stress tolerance and yield. In addition, stress tolerance is believed to be a polygenic trait which involves complex gene networks, and the molecular signature of combinations of stresses are distinct and do not correspond to the additive effects that the individual stressors pose. Additional reasons for the lack of progress are: (1) the protocols that are used for stress application and (2) analysis of the results are diverse and not standardised within the community, preventing accurate comparison of different studies, (3) many efforts to determine tolerance, even in genetic screens, are focused on laboratory experiments, results of which are difficult or impossible to apply in field conditions, (4) description of the stress response is typically single-faceted, focusing on a single –omics but ignoring the rest, (5) there is lack of interdisciplinary approaches in experimental design and data analysis, (6) particularly in non-cereals (e.g. fleshy fruits), tolerance traits consider on specific tissues (e.g. roots and leaves) and a direct link to yield is ignored. (7) scarce efforts on molecular genetic approaches such as CRISPR due to discouragement from the current European legislative framework. However, there are efforts in Europe to explore the support of sustainable agriculture through genome editing technologies such as the initiative SAGE (www.eu-sage.eu) and stay on the frontline of global efforts for crop improvement (https://www.eu-sage.eu/index.php/genome-search).

The timely delivery of resilient germplasm within **less than the time span of a generation** requires the cooperation and collective efforts by researchers of all relevant scientific disciplines (agronomy, physiology, cell and molecular biology, genetics, mathematics, bioinformatics, data science) but also the active participation of stakeholders (breeding/seed companies, policy makers, consumer and farmer associations, etc.). RECROP is a platform to bring together all relevant parties with **three major aims**:

- 1. Identify genetic, molecular, and physiological makeup of reproduction sensitivity of crops under different abiotic stress scenarios and indicate the weakest links and targets for improvement
- 2. Create a roadmap for the generation of resilient crops
- 3. Provide guidelines of exogenous treatments with biostimulants to increase resilience in a sustainable manner and push the limits of the genetically inherited stress tolerance.



RECROP will primarily focus on fruit and grain crops such as tomato, maize, wheat, barley and rice, however the pipelines that will be generated will be suited for other crop species as well. Through direct interactions we aim to create a Pan-European community (and beyond) that will share expertise, data, and viewpoints to:

- 1) Develop standardized protocols for tailored-based experimental setup for various crops and climate scenarios, and to formulate the requirements and quality control aspects for data analysis and interpretation.
- 2) Bring together disciplines that are not accustomed to working together but whose collaborative research is crucial towards understanding stress response and resilience in reproduction of crops.
- 3) Provide training on state-of-the-art methods necessary to investigate response of reproductive tissues to high temperatures in both a qualitative and quantitative manner.
- 4) Create repositories for phenotypic traits and genetic material sharing to boost scientific exchange and collaborations and support progress in applied and basic research.
- 5) Propose specific strategies for crop improvement via different approaches (modern breeding or biotechnological/CRISPR).
- 6) Propose farming practices including the use of biodegradable chemicals and biostimulants to increase resilience to single and multiple stresses in a sustainable manner.
- 7) Communicate current pitfalls and future directions to policy makers, private sector, consumers and the general society to increase awareness and stimulate discussions to support food security.

# 1.2. PROGRESS BEYOND THE STATE-OF-THE-ART

1.2.1. APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE OF THE ART

By implementing the following measures, we aim to provide progress beyond the state-of-art:

- Progress of basic research on understanding stress response and abiotic stress resilience of reproductive tissues and yield. Experts on plant reproduction will combine the state-of-the-art on different crops and model plants regarding stress response and resilience on cellular, tissue and organ levels. Stress resilience traits will be described for different crops. Less thoroughly investigated aspects of resilience of plant reproduction to single or combination of abiotic stresses will be determined and specific priorities for future research directions will be defined.
- 2. Develop/improve protocols for stress application and analysis for lab-to-lab and lab-to-field translatable results, including commonly accepted quality standards, and pipelines for analysis of phenotypic outputs. We will define guidelines for application of stress (single and multiple) with respect to naturally occurring adverse environmental conditions, to increase the relevance of the findings to climate models for different regions. A major aspect is the integration of high-throughput phenotyping methods to minimize the effect of man-made errors in the analysis. RECROP will introduce modern techniques for the evaluation of stress-related traits, e.g. pollen viability and germination capacity, flower volatile production, seed and fruit set under laboratory and field conditions. Another important aspect is the lack of access to already characterized germplasm that can be used as an experimental control or background for crop improvement. We aim to create a platform for description of relevant genotypes for different crops (species, accession, ecotypes, varieties, mutants, transgenic lines) to stimulate exchange and collaborations among partners.
- 3. Create a framework for the integration of global –omics and phenotyping approaches and Big Data analysis platforms. RECROP will develop ML strategies for network construction in molecular responses and phenotyping. Together with mathematicians and bioinformaticians the principles and requirements for the experimental design and implementation for laboratory and field investigations will be defined. Available datasets on different –omics levels regarding abiotic responses of plant reproductive and vegetative tissues will be collected to a single database to allow thorough and efficient data mining for the identification of key elements for stress response and resilience. A common language for data generation, analysis and interpretation of results will be defined to stimulate comparative analysis across research groups. The database will conform to the standards of Open Science policy.
- 4. Design specific guidelines for crop improvement via different genetic approaches. Using a crop specific approach and based on the peculiarities of each crop, RECROP will provide the framework for the introgression of stress tolerance traits in sensitive varieties either via breeding, or generation of transgenic lines and gene editing technologies like CRISPR. We will exploit various possibilities, define advantages/disadvantages and their perspective and feasibility for crop reproductive stress



resilience improvement considering the peculiarities and tools available for each species considering the constantly changing legislative environment.

5. Increase public awareness for the sensitivity of plant reproduction to environmental conditions and the impact of global warming on food production, and engage policy makers, private enterprises including Small and Medium-sized Enterprises (SMEs), farmers and breeders in the discussion on crop yield improvement in the era of global warming. Communicate the key position of New Genome Editing Techniques (NGT) in achieving our goals, particularly now, when the communication about the future regulation of NGT at the EU level is in full swing.

# 1.2.2. OBJECTIVES

#### 1.2.2.1. Research Coordination Objectives

The specific outputs of the COST-ACTION will be realised through the following Research Coordination Objectives that will stimulate the knowledge exchange and generation of synergies. COST-ACTION will:

1. define standards for experimental setup, data collection, analysis and result interpretation, including both wet lab and in silico methodologies and determination of basis for integration of interdisciplinary approaches from small to large scale on different levels, and further to Big Data analysis and modelling. A challenging but very important aspect is the experimental scalability that needs to integrate standards for the translation of lab results to the field. This objective requires the expertise of plant biologists, geneticists, mathematicians, bioinformaticians and agronomists. The input from private sector (SMEs such as breeding or biotechnology companies) will support the generation of widely accepted protocols and guidelines.

2. coordinate the collection of state-of-the-art data on response and resilience of plant reproduction to high temperatures, drought and flooding as major stress factors in the era of climate change and build up-to-date models regarding response description and prioritize specific genes/pathways as targets for crop improvement.

3. coordinate experiments across different laboratories for crop model validation and hypothesis testing and organise benchmarking of pipelines and workflows across different research groups with various expertise. Benchmarking will be based on own funding for each laboratory and COST will have a advisory role.

4. define standards and requirements for integration of interdisciplinary approaches on different scales (from single cell to tissue/organ, and from single molecule to global –omics) to build models for stress response.

5. bring together expertise, share of knowledge on experimental design and execution, data collection, analysis and result interpretation in a multidisciplinary scale, share infrastructure and instrumentation, datasets and molecular and genetic tools to expand the accessibility of partners to a wider range of resources.

6. provide input to stakeholders (e.g. standardization body, policy-makers, regulators, users) as results related to current status and future projections of yield for important crops, guidelines as solutions to improve thermotolerance and yield to assist constructing a roadmap for strategic collaborations and future investments on national and international levels.

7. coordinate discussions with private enterprises (e.g. breeding and biotechnology companies) on short and long-term requirements of the market regarding resilient germplasm and support of crop improvement, map short- and long-term R&D priorities and the strategic collaborations that need to be built.

8. disseminate up-to-date models, future guidelines for improvement of crop resilience, standardise methods and pipelines as well as the principles, prospective and workflow for interdisciplinary approaches on exploring and improving stress resilience of plant reproduction and yield.

9. Inform the public (e.g. consumers, students) on the relevance of stress resilience for food production, current threats and strategies to tackle the problem.

# 1.2.2.2. Capacity-building Objectives

The aims of the RECROP will be achieved by creating a network of European experts that will stimulate progress on stress resilience in plant reproduction and yield, through five Capacity Building Objectives. We will:



1) Promote the exchange of knowledge through a Pan-European network of scientists from universities, research institutes, SMEs, farmer and consumer associations to tackle the problem of the effect of global warming on crop yield, public awareness and strategic solutions.

2) Engage scientists from different research disciplines (biologists, geneticists, biochemists, mathematicians, bioinformaticians, big-data scientists, botanists, agronomists, engineers) to develop holistic approaches to tackle the description of stress response and develop complex and robust solutions to enhance yield in the era of global warming.

3) Create a central hub for a network of scientists aiming to understand crop thermotolerance and offer solutions for crop improvement via enhancement of the resilience of plant reproduction through innovative approaches.

4) Foster a group of early stage researchers and group leaders, particularly female researchers and with respect to diversity, equity and inclusion, who will be trained in various aspects of plant biology, – omics, and Big Data analysis, and allow them to build a network that will stimulate their career and prospects for further involvement through collaborations.

5) Promote the interactions between public research bodies and private sector particularly SMEs from different European regions with different climate types and predictions on global warming effects, to define prerequisites and priorities towards the development of resilient germplasm.

# 2. NETWORKING EXCELLENCE

# 2.1. ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Due to their sessile nature, plants are exposed to stressful conditions by definition. This pressure is intensified due climate change and therefore the effects of abiotic stresses on both agricultural and natural ecosystems are of major interest for research communities. While it is widely accepted that the reproductive development is the weakest link of stress resilience, only few consortia have up till now focused on this aspect. From the ongoing COST-Action projects, ROXY deals with oxygen sensing and oxygen species signalling in commodities, focusing on postharvest biology, PlantEd focus is more on gene editing technologies in plants, and EPICATCH on the epigenetics regulation in plant abiotic and biotic stress responses. Therefore, none of them focuses on reproduction and they do not consider crop improvement in terms of yield as a primary objective. Nevertheless, in a broader perspective RECROP can benefit from interactions with these Actions and therefore members of these consortia will be invited to scientific meetings for knowledge exchange. From the newly approved COST initiatives, CA21138 deals with the effect of climate extremes on forests, while concluded Actions such as PlantMetals (CA19116) focused on the effects of metals on plants. FP1106 STReESS focused on stresses in forest trees, FA1306 on plant phenotyping technologies, CA21142 on genome editing methods, and INDPETH (CA16212) focused more on the nuclear processes underlying plant response to external stimuli, such as nuclear architecture, chromatin organization and gene expression. In the past, a Marie-Curie ITN has addressed pollen thermotolerance in tomato (SPOT-ITN concluded in 2016), however that project did not consider more stresses and crops, and especially various reproductive tissues and their differential and specific response and sensitivity. Similarly, TomGem and TOMRES focused on interaction of tomato with the environment and did not address reproductive performance per se, nor other crops. SUSCROP ERANET project BRACE addresses wheat fitness under adverse environmental conditions based on screening a nested association mapping population. In summary, several projects in the past or currently running have addressed the individual questions of crop reproductive resilience but not in a holistic manner like RECROP proposes.

# 2.2. ADDED VALUE OF NETWORKING IN IMPACT

# 2.2.1. SECURING THE CRITICAL MASS, EXPERTISE AND GEOGRAPHICAL BALANCE WITHIN THE COST MEMBERS AND BEYOND

RECROP will follow the guidelines for excellence and inclusiveness in science by generating an interdisciplinary consortium that will promote cooperation opportunities for researchers of all career stages across Europe and neighbouring countries. We will particularly encourage researchers from less research-intensive RECROP Members to participate and have an active role in administrative management and organization of events (meetings, conferences, webinars) as hosts. RECROP aims to at least 50% participation of female members and at least half of the Working Group leadership positions will be filled by female scientists. As many of the top scientists in the fields relevant for RECROP are women, we do not envisage any obstacle to achieve this ambitious target.



RECROP members are among the top scientists in Europe with complementary expertise in the fields of cell and molecular biology of plant reproduction, genetics of crops, –omics technologies and analysis, phenotyping from cell to plant levels, bioinformatics, modelling and Big Data science. In addition, the participation of members from the private sector (e.g. breeding companies, companies specialized on genomics) will ensure the establishment of an extended R&D Pan-European network that will bridge the gap between basic and applied science. Companies will contribute by introducing cutting-edge technologies to the consortium related to breeding, –omics analysis, phenotyping, and data analysis. We will promote discussions between research groups and private companies including SMEs to promote new possibilities for innovation and application. The consortium will ensure the access of all groups to new areas of research and technology and will stimulate the formation of new collaborations. This will be particularly promoted for groups and companies from NNCs. Specifically, RECROP will:

- 1) Promote the transfer of knowledge and expertise among the best research groups in Europe in the respective fields.
- 2) Build connections with Third States (IPC), Near Neighbouring Countries (NNC) and other organizations such as NGOs, as representatives will be members but also via direct invitations to round tables, conferences and meetings.
- 3) Provide a platform for training for all members and particularly NNC on advanced methodologies, highly specialised techniques and infrastructure via STSMs.
- 4) Promote the direct dissemination of information through round tables, meetings, conferences, webinars, workshops, and publications, as well as presenting a roadmap to address crop improvement under changing conditions.
- 5) Organize conferences that will be open to researchers that are not COST-X members.
- 6) Foster new synergies between universities and research institutes through existing student exchange programs to stimulate long-term and beyond the RECROP collaborations (e.g. establish bilateral exchange agreements such as ERASMUS).
- 7) Explore synergies and knowledge exchange beyond COST-ACTION borders (international conferences, webinars, etc) particularly exceeding scientific community to involve stakeholders and policy makers (see Section 2.2.2).

# 2.2.2. INVOLVEMENT OF STAKEHOLDERS

RECROP will include researchers from institutes and universities, representatives from private enterprises and particularly R&D departments from SMEs, and will establish communication channels with public officials from institutions such as farmer and consumer associations, funding agencies, climate and agriculture NGOs (e.g. CropTrust), policy makers from international organizations (e.g. Consultative Group of International Agricultural Research, Institute for Agriculture and Trade Policy, European Food Safety Authority, European Central Bank, EU and UN), EU-SAGE (European Sustainable Agriculture through Genome Editing) network and national and regional administrations. RECROP will ensure the active participation of stakeholders and stimulate discussions by:

- 1. Recruiting researchers with experience in the policy sector and academics who are active in industry.
- 2. Including from the very beginning of the COST stakeholders from a wide range of sectors, such as policy advisers, evaluators, funders, civil society, as key elements of generating future perspectives and approaches.
- 3. Organising a Kick-off workshop within the first two months of the Action start where the problem of climate change and crop yield will be discussed and each participant will contribute to drafting a white paper/roadmap to identify and describe the problem and will propose key steps that communities should take to tackle crop productivity in the era of global warming. This roadmap will be published on the website of the Action and will be disseminated by various means (WG4 activities) to maximize outreach.
- 4. Bridging communication channels and set the basis for discussions between researchers and policy makers through the agreement on a common language that will be outlined in "Management Committee Agreement".
- 5. Organising regular annual policy meetings throughout the duration of the Action to sustain discussions, update the roadmap and adopt positions based on progress and development. We will maintain active discussions by writing interim papers and recommendations after each meeting and include a final conclusion in the end of the COST that will be included in the final report.
- 6. Promoting meetings with stakeholders in workshops, conferences and various other meetings on national and international media (TV, radio, newspapers, magazines), social media (e.g. LinkedIn, Twitter, Facebook, Instagram) and podcasts.



7. Communicating results from the ACTION to international EU and national/regional administrations and provide the scientific results for use by policy makers.

Creating concrete interactions with stakeholders that will be sustained beyond the funded period of the COST, as for example long-term collaborations of public research institutes with breeding companies for crop improvement. This will include active participation of the ACTION members in international advisory organizations (Biovegen, EU-SAGE, EASAC – European Academies Scientific Advisory Council).

# 3. IMPACT

- 3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAK-THROUGHS
- 3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

RECROP addresses a major scientific and socioeconomic challenge: the generation of abiotic stress resilient crops to ensure food security. Consequently, our consortium will have an impact on the scientific community, the private sector, policy makers and the general public on several levels:

# Impact on the scientific community

RECROP will:

- 1) Develop and provide commonly accepted standards for experimental design and execution, data collection and analysis, and result interpretation. Particularly we will focus on the relation between lab-to-field results, and their translatability to yield and crop performance.
- 2) Include new methods or underrepresented techniques in current studies, and introduce new levels of information that are critical for understanding the basis of stress resilience.
- 3) Provide a roadmap for the inclusion of holistic approaches
- 4) Introduce the concept of ML in data analysis (from molecule to phenotype), and develop models for yield prediction.
- 5) Provide updated models for response of different species and tissues/organs within their reproductive apparatus to elevated temperatures, as single stress factor or in combination with other stressors such as drought and flooding, thereby we will uncover specific and conserved aspects of single and combinatory responses across different species and environments.
- 6) Create an open access repository with thermotolerant varieties/accessions, mutants and transgenic lines for different crops that will be accessible to the community.
- 7) Create an open access platform with –omics datasets to enable efficient and productive data mining, conforming to Open Science standards.
- 8) Identify and define the most important stress response pathways for thermotolerance and propose specific actions for crop improvement under stress conditions.

#### Impact on the private sector:

Breeding companies and farmers, as well as technology and analytics enterprises through RECROP will be able to:

- 1) Validate models in own germplasm and explore thermotolerance limits of different varieties and hybrids under different climate scenarios using the standardised pipelines designed by the COST.
- 2) Use innovative technologies, tools and knowledge from the COST to support and enhance existing and initiate novel breeding programs for crop improvement.
- 3) Provide guidelines for agricultural practices and plant treatments to mitigate the impact of environmental stresses on crop yields.
- 4) Gain access to stress resilient germplasm that can be used for breeding tolerant varieties development.
- 5) Establish knowledge-transfer schemes that will facilitate breeding programs for stress resilient new crop varieties and hybrids.
- 6) Develop products through discussions with the scientific community for current and future needs on analytical methods and services.
- 7) Introduce new customized products (innovation) into the scientific community but also to breeders/farmers as end-users.

#### Impact on policy makers in national and international regulatory bodies: RECROP will:

1) Give access to data generated by the COST to use in strategic decisions for funding and agricultural policies.



2) Evaluate the European competitiveness in the agriculture sector in the era of climate change

3) Update policies on food security and future relevant societal impacts of climate change based on progress of crop improvement

- 4) Provide experimentally based data on the potential impact of various genome editing technologies to assist in legislation discussions.
- Actively participate in international advisory organisations and be involved in preparation of their advisory statements and publications presented directly to policy makers at the EU level (EU-SAGE, EASAC)
- 6) Broaden the communication channels with stakeholders and policy makers by seeking and promoting the active in person participation of their representatives at the ACTION meetings.
- 7) Assist in creating management strategies to avoid risk associated with climate change.

#### Impact on the general public:

1) The ultimate goal of the COST is to promote the production of crop varieties and hybrids that are more stress resilient, to ensure high production of food particularly in regions that are or predicted to be heavily affected by climate change.

2) Educate the public and increase awareness for the threat of climate change on food security. Members of RECROP will create a website dedicated to educational purposes, describing the effects of global warming and climate change in general on plant reproduction and crop yield describing the subject in layman's terms. This information will be important for the dissemination of the problem of food security to school pupils, higher education students and the general public, therefore it will be offered in the most used European languages and will be communicated through Education programmes and local administrations to schools, social media, etc.

# 3.2. MEASURES TO MAXIMISE IMPACT

# 3.2.1. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

RECROP aims to elucidate the basis for the sensitivity of plant reproduction to high temperatures, drought and flooding, identify the critical elements for stress resilience for single and combinations of stresses and develop tools for the generation of elite crops that will be able to perform at high yields under more unfavourable environmental conditions. Currently several groups in Europe work on these topics, RECROP will support existing collaborations among members and promote new collaborations that will boost the progress of several projects related to resilience in plant reproduction and yield. One of the main weaknesses in the field is the lack of commonly accepted, standardized protocols. RECROP will take advantage of the involvement of groups with different expertise on the wider sense of the topic of stress resilience in plant reproduction to agree and publish commonly accepted standards for experimental setups, data analysis, and result interpretation. These guidelines will be available via an open access peer reviewed publication to maximize access by the scientific community but also the private sector. Another important weakness in the field is the lack of interdisciplinary approaches, which frequently results in fragmented information. Modern research requires more holistic approaches, which RECROP will promote through the interactions of different research groups that will encourage the introduction of new methods and approaches to existing projects, allow access to infrastructure to many groups that will overall have a stimulatory, synergistic impact on the quality of projects and increase their relevance and chances of success. Therefore, we foresee that all these interactions will create novel knowledge and will accelerate the efforts to develop stress resilience crops and secure food production. RECROP will also encourage the interaction with other European networks such as COST Actions (e.g. PlantEd, Epi-CATCH) to stimulate knowledge exchange, through invitations to workshops and conferences and the organization of joint events.

We will foster knowledge transfer among members of RECROP by organizing **training schools** with hands-on sessions on both *in silico* and wet lab methods. The training schools will be designed to cover the needs for both inexperienced and more advanced PhD students and young postdocs. We will host two in-person training schools per year focusing on stress application and phenotyping, various –omics methods, big-data analysis, and modelling. We will also provide a series of **Massive Online Open Courses (MOOCs)** on theoretical background and practical aspects of molecular and physiological aspects of stress response and tolerance, various techniques for phenotyping and –omics to maximize outreach. Every three months we will offer a **webinar** where experts from each field that is relevant for RECROP will provide a theoretical and a practical session on methodologies which will be attended by a high number of RECROP members offering the chance for a live Q&A session. Furthermore, we will encourage private enterprises and particularly SMEs to provide the latest updates on their portfolio that is relevant for the RECROP. Updates on ongoing research activities will be provided in monthly online



seminars "**RECROP Monday Update**" where each member of the group will have the chance to present the state-of-the-art of the research group and newly published papers on crop thermotolerance. These seminars will be particularly directed to post- and undergraduate students. RECROP will organize annual **international conferences** with the participation of top researchers from around the globe which will promote networking, foster discussions on state-of-the-art in the field, and encourage new collaborations beyond the borders of Europe. RECROP members will also offer more **focused workshops** on specific topics that are relevant for the consortium. Three workshops per year will be planned in which PhD students and young postdocs will participate. One of the first workshops will also focus on **Intellectual Property Rights (IPR)** which will stimulate researchers from academia and public institutes to think in this direction.

RECROP will foster the **career development of Early Career Investigators (ECIs)** who will benefit from direct interactions with various research groups. ECIs will promote their work through workshops and conferences, share expertise via the training schools and support collaborations via STSMs which result in collaborative publications and will participate in the organization and writing of review articles on relevant topics. We will particularly encourage the participation of female ECIs and ECIs in general from NNCs, especially from regions that are heavily affected by global warming such as North African countries. We will also provide COST Animation - Inclusiveness Target Countries (ITC) Conference Grants to members to attend high profile international meetings and increase their visibility and expand their professional network. The active participation of early career researchers will strengthen their leadership skills and boost the experience of less-connected researchers.

3.2.2. PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

# DISSEMINATION

The results obtained by RECROP research coordination activities as well as the achievements from interactions with stakeholders will be disseminated through:

1. **Publications in peer reviewed journals** (original articles, reviews, opinions, perspectives in Open Access format). We will publish: (a) manuscripts on commonly accepted guidelines for experimental setup, data collection and analysis and results interpretation; (b) a manuscript with updated models on crop thermotolerance within the first 6 months of the Action and another one at the end of the Action with the contribution of all members, where developments during this period and further perspectives will be summarised; (c) original articles with novel results as outcomes from collaborations among RECROP members.

2. Articles in magazines and websites related to agriculture with information that is relevant for farmers and breeders and science magazines with broader readership: (a) effects of global warming on crop yields and projections for the future, (b) technologies for monitoring yield, (c) genetic approaches for developing stress resilient crops. In the same articles the scope of RECROP will be described and possibilities for interactions with stakeholders will be provided.

3. **Annual international conference** with sessions covering all disciplines that are relevant for RECROP, which will bring together top scientists from all around the world.

4. **Workshops** focused on specific topics related to RECROP, in which expert members of the action will provide update on crop stress response and resilience of reproduction.

5. **MOOCs, Training schools** and **webinars** on stress application and phenotyping, various –omics methods, big-data analysis and modelling.

6. Disseminate activities via **Social Media** (Twitter, Facebook, Instagram), **Professional Platforms** (e.g. LinkedIn, ResearchGate) which will also be effective communication with people beyond RECROP

7. Present the activities of our consortium in **public events** like "Fascination of Plants Day" organized under the umbrella of the European Plant Science Organisation (EPSO), as well as in **local events** organised Europe-wide which promote access of the universities to public (e.g. Night of Science, Science Festivals, etc.).

8. Update on activities and achievements of RECROP on the website and through a **Newsletter** for registered users of our website.

#### **EXPLOITATION**

- 1. Annual round table discussions with private sector to share views on market oriented products
- 2. Provide lists with **novel genetic targets** for crop improvement for European breeders.
- 3. Development of **new sustainable agricultural practices** such as the use of biostimulants to maximize protection of crops against abiotic stresses.



- 4. Development of **customized innovative products** with technology companies for phenotyping stress resilience in reproductive tissues for laboratory and field trials.
- 5. RECROP will encourage Open Innovation approach for Intellectual Property (IP) management, but we will also support IPR in line with the principles set out in the *Rules of Participation in and Implementation of COST Activities* as defines in COST guidelines.

#### DIALOGUE

- 1. **Annual meetings with policy makers**, for round table discussions. After each meeting we will write interim papers and recommendations and will include a final conclusion at the end of the COST that will be included in the final report.
- 2. **Reports of achievements and activities** that are relevant for policy makers (available in the website of RECROP).
- 3. **Preparation of results on slides for customized presentations** for policy makers and publication through the website of RECROP.

# 4. IMPLEMENTATION

# 4.1. COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

# 4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

To address the three goals presented in section 1.1.2 and achieve the research and capacity-building objectives, RECROP is organized in **four interlinked Working Groups (WGs)**. WG1-3 are interconnected as information generated from one WG will be used by the others, e.g. we will use tools from WG1 to identify important genes for stress resilience in WG2 and then utilise them as targets for the generation of strategies for crop improvement in WG3. Therefore, members of RECROP will be encouraged to participate in more than one WG. WG4 is directly related to the outcomes of WG1-3 to build a platform for effective dissemination of information and transfer of knowledge among different groups by training and joined research activities.

#### WG1: Tools to decode stress response and tolerance of crop reproduction

A flower is an anatomically complex organ containing tissues of different identity which undergo distinct developmental programs but which have to be synchronized to ensure successful gamete formation and fertilization. Stress conditions have diverse effects on different tissues, but also the sensitivity for a specific tissue or cell type can vary among the different developmental stages.

One of the major challenges in the field of stress resilience in reproductive tissues is the dissection of the phenotype on the tissue and cellular level. This information is important for the characterization of the basis of the sensitivity and the selection of specific targets (e.g. cell types or tissues) for improvement. Due to the easier access to pollen, stress related studies have mainly focused on the resilience of the male gametophyte, often ignoring the female counterpart or even the vegetative tissues of the flower organ. As a consequence, a detailed picture of the effects of abiotic stresses both on the response and the tolerance of the different flower tissues is absent.

WG1 will lead the efforts to unify, optimize and standardize protocols used for studying stress resilience and response in reproductive tissues in various model and crop plants, propose novel methods for phenotyping and create tools for –omics data acquisition and analysis.

**TASK 1.1: Determine the critical information required to decipher stress response and resilience in reproductive tissues.** State-of-the-art –omics and phenotypic techniques need to be utilised in order to provide an in-depth description of the response of different male and female reproductive tissues to different and combined stress scenarios. Only a basic set of such techniques has been used so far and therefore the picture of stress response is far from being complete. Experts from different fields of stress biology and different –omics strategies (including GWAS - genome-wide association studies) will describe the missing levels of information and the steps that need to be taken to establish a comprehensive description of the stress response. In addition, members of WG1 will define the limitations in the current approaches for stress resilience determination in plant reproduction and will propose novel techniques to address resilience under laboratory and field conditions, both for reproductive success and yield.

TASK 1.2: Optimize and standardize methods for monitoring stress response and resilience in reproductive tissues in major crops. Currently different laboratories use different methods to define stress tolerance in reproductive tissues. Members of WG1 will define a commonly accepted set of methods that can be used to verify stress resilience in reproductive tissues and yield across different levels of experimental scales, including laboratory and field trials. These methods will ensure



reproducibility, and minimize man made error in data collection and analysis. Pitfalls and limitations of these techniques will be identified and measures for their improvement will be determined. For each crop, stress resilience thresholds will be defined under different conditions, which will allow a direct comparison of results across different laboratories. By this, we aim to create a common experimental "language" that will boost cooperation, cross-validation, and progress in the field. The improved protocols will be benchmarked by different groups and data will be submitted for evaluation using a RECROP platform based on a blind analysis approach. By this RECROP will develop pipelines for stress application, data collection and analysis and result interpretation. The pipelines and the results from the benchmarking will be reported in the meetings and conference organized by RECROP, and will be published as a peer reviewed open access method manuscripts.

TASK 1.3: Advanced imaging techniques to dissect the effects of abiotic stresses on reproductive tissues at the cellular level. Improving resilience either by genetic approaches or agricultural means requires a prior knowledge of the basis of sensitivity. Therefore, it is of utmost importance to define on the tissue and cell-type levels the effects of different stress combinations, as sensitive cells can be targeted for genetic improvement. RECROP will provide a framework of various bioimaging techniques for the 3D analysis of stressed floral meristems. RECROP will include photon-based tomography techniques such as confocal, light sheet and super-resolution microscopy, X-ray microscopy (XRM) and Magnetic resonance imaging (MRI) to create 3D images of stressed flowers to capture defects on reproductive tissues based on deep learning approaches that will provide robust automated algorithms to develop a 3D Digital Tissue Atlas for different crops exposed to different stresses. Such analysis will allow us to decipher the most sensitive cells and tissues during reproductive development under different stress conditions.

TASK 1.4: Create a common platform for integration and analysis of –omics datasets from abiotic stress studies. WG1 will coordinate the integration of existing and upcoming –omics datasets in a single platform accessible through the RECROP website. The platform will operate under a user-friendly interface for biologists and non-experts and will provide comparative analysis of RNAs, proteins, and metabolites between different conditions, tissues and species as well as multilevel analysis of specific pathways. New data generated during the RECROP will be integrated into the platform to increase statistical power. WG1 members will create a commonly accepted pipeline for data acquisition, quality check, integration, and analysis. The platform will allow the easy mining of the results which will be visualised as ready to use images for publications or presentations. The platform will be presented in COST meetings, conferences and a publication, to encourage more researchers to deposit relevant data. Students and COST-members will be trained on the use of the platform through workshops and webinars. We envision that the platform will operate beyond the funding period of RECROP.

**Milestones WG1:** M1.1 (Y1M12) List with methods and layers of information required to characterize crop stress resilience; M1.2 (Y2M12) Agree on commonly accepted and optimized protocols; M1.3 (Y3M3) Publication of method article on bioimaging and 3D modelling of floral meristem tissues; M1.4 (Y3M3) Online and fully operating platform for integration of –omics datasets from different crops; M1.5 (Y4M2) benchmarking and open access publication of the platform on a scientific journal

# WG2: Description of the effects of abiotic stresses on reproductive tissues and their relevance for resilience and yield

The improvement of stress resilience and yield requires a detailed understanding of the effects of stress on plant reproduction. To achieve this, four important questions need to be discussed:

- 1. How do different environmental scenarios affect sexual reproduction and yield in different crops?
- 2. Which cellular processes in addition to meiosis are the most vulnerable to stress during sexual reproduction?
- 3. How do individual tissues and cell types respond to different stress conditions at the molecular level?
- 4. What are the critical defence mechanisms for survival and recovery from stress?
- These four questions will be addressed by three specific tasks:

**TASK 2.1: Determine the effects of abiotic stresses on crop reproduction.** Typically, in nature abiotic stresses coincide, e.g. heat and drought. Up until now, the focus has been placed on deciphering the effects of individual stresses on plant performance, mainly in laboratory experiments. The goal to improve stress resilience of reproductive tissues and thereby yield, requires the fundamental description of the effects of different stress combinations on the physio-morphological phenotypic traits of reproductive tissues. More specifically, we will **generate a phenotypic inventory** including quantitative data and image compilation as a textbook for the impact of various abiotic stress scenarios on reproductive tissues, e.g. pollen viability and germination, morphological alterations in female and male



organs, for different crop species. This will allow the identification of the most stress sensitive traits and the determination of the key targets for improvement of stress resilience.

**TASK 2.2: Describe the response of reproductive tissues under stress combinations.** The survival of a plant exposed to a stress incident depends on the activation of defence mechanisms that are generally called stress responses. Stress responses induce changes in all levels of regulation of gene expression which in turn ensure the synthesis of molecules (e.g. proteins, metabolites) with protective functions for macromolecules and cellular structures and which stimulate physiological reactions that minimize stress costs. Members of WG2 will compile information on the effects of different stress scenarios on the response of reproductive tissues, based on existing data derived either from – omics studies or studies on specific pathways (e.g. transcription factors, metabolic pathways, etc.).

**TASK 2.3: Determine the relationship of reproductive and vegetative stress resilience** While an abiotic stress incident can have a direct effect on plant reproduction by affecting male and female developmental processes, stress tolerance is also dependent on the physiological status of vegetative organs. Traits that contribute to the regulation of water uptake such as root angle, primary root length, lateral root formation and root diameter can affect drought tolerance which can be exhibited in reproductive fitness, while physio-morphological adaptations of leaves and regulation of physiological processes such as transpiration and photosynthetic activity can also affect reproductive fitness under stress conditions. Members of WG2 will determine the relation of traits of vegetative tissues to the abiotic stress resilience of reproduction of crops.

**TASK 2.4: Identify key elements of stress resilience and their contribution to yield.** WG2 will identify metabolic pathways, cellular and biological processes that are critical for stress response and tolerance and pinpoint to specific molecules across different scales (from metabolites and hormones to DNA, RNA and proteins) that play pivotal role in stress resilience during plant reproduction and yield. WG2 members will write a review in an Open Access journal regarding the molecular responses of reproductive tissues of crops on different abiotic stress combinations. The contribution of these elements for the resilience to different stress scenarios will be examined using existing or newly generated lines where their biosynthesis will be either enhanced or repressed, for example by ectopic expression of a gene or knockout mutation via CRISPR. Research groups along with private enterprises will collaborate to challenge the importance of these elements through joint experiments. We recognize that while some genes or metabolites have a universal positive impact on stress resilience across different crops, species-specific adaptation strategies require more specialized responses as well. Therefore, RECROP subgroups will join to define crop-specific responses and disseminate the results in more focused publications. A list of genes and molecules as potential primary targets for the improvement of the resilience of reproductive tissues of crops will be will be will be published in the webpage of RECROP.

**Milestones WG2:** M2.1 (Y2M3) Online phenotypic repository with stress resilient traits; M2.2 (Y3M6) Definition of important molecular responses that are related to crop reproduction resilience based on – omics datasets; M2.3 (Y4M2) Determine the stress tolerance traits of vegetative tissues that are related to reproduction resilience; M2.4 (Y4M6) Validation of functional relevance of selected genes or regulatory pathways for reproductive resilience by reverse genetics approaches by different groups; M2.5 (Y4M7) Submission of manuscript(s) with a list of genes/molecules as primary targets for crop improvement.

# WG3: Improvement of crop yield under suboptimal environmental conditions using genetic approaches.

The ultimate goal of RECROP is to propose and implement different strategies for the improvement of crops resilience under different stress scenarios. For this purpose, experts from different fields will propose the most up do date methods for crop improvement via (I) Breeding, (II) Gene editing techniques such as CRISPR and (III) transgenic lines. We foresee that at least some of the proposed strategies can be implemented by members of RECROP. In this task, plant breeding companies will be encouraged to be involved in the strategic planning as well as in the generation of new lines through collaborations with research groups from institutes. Upon publication, the lines will be deposited in the online repository. We will encourage the simultaneous testing of new lines by multiple partners, for cross-validation of the stress resilience, using the methods described in WG1-2. We will further encourage the evaluation of the performance of these lines in combination with chemical treatments described in WG2. Currently, several research groups involved in RECROP have in hand lines from breeding populations, mutants and transgenic plants from different crops that show enhanced tolerance to abiotic stresses. RECROP members will encourage the sharing of this valuable genetic material in the frame of bi- or multilateral collaborations for further improvement. To stimulate material exchange and progress, RECROP will create a platform where a description of the genetic background and the



traits for each line will be displayed. We believe that this step will accelerate crop improvement efforts for high yield under suboptimal conditions.

**TASK 3.1: Create a repository with stress tolerant genotypes.** RECROP members are engaged to scientific exchange and research progress. A key aspect for RECROP community is that scientists bring in complementary skills and come from laboratories with a variety of expertise in different aspects that are relevant and essential to achieve the goals of RECROP. Several members of RECROP possess varieties and hybrids, mutants and transgenic lines from different crops that exhibit enhanced resilience to abiotic stresses. RECROP will encourage the sharing of this material, by creating an online repository, in which different lines will be presented along with their genetic background, description of important traits and contact information with the research group that own the material. By this, we will stimulate the phenotypic and molecular characterization of promising lines using the approaches described in WG1 and 2. We also envision that such lines can be used as starting material for pre-breeding but can be also be subjected to further improvement by genetic manipulation, e.g. to reduce trade-offs. We will encourage through bilateral agreements the use of this material by breeding and biotechnology companies and in collaboration with the research groups to exploit them as basis for crop improvement. The website will be integrated within the RECROP webpage.

TASK 3.2: Enhancing reproductive success under different stress scenarios using state-of-the art genomics-based breeding approaches The development of climate-resilient crops can be accelerated by the utilization of integrative genomics approaches. RECROP will take advantage of the existence of seed collection as well the rich genomic resources for major crops (e.g. wheat, rice, barley, maize, tomato) to mine stress resilience related traits with the use of ML. Members of the WG3 will design adaptive introgression of genes from crop wild relatives, that may have been lost during long period of conventional breeding, a source that is rich for adaptive traits, to elite cultivars. Furthermore, we will explore the possible use of available pangenomes to link traits related to reproductive resilience to abiotic stresses to single nucleotide polymorphisms (SNPs), mutations and genes harbouring structural variants (SVs). SVs although they represent complex variations that are difficult to detect and therefore largely unexplored compared to SNPs, they are considered as important genomics resources for developing new crops with desired adaptive traits. We will encourage the active involvement of experts in Deep Learning genomics mining, to create high confidence pipelines for the discovery of SVs.

TASK 3.3: Design and implement strategies to improve crop reproductive resilience by gene editing approaches. Gene editing is a powerful tool to study gene function and targeted mutations in specific genes have been proven to have stimulatory effects of stress resilience. Currently CRISPR-based mutagenesis is considered as a fast-forward molecular engineering technique for basic research as commercial use due to European legislation is still regulated. However, using CRISPR or other gene editing techniques such as TALENs can provide information on genetic variations and gene functions that can be valuable for the development of breeding strategies. Gene editing can accelerate de novo domestication of a wild crop relative. Therefore, based on information derived from WG2, RECROP will create a list of genes that their mutation can cause enhanced resilience of reproductive tissues to different stress scenarios. Several of these genes will be mutated in different crops by members of RECROP and their resilience will be evaluated by the methods presented in WG1.

**TASK 3.4: Using synthetic biology to introduce novel entities for trait improvement.** Stress resilience often is associated with trade-offs, as typically stress response mechanisms can interfere with growth and development, and it is frequently manifested as disturbances in reproductive development. This can be a major obstacle for the generation of stress resilient germplasm through breeding approaches or even gene editing. To overcome this problem, synthetic biology approaches can be implemented which allow the tight regulation of gene expression in time and space (e.g. specific cell types) and genes can be modified in order to produce proteins with customized activities. Members of WG2 and 3 will design strategies for synthetic genes that could target the improvement of specific traits. Several of these genes will be transformed in different crops to evaluate the validity of the proposed strategy.

**TASK 3.5: Identify exogenous treatments with chemicals that can stimulate the genetically inherited capacity of crops to tolerate stress** Exogenous chemical treatments with plant-based biostimulants can reduce the negative impacts of stresses in crops and have a positive impact in yield [9]. WG3 will establish guidelines for the exogenous application of such compounds, their benefits for sustainable agriculture and their suitability for impacting yield under different stress scenarios. Furthermore, WG3 will examine the effects of these treatments on plant reproductive tissues and whether they can reduce the negative impacts of stresses on specific traits. We envision that the genetically imprinted tolerance limits of even resilient varieties and hybrids can be further boosted when combined with such exogenous treatments, and therefore this can be a powerful tool for the farmers in



the future. This task will be carried out in cooperation with private enterprises and growers to ensure that the guidelines reflect the newest advances in agricultural practices and therefore can be exploited by farmers. WG3 members will test these treatments in different crops and under different scenarios using the commonly established protocols (WG1, Task 1.2) and the results will be presented in MC meetings, conferences, meetings with various stakeholders including private companies and farmer associations.

**Milestones WG3:** M3.1 (every year month 1) Determine meetings and topics to discuss for WG3 members; M3.2 (Y2M9) Platform with genetic repository online; M3.3 (Y3M9) Define breeding strategies for crop improvement; M3.4 (Y3M9) Determine specific strategies to enhance reproductive resilience through gene editing approaches; M3.5 (Y3M9) Define synthetic biology approaches to improve stress resilience of different crops; M3.6 (Y3M9) Define specific treatments and agricultural practices to improve stress resilience.

# WG4: Dissemination, Training and Stakeholder Engagement

TASK 4.1: Coordinating the development of the Action Science Communication Plan;

TASK 4.2: Creation and implementation of the RECROP Website;

TASK 4.3: Coordinating the publication of review, opinion, method and original papers

TASK 4.4:Coordinate with the WGs the organisation of **Training Schools** 

TASK 4.5: Organize meetings with Policy makers

**Milestones WG4:** M4.1 (every year M1) Development and annual revision of the Science Communication Plan; M4.2 (Y1M6) Fully operating website; M4.3 (Y1M2) Fully operating communication media; M4.4 (Y1-Y3M12, Y4M9) Submission of review, opinion and method articles; M4.5 (every year M4) Schedule training schools and workshops;; M4.7 (every year M7) Schedule meetings with policy makers.

# 4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

Abbreviations: D: Deliverable; Y: year, M: Month

#### WG1 Deliverables:

- D1.1 (Y1M12): Publication with state-of-the-art techniques for the description of stress response in reproductive tissues
- D1.2 (Y2M12): Collection of standardized protocols for analysis of stress resilience of reproductive tissues
- D1.3 (Y3M3): Operating online platform for –omics analysis via RECROP website
- D1.4 (Y3M6): Open access peer-reviewed publication about the multi–omics platform
- D1.5 (Y4M12): Publication on new technologies for deciphering stress response and resilience in reproductive tissues of crops

# WG2 Deliverables:

- D2.1 (Y2M3): Publish an inventory with stress sensitive phenotypes related to reproduction and yield for different crops via RECROP website
- D2.2 (Y3M6): Publication with a comprehensive description about the molecular changes occurring in reproductive tissues
- D2.3 (Y3M9) Publish a list of primary targets for the improvement of the resilience of reproductive tissues for different crops and stress scenarios
- D2.4 (Y4M2): Publication on phenotypic traits in vegetative tissues that are related with reproductive resilience

# WG3 Deliverables:

- D3.1 (Y2M7): Fully accessible and operating germplasm repository via RECROP website
- D3.2 (Y3M6): Report with a priority list of genes as targets for genetic improvement
- D3.3 (Y4M9): Publication on mutant and transgenic crop plants with enhanced resilience in reproductive success under stress conditions
- D3.4 (Y4M9): Publication of guidelines for boosting stress resilience increase by treatment with chemicals with biostimulatory activity

# WG4 Deliverables:

• D4.1 (Y1M12): Online fully operating RECROP website



- D4.2 (every year, M12): At least 4 open access joint review articles in scientific journals on topics related to crop stress resilience (at pert year)
- D4.3 (Y2M2, Y3M2, Y4M2): Method papers on the pipelines, platforms and protocols generated by RECROP
- D4.4 (every year, M12): Annual white paper/reports on discussions with policy makers
- D4.5 (Y1M3): Operating communication platforms (e.g. social and professional media)

# 4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

Potential risk	Mitigation strategy
Low participation of members from inclusive countries	Reach out for members from inclusiveness countries through their national research organizations, societies, etc
	Communicate directly the scope and activities of the Action to private enterprises, discuss and find solutions for grounds of hesitance
Low engagement of ECIs	Make events specifically targeted to ECIs, involve them more to planning and decision making
Difficulty to agree on common protocols	Include different approaches but highlight advantages and disadvantages
Limited interest for STSM	Engage direct communications of senior with young researchers such as PhD students or ECIs to promote ideas for collaboration
No physical attendance to meetings due to public restrictions (e.g. pandemic) or political situation	Make hybrid seminars, meetings and conferences
No feasible generation of stress resilient plants within the time frame of the Action	Identify bottlenecks and agree on alternative future strategies

# 4.1.4. GANTT DIAGRAM

		Year 1				Year 2	?			Year 3	1			Year 4			
WG	Deliverables and Main Milestones	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WG1	D1.1 Publication with techniques																
	D1.2 Collection of standardized protocols																
	D1.3 Operating online platform for –omics																
	D1.4 Open access peer-reviewed publication																
	D1.5 Publication on new technologies																
	Main Milestones WG1				M1.1	M2.2	M1.3				M1.4			M1.5			
	D2.1 Publish an inventory with stress sensitive phenotypes																
	D2.2 Publication on molecular changes																
WG2	D2.3 Publish a list of primary targets																
	D2.4 Publication with list of phenotypic traits in vegetative tissues																
	Main Milestones WG2					M2.1	M2.3				M2.2, M2.3				M2.4	M2.5	
	D3.1 Fully accessible and operating germplasm repository																
	D3.2 Priority list of genes as targets																
WG3	D3.3 Publication of mutant/transgenic plants with enhanced resilience	2															
	D3.4 Publication on biostimulants																
	Main Milestones WG3	M3.1				M3.1				M3.1		M3.2- M3.6		M3.1			
	D4.1 Online fully operating INCRREASE website and data platforms																
	D4.2 Review articles																
	D4.3 Method papers																
WG4	D4.4 White paper/reports on discussions with policy makers																
	D4.5 Communication platforms																
	Main Milestones WG4		M4.2, M4.5, M4.6	M4.7	M4.4	M4.1, M4.5, M4.6	M4.6	M4.7	M4.4	M4.1, M4.5, M4.6	M4.6M 4.7		M4.4	M4.1, M4.5, M4.6	M4.6	M4.4, M4.7	



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