

REVIEW

INCREASE – INTELLIGENT COLLECTIONS OF FOOD LEGUMES GENETIC RESOURCES FOR EUROPEAN AGROFOOD SYSTEMS - PROJECT OVERVIEW

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INTRODUCTION

Leguminosae family comprises 800 genera and 20,000 species (Lewis, 2005) being considered the third largest family of flowering plants. The family includes species considered weeds and major grain crops known as pulses or grain legumes, being featured by a range of valuable traits for human and environment health. The enormous importance of plant foods is now recognized all over the world, especially in the EU, where the impact of plant foods on human health and the increased appreciation (European Commission Communication 2010). There is also definitely growing interest in higher process quality in the agri-food sector, particularly reduced negative environmental impacts (Murphy-Bokern and Kleeman, 2015). Moreover, since the COVID 19 became a pandemic, consumers started buying much more convenient and healthy products, such as beans, chickpea, lentil, rice and pasta, trying to stay healthy. While restaurants and bars have closed in most countries in Europe, the demand for grain products and pulses is doing well in retail. The moisture contents of all the dry legumes are in the range of 9-13% making them favorable for long storage. In this context, food legumes and legume-inclusive production systems can play important roles by delivering multiple services in line with sustainability principles (Stagnari et al., 2017). There is a great interest at EU level and significant investment in research aimed to breed new varieties and to develop new technologies with benefits at different levels and industries. New value chains, such as those exploiting biodiversity for food uses and clean environment, may synergize with each other. In this context, grain legumes make a major share of the human diet and play a significant role in the human nutrition, especially as source of protein, vitamins, minerals, dietary fiber, and folic acid (Singh, 2012). Globally, grain legumes are the most relevant source of plant protein, especially in many countries of Africa and Latin America, but there are some constraints in their production, such as a poor

adaptation, pest and diseases and unstable yield. (M. De Ron, 2015). EU developed a new protein plan and its implementation will be largely based on traditional and innovative uses of food legumes. Entire plan reflects the strong interest of the agrofood sector for development of products and technologies to meet consumer requests for healthful diets (<https://eur-lex.europa.eu>), in the context of increased population and changing climate conditions. Globally, diets have changed considerably, for many countries studied, more calories are being consumed per person, and the proportion of fat and animal protein consumed has increased significantly with wealth (Williams, 2020). Grain legumes are featured by superior quantity of protein comparing with other plant foods and have twice the dietary protein content of cereal grains (Kouris-Blazos and Belski, 2016), having strongly perspective to be exploited against malnutrition and generally in food sector. Recent investigations suggest that grain legumes may contribute to human health and wellbeing, mostly through prevention of chronic diseases such as coronary heart disease, hypertension, cancer, diabetes, and obesity. Consumption of legumes reduces the risk of cardiovascular disease, some cancers and helps to manage body weight due to its satiety value (Kamboj, 2017). Few EU projects funded for this aim are: LEGATO, EUROLEGUME, LEGVALUE, PROTEIN2FOOD, BRESOV, ECOBREED. INCREASE is a new H2020 launched in May 2020 and has a complex character being designed to exploit the grain legume diversity in order to achieve benefits in agro food chain and to develop a comprehensive strategy for GenRes conservation for valuable utilization and long-term impact. Human plant protein intake is on the rise in many EU regions (EC Report COM (2018) 757 final). This implies that the main crucial aspect leading to added value to European primary production will be improvement of nutritional and quality traits.

The work presents the objectives and tasks of INCREASE project funded by EU in frame of

Horizon 2020 research and innovation program under Grant Agreement version final - 15/09/2020. SCDL Bacau acts as partner and develops researches related intercropping, phenotypic characterization, seed production aimed to increase the seeds yield and seeds quality (figure 1).

- Despite some approved beneficial aspects of intercropping previously reported by different authors, such as better pest control (Lopes, 2016), pollution mitigation (Luo, 2016), competitive yields with reduced inputs (Monti 2016, Tosti 2010), more stable aggregate food or forage yields per unit area (Smith, 2013), there are a number of constraints that make intercropping not common in modern agriculture, such as example the request of a single and standardized product and the suitability for mechanization or use of other inputs as a prerogative in intensive farming system (Brooker, 2015). It is therefore necessary to optimize intercropping systems to enhance resource-use efficiency and crop yield simultaneously (Li, 2014) while also promoting multiple ecosystem services (Stagnari, 2017).

- A common phenotyping kit will be used by partners in different locations; The protocol is designed according to different priorities based on commonly accepted descriptors.

- Related seed increase it is well known the fact that grain legumes are not quite common in high-input cropping systems, due to their low and unstable yields (Cernay, 2015) (Reckling, 2015). Not proper designed policy support (Bues, 2013); lack of systematised data on long-term benefits of legumes within cropping systems (Preissel 2015).

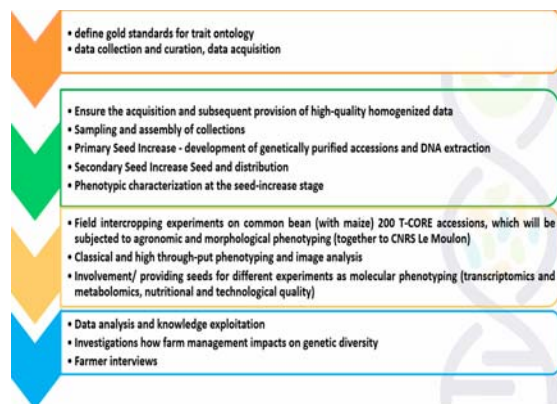


Figure 1 SCDL Implication in INCREASE project

Twentyeight international partners from fourteen different countries launched this year the new EU research project INCREASE - looking into the status of plant genetic resources of four important traditional European food legumes (chickpea, common bean, lentil and lupin), the consortium aims to develop efficient and effective conservation tools and methods to foster agricultural biodiversity in Europe, for a period of five years.

INCREASE will enhance the management and use of Genetic Resources on food legumes, which are crucial for sustainability, food security and human health, as recognized by the new European Protein plan for the innovation of this sector in Europe. To meet this challenge INCREASE will expand the utilisation of food legumes Genetic Resources targeting users' needs in terms of accessibility, quality, and quantity of information available. INCREASE, working with four important food legumes (chickpea, common bean, lentil, lupin) with significant value for the innovation of EU agriculture and food industry, will be based on four pillars: i) innovative data management solutions to develop gold standards for data sharing and integration into the central infrastructure, with decentralised data input, defined methodologies and best practices for exploitation of the novel information produced as well as the development of user friendly visualization tools; ii) developing novel tools and principles for germplasm management, based on the development of "Intelligent Collections" as a set of nested core collections of different sizes representing the entire diversity of each crop; iii) adoption of cutting-edge technologies for genotyping and phenotyping combined with the potential of Artificial Intelligence focusing on traits of interest for users; iv) international effort with the involvement of non-European partners and international organization to expand the scope and ambition of INCREASE, by facilitating the integration of available data and available Genetic Resources for European users. We will develop a citizen-science experiment, primarily aimed at dissemination of the project to stakeholders and citizens. Overall, INCREASE will strengthen Europe in the field of legumes Genetic Resources and simultaneously it will represent an important model and tool for all crop Genetic Resource.

MATERIAL AND METHODS

Biological material - INCREASE partners have a wide collection of chickpea, common bean, lentil and lupin, and two type of accessions will be available: heterogeneous accessions, which refer to accessions stored in genebanks (collected as populations and not as individuals, meaning the genotype and phenotype within an accession can be vastly different from plant to plant) and Single Seed Descent (SSD) lines (seeds derived from a single plant and maintained as pure lines by selfing cycles). All material is described, including biological status, georeferentiation, countries of origin, phenotypic and genotypic data, in case are available. Heterogeneous accessions: most of them have phenotypic information (especially few classical phenotypic traits, such as seed color, size and weight) and a near complete lack of genotypic data. SSD lines were

collected for chickpea, common bean, lentil, and lupin.

Data storage, management and sharing - the project is characterized by a wide variety of data from different domains, different phenotypic data and related ontologies and controlled vocabularies, genotypic data, and passport data. This challenge will be addressed by a powerful data management combining a central data management system (LIMS-based) and integration with established domain-specific repositories. Using repositories such as e!DAL (Arend et al. 2014) will meet the requirements for data management in accordance with the FAIR principles (i.e., Findable, Accessible, Interoperable, Re-usable). The unique identification of GenRes entities, which is important in distributed solutions, is considered by an assignment of DOIs integrated within the workflow. A decentralized data entry mechanism will enable the project partners to transfer the collected data in a more structured form. Dedicated curation will ensure the desired high quality of the data. At the same time, the central data management represents a hub for communication and support for the project partners. By providing interfaces based on existing standards such as MCPD, MIAPPE or BrAPI, connectivity to existing and future systems is ensured. Web-based user interfaces will be developed which will be available not only to expert users (e.g. breeders), but also to farmers and consumers involved in conservation and evaluation activities. For these reasons, INCREASE will be open to contributions of different stakeholders. Furthermore, the goal is to test a novel data production and sharing architecture also using a blockchain-based authentication model that allows users to truly identify and confirm each other. The final output will be a blockchain-based shared and secured database that is not controlled by a single institution, but by the network of the whole community.

Criteria of selection of Intelligent Collections INCREASE will develop Intelligent Collections the selection of lines to be part will include the following steps: (1) removing as much as possible redundancy of accessions: data (e.g. passport information will be used to remove putative duplicated accessions). (2) selection of different collection as reference core, hipcore aimed to develop a world-wide sample of wild forms, landraces, as well as cultivars and improved varieties of different market classes and origins, including landraces cultivated on Europe, Asia, Africa, Oceania, America, commercial varieties provided by seed companies to have availability of enough seeds to be distributed to citizens.

Genomics and genotyping the most recent and advanced sequencing technologies will be applied to sub-samples of genotypes in order to develop pan-genomes of common bean and lentil and obtain new de novo Platinum genomes (i.e. a contiguous, haplotype-resolved representation of the entire

genome) for common bean, lentil, two for chickpea and two for lupin. Overall, INCREASE will sequence about 20-30 de-novo Platinum Genome of which four will be for lentil that has a much larger genome size compared to the other legume species. Regarding genotyping, the SSD lines for all the Intelligent Collections will be subjected to different levels of genome sequencing,

Phenotyping - multilocation field trials (MLFT) and intercropping study: comprehensive agronomic and morphological phenotyping under field conditions using standard phenotyping to assess the adaptation and the agronomic performances of the genotypes of the four species along with the effect of GxE interaction. Field trials will be conducted for each species in different locations. Phenotyping for root traits, disease and symbiosis: a specific test will be conducted under controlled conditions for root traits. The same set of lines will be evaluated under control conditions for disease resistance to anthracnose, white mold, powdery mildew and ascochyta to combine association genetics and R gene enrichment sequencing. Ability to nodulate, efficiency of biological nitrogen fixation and competitiveness for nodulation will be assessed in controlled environmental conditions, and in the field. Drought study under controlled conditions: a proper understanding of the mechanisms that govern drought tolerance in plants is therefore of the utmost importance in the context of climate change mitigation. INCREASE will study drought tolerance in chickpea and common bean under controlled conditions. A pre-experiment will be conducted to test a suitable drought setup that will consider two treatments (control and drought). A high-throughput phenotyping system with automated imaging and watering of plants will be used. In these experiments several phenotypic and morphological traits will be scored (e.g., days to flower and maturity, growth habit, plant height, seed number and weight, flower color, etc.). In addition, leaf samples for metabolomics and transcriptomics analysis will be collected as described for field trials. Further, we will evaluate effects of water stress on plant and leaf photosynthesis by measuring photosynthesis parameters. Metabolomics and transcriptomics (molecular phenotyping): we will conduct a large scale and comprehensive metabolic profiling across several legume species and many genotypes across different environmental conditions on both leaf and seed tissues. The transcriptomic and metabolic analysis will provide essential information to be integrated to genomics to understand the molecular mechanisms associated to phenotypic responses and GxE of GenRes. In addition, combine genome sequence data will be combined with metabolomics and transcriptomics to highlight quantitative loci underlying metabolic and transcriptomic variation and perform network analyses. Identification of transcripts strongly correlated with the abundance of

given metabolites across tissues and genotypes will allow correlation of metabolite content with physiological data, to define the positively and negatively correlated phenotypic traits with signal metabolites. The metabolite data collected from different environments as biomarkers will be used to predict plant growth and behavior. Finally, transcriptome/metabolome data will be used to guide gene functional annotation and elucidation of species- and/or tissue-specific metabolic pathway structures. Nutritional and technological quality traits will be evaluated, since crop plants produce compounds that affect their market quality, taste and nutritional value, as well as numerous compounds that contribute to human health. Therefore, in addition to metabolomics and transcriptomic analysis, we will determine several quality traits, antioxidant, and healthy related compounds as well as anti-nutritional such as alkaloids in the seeds. Quality is an important issue to be considered to design better breeding programmes aimed at higher quality products and healthier plant foods. Within the objectives of INCREASE the aim is to elucidate the underlying cellular, biochemical and molecular mechanisms associated with nutritional quality aspects under different environments; the candidate genes will be identified in addition to rational strategies for the development and selection of genotypes with high nutritional properties.

RESULTS AND DISCUSSIONS

INCREASE thought its tasks will improve the sustainable use of plant genetic resources in food and agriculture by enhancing, in food legumes, the genotypic and phenotypic characterization of GenRes and by developing efficient and effective conservation tools and methods in order to promote agrobiodiversity and its use in Europe.

INCREASE will enhance the status of the GenRes of four important food legumes (chickpea, common bean, lentil, lupin), and promote their conservation and use. This will contribute to development of methodologies and tools that can be applied to, and should benefit, all kinds of GenRes.

This will be achieved by:

- Improvement of food-legume GenRes data management and sharing, by development of optimised databases, data-management solutions and web-based searching and data visualisation tools to promote with much more useful and easily accessible information the access of stakeholder to GenRes;
- Massive production and use of high quality genotypic and phenotypic data using cutting-edge methodologies.
- Development of Intelligent Collections and facilitate the access to users through a participatory strategy that will be used to design innovative conservation management approaches.

- Development of new knowledge (e.g. gene discovery, genomic prediction) that will be easily available for stakeholder by user's friendly web-based searching and visualization tool. This will promote and enhance the sustainable use of GenRes providing an efficient possibility to identify the appropriate source of germplasm with minor effort and to meet manifold requirements of users, stakeholders and European citizen, by targeting traits of interest for adaptation to European agroecosystems and for the agri-food and non-food industries, and by participatory approaches.

- Development, testing and dissemination of best practices for dynamic management of GenRes across European and non-European institutions and initiatives, involving data integration, sharing and traceability, and facilitating integration between ex-situ/static and on farm/dynamic conservation strategies.

- Develop decentralised information technology approaches for data sharing and germplasm conservation, using innovative approaches for distribution and co-production using blockchain approach, with strong potential for delivering groundbreaking results in GenRes conservation field. Activities will enhance the status of GenRes and increase effectiveness of conservation efforts, in Europe:

- by improving data management (improvement of EU genebanks standards for data management, development of a central data management infrastructure, and an expert portal,

- by connecting GenRes with precise and high quality genotypic and phenotypic information along with validated passport data,

- by developing GenRes exploration tools available for users and by applying innovative approaches for data sharing and collection

- Establishment of an open space for an efficient and effective conservation and use of food legume GenRes. Establish high quality, harmonised standards for the management and description of GenRes across Europe (and beyond): will test innovative solutions and develop best practices for dynamic management of food-legume GenRes across European and international organisations and initiatives that will be extendable towards all kinds of GenRes. A wide range of users and stakeholders will be involved: from breeders, experts, genebank curators, to farmers, agri-food and non-food industry and consumers. Increase the quantity and quality of data in established information systems for crop GenRes - massive, new and already available genotypic and phenotypic information will be generated, analysed and integrated, with development of improved databases and innovative visualisation tools.

- Integration of the available information and germplasm from different sources, and will develop novel phenotyping, genotyping, and annotation data.

Phenotyped and genotyped collections will be made available as data and as germplasm for user exploration and resources exploitation. Promote innovative ways of sharing resources and services between genebanks/ in-situ conservation sites in Europe and beyond: INCREASE will apply a centralised approach with decentralised inputs for data management and sharing resources, with the development of web-based user tools available not only for experts (e.g. breeders, scientists), but also for farmers and consumers involved in conservation and evaluation activities. A fully decentralised system for data integration across different genebanks will be also tested using a citizen science experiment. All our approaches can be extended to other crops and other European conservation initiatives (e.g. genebanks). Develop methods and tools for greater insight into the characteristics and value of collections - Intelligent Collections will be developed, starting from germplasm available from genebanks, and from on farm conservation activities and initiatives. Nested core collections of inbred lines will be generated and deeply genotyped and phenotyped. Phenotyping and gene discovery activities will meet the needs of various actors (e.g. breeders, scientists, farmers, agri-food and non-food industry, consumers) and will include not only 'conventional' traits (e.g. morpho-agronomic traits), but also adaptation to abiotic and biotic factors (pathogens and symbionts) and key features often ignored that are related to the value chain (e.g. food nutritional value, quality - that will also exploit the massive metabolomics analysis of the seeds), to agroecosystem characteristics (e.g. intercropping), and to artificial intelligence and smart tools (e.g. seed image recognition) Create novel services for users within and outside the conservation communities - a central database for data storage will be developed within INCREASE, along with decentralised data-import procedures. INCREASE will implement two data-management models: one for integration of material, traits, data generated (mainly based on expert data contributions), and one based on blockchain data integration and certification, mainly focused on user data collection and use. Participatory and open-science approaches will be implemented in INCREASE, and an open-science experiment will be carried out with stakeholders and citizens that will contribute in conservation, evaluation and distribution of food legume GenRes. INCREASE will develop and test, with the citizen science experiment, a fully decentralise blockchain based structure coupled with image analysis for sharing data and germplasm that will include SMTA generation and reporting functions and digital agreements along with the FAO ITPGRFA. This solution, designed for general public, will make easier sharing seeds in a legal framework and could be also adopted by expert users that often find the

actual SMTA procedures uneasy and time consuming.

CONCLUSIONS

Consortium carries out interdisciplinary activities to discover new valences to be exploited in new products, technologies with positive impact on the environment, economy, human health. The implementation is feasible and has as main straight the exploit and valorization of knowledge and previously developed varieties, methods, and technological links with the aim to develop a higher technological maturity level technology. Through this activity, Romania will have a valuable and enriched collection as part of European Collection, maintained physically in country and virtual accessed as genebank, system. Responsibilities for the long-term conservation and contribute to the sustainable use of these precious resources, the unique and important germplasm accessions in Europe. SCDL Bacau and other research institutes involved in germplasm management Suceava Gene Bank, can become part of a truly European system that is jointly operated and governed. Such an approach enables us, as country to make use of our strengths and to gradually eliminate the weaknesses, through capacity-building and specialization, related germplasm use. This is a true benefit to breeders, germplasm curators and policy-makers. The alignment to established standards guarantees the delivery of a high-quality product, together with the related information obtained directly from the most relevant source, conducted research.

ABSTRACT

The general objective is to enhance the management and use of Genetic Resources on food legumes, which are crucial for sustainability, food security and human health. The project will expand the utilisation of food legumes genetic resources targeting users' needs in terms of accessibility, quality and quantity of information available. INCREASE, working with four important food legumes (chickpea, common bean, lentil, lupin) with significant value for the innovation of EU agriculture and food industry, will be based on four pillars: (i) innovative data management solutions to develop gold standards for data sharing and integration into the central infrastructure; (ii) developing novel tools and principles for germplasm management, based on the development of "Intelligent Collections"; (iii) adoption of cutting-edge technologies for genotyping and phenotyping combined with the potential of Artificial Intelligence; (iv) integration of available data and available genetic resources for European users.

INCREASE will strengthen Europe in the field of legumes genetic resources and

simultaneously it will represent an important model and tool for all crop genetic resources

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