Introduction to EUCLEG

Bernadette Julier

EUCLEG project "Breeding forage and grain legumes to increase EU's and China's protein self-sufficiency" (www.eucleg.eu).

The application of genomic technologies in the breeding of legume species

Techical booklet based on the EUCLEG online workshop on held on the 30th September and 1st October 2021

Thank you to the organisers, contributors, and sponsors of this event

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This project EUCLEG was prepared after a call launch by the EU in 2016 entitled "a joint plant breeding programme to decrease the EUs and China's dependency on protein". The call included many important words requesting consideration of many topics such as forage and animal feed, productivity, climate change, diversification, stresses etc. and also called for collaboration with Chinese colleagues.

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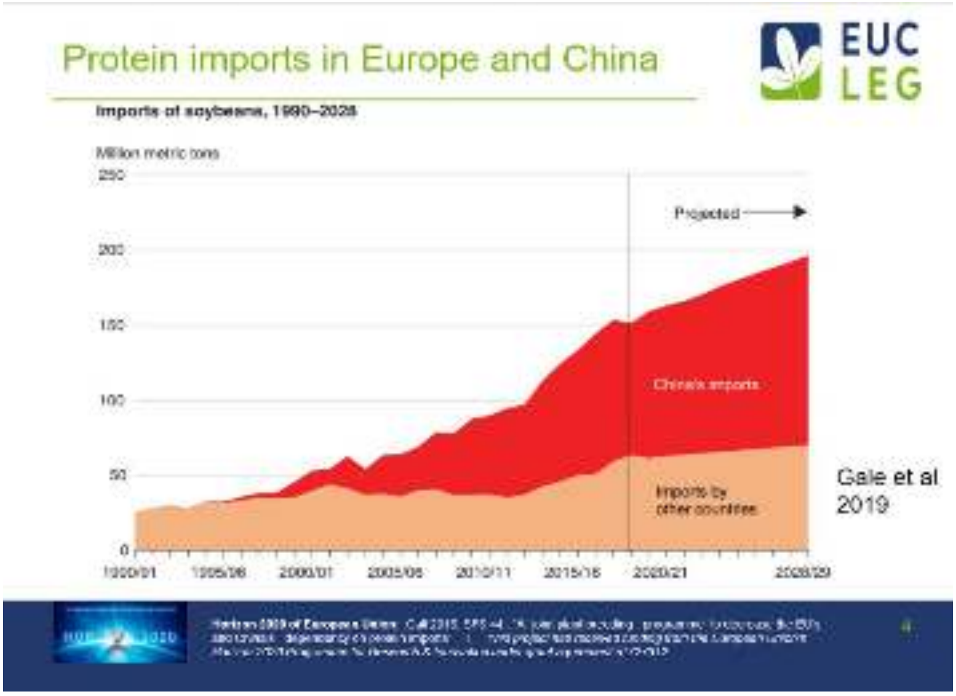
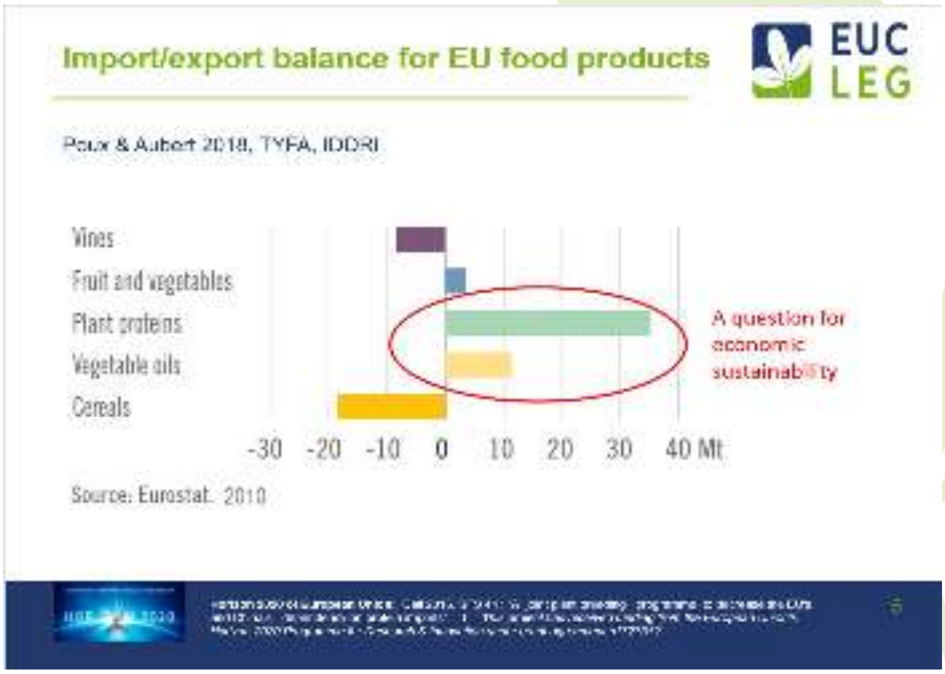


What is the problem with protein in Europe and China? Protein dependency in Europe is about 69% and China imports around 60% of the soybean world market trade. Most of the soybean is imported from the USA and Brazil, or South America. The dependency is quite different in Europe and China. In Europe the imports are more or less stable, but important and in China the imports began about 20 years ago to feed animals. and the trend is for a strong increase.



Seen from an economic point of view, proteins are part of the section of plants that are largely imported in the EU and it is an economic problem compared to other crops.

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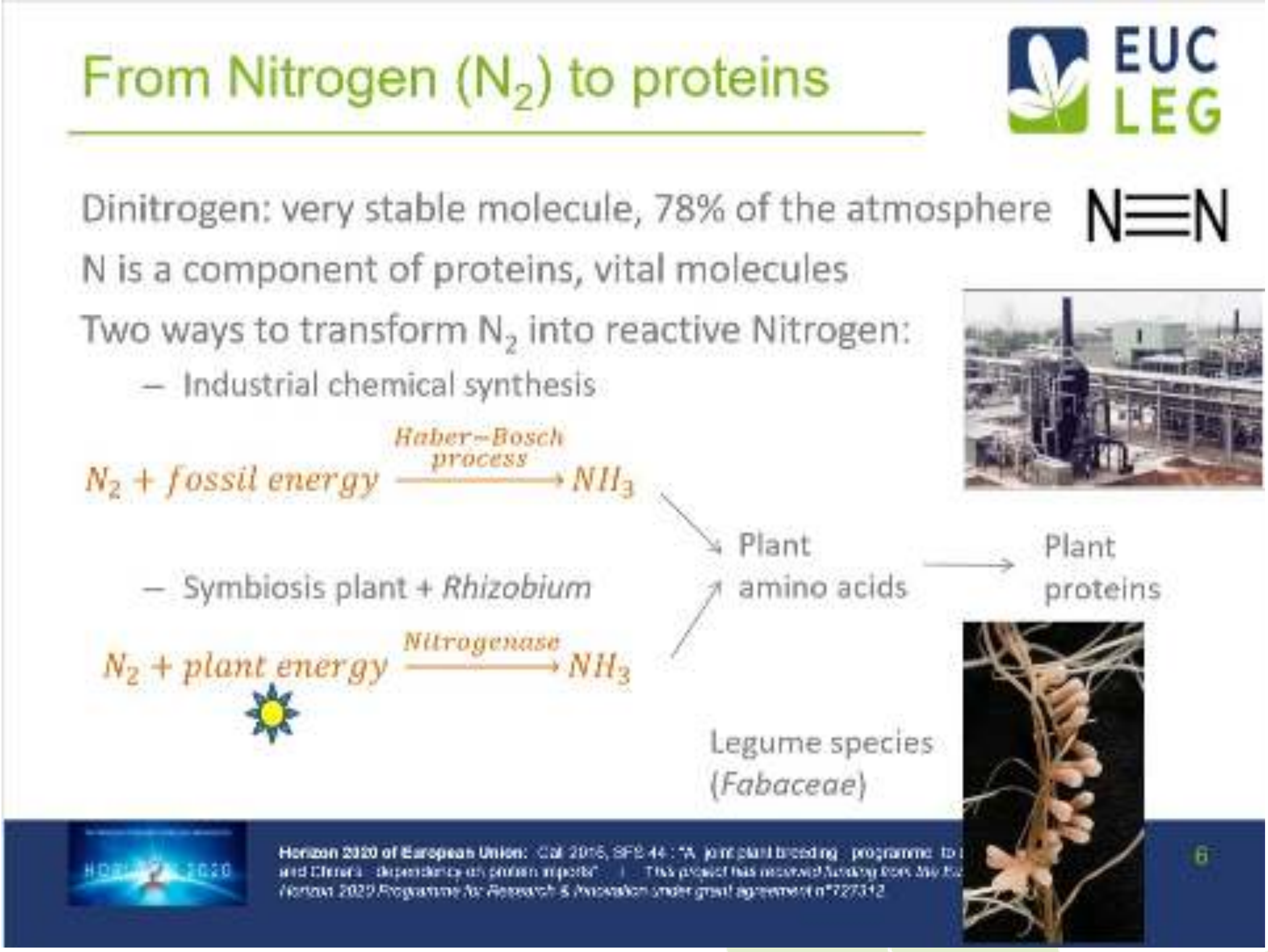

What about the proteins? In fact the question here is how to transfer atmospheric nitrogen gas (more exactly dinitrogen) into protein. Dinitrogen is a very stable molecule and makes up 68% of the atmosphere. It is a molecule composed of 2 atoms of nitrogen which bond with 3 very strong covalent bonds. Nitrogen is a component of proteins. There are two ways to transfer dinitrogen into reactive nitrogen, the first one is chemical synthesis carried out in industries, the process turns dinitrogen into ammonia by using large quantities of fossil gas energy. The second way is to use the symbiosis between legumes and specific soil bacteria called *Rhizobium* that are able to carry out the same reaction with the help of the nitrogenase enzyme. Plants are then able to assimilate and absorb the ammonia and transform it into amino acids and then plant proteins.

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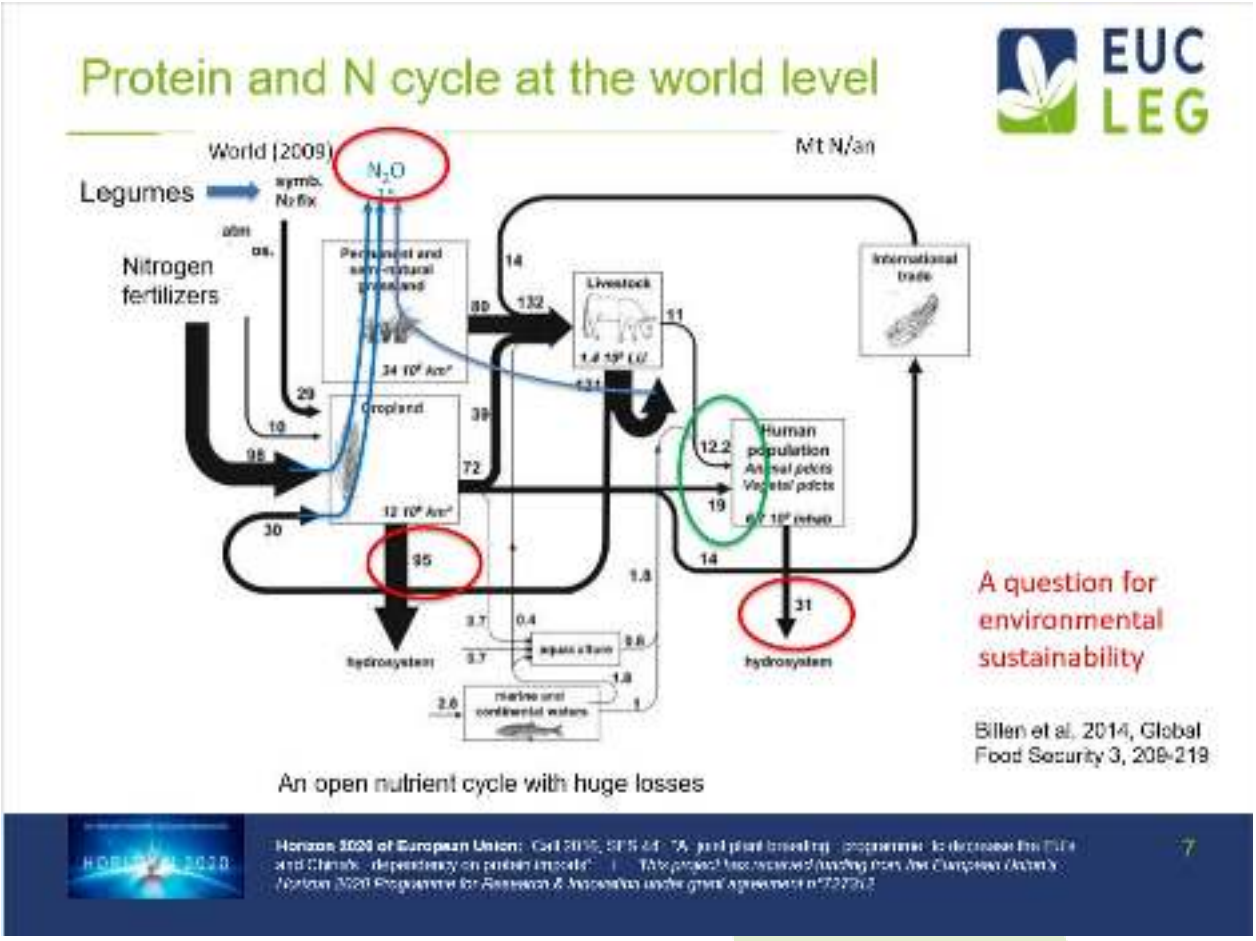

We also have a problem with the nitrogen cycle at a world level. Nitrogen enters into the plant system, mostly from nitrogen fertilisers that are applied on both crop and grasslands (72%). The second way that nitrogen enters the system is from nitrogen fixation with legumes, but this represents only 21%, and the third way is from acquisition from the atmosphere (7%). By using this nitrogen, the plant grows to produce grains, forages, fruits or vegetables but there is important leaching towards the hydrosystem. The plant products are eaten either by livestock or the human population and again there is some waste from livestock or human effluents. As a whole, the nitrogen cycle is completely open with huge losses that cause pollution and question environmental sustainability of agricultural system. We also see here the part that the entrance of nitrogen into the system of crop production plays and the small part of nitrogen currently coming from plant based nitrogen fixation.

As a consequence, the current situation indicates that we need to increase atmospheric nitrogen fixation by expanding legume cropping and produce more plant proteins that originate from nitrogen fixation.

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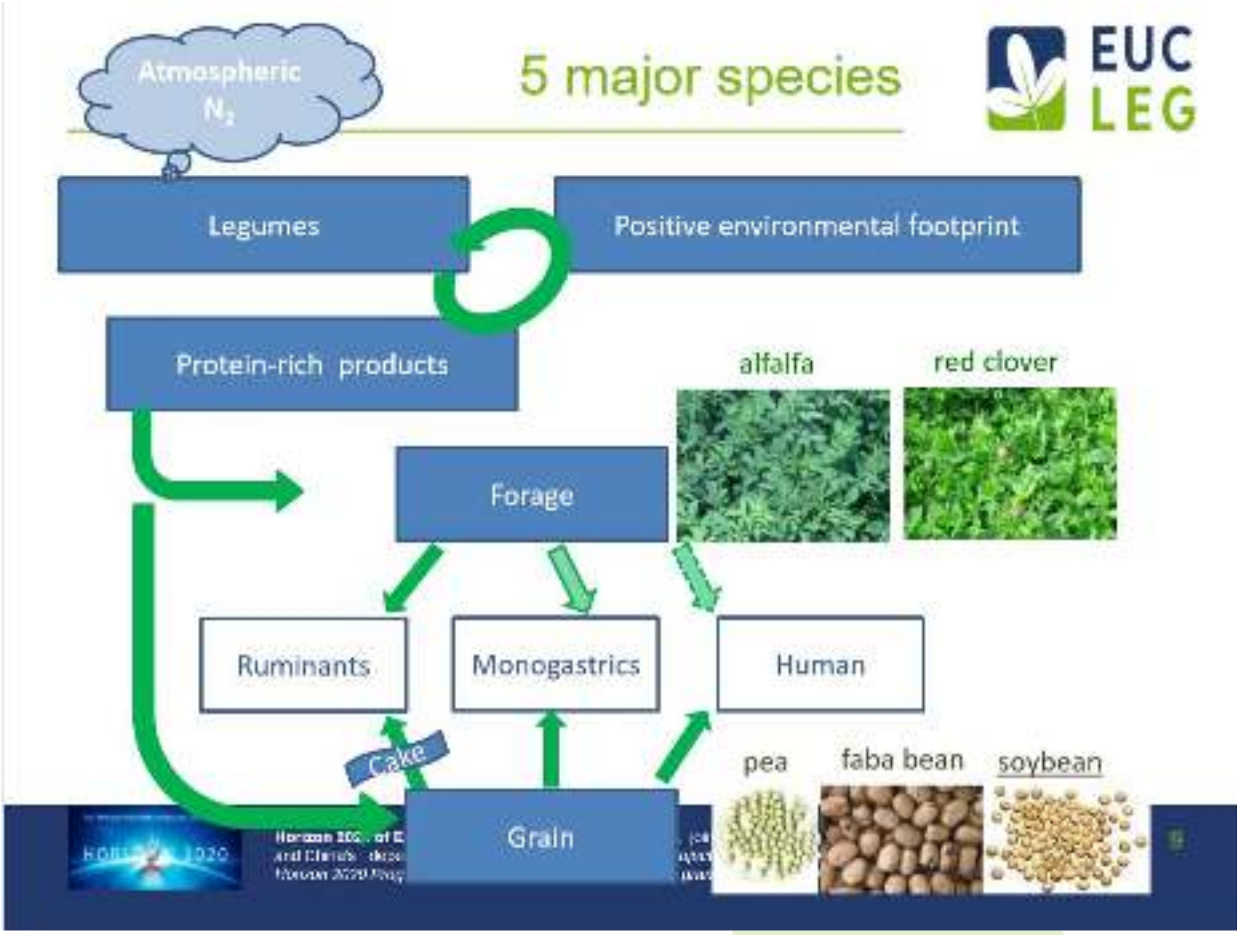

In EUCLEG, we have focused on the five major agricultural legume species in Europe. These species are able to produce protein rich products, either forage or grain, whilst providing a positive environmental footprint. We have studied alfalfa (or lucerne) and red clover as forage crops, pea, faba bean and soybean as grain crops. Our Chinese colleagues have worked on alfalfa and soybean, these two species are the most important legume crops in China.

Forages are used to feed ruminants mostly, monogastrics marginally and very rarely humans. Grain legumes are used to feed monogastrics and human and partly ruminants, mostly as soybean meal.

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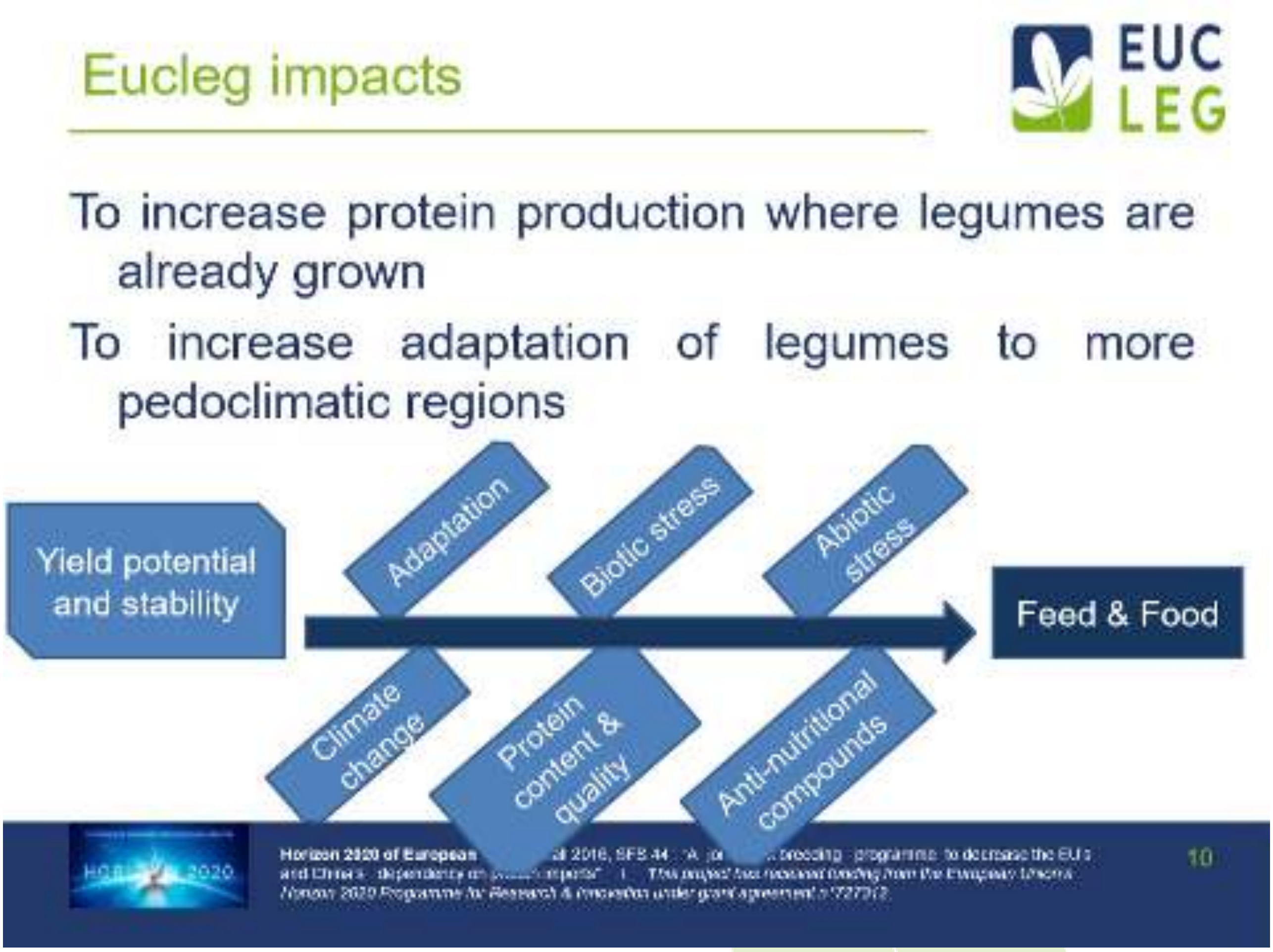

The expected impact of EUCLEG is to increase protein production in the regions where legumes are already grown, but also to increase the adaptation of legumes to regions where we are not currently able to grow legumes at a competitive level. At the end, the production of feed and food must be achieved with an improved yield and yield stability. This requires many improvements related to general adaptation, resistance to biotic and abiotic stresses, adaptation to climate change, also quality traits that, depending on the species, refer to protein content and protein composition, forage quality and anti-nutritional components.

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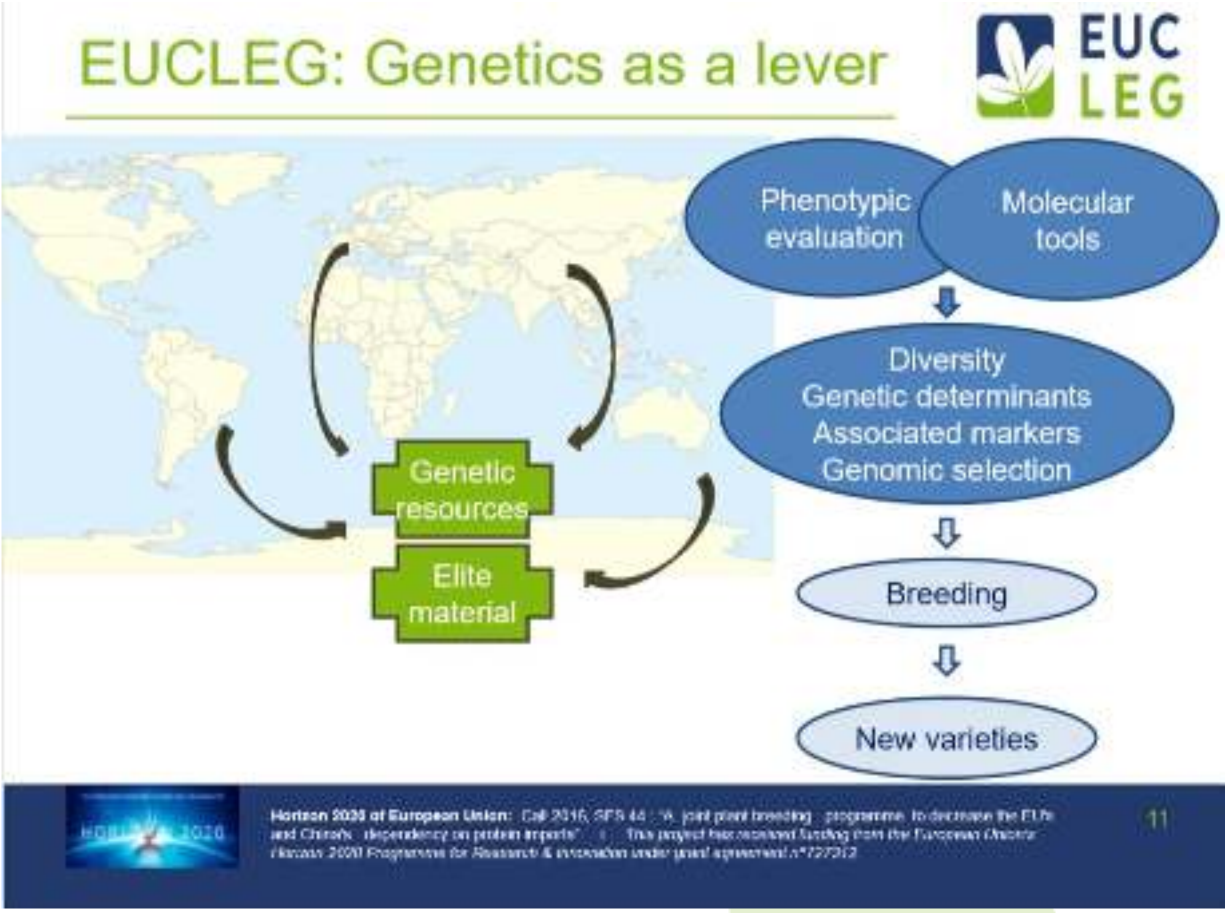

EUCLEG uses genetics as a lever to achieve these goals, it is based on the use of genetic resources and also elite material coming from worldwide sources, with an emphasis on material from Europe and China. A large phenotypic evaluation was carried out with a long list of traits established by the species experts. We have also developed molecular tools, with which the accessions of the five species were genotyped. We have studied genetic diversity, genetic determinants of traits and looked for markers associated with trait variation i.e quantitative trait loci (QTL). We have also worked on the potential of genomic selection to improve breeding programme efficiency. The outputs and impacts will be for breeding of new varieties in the future.

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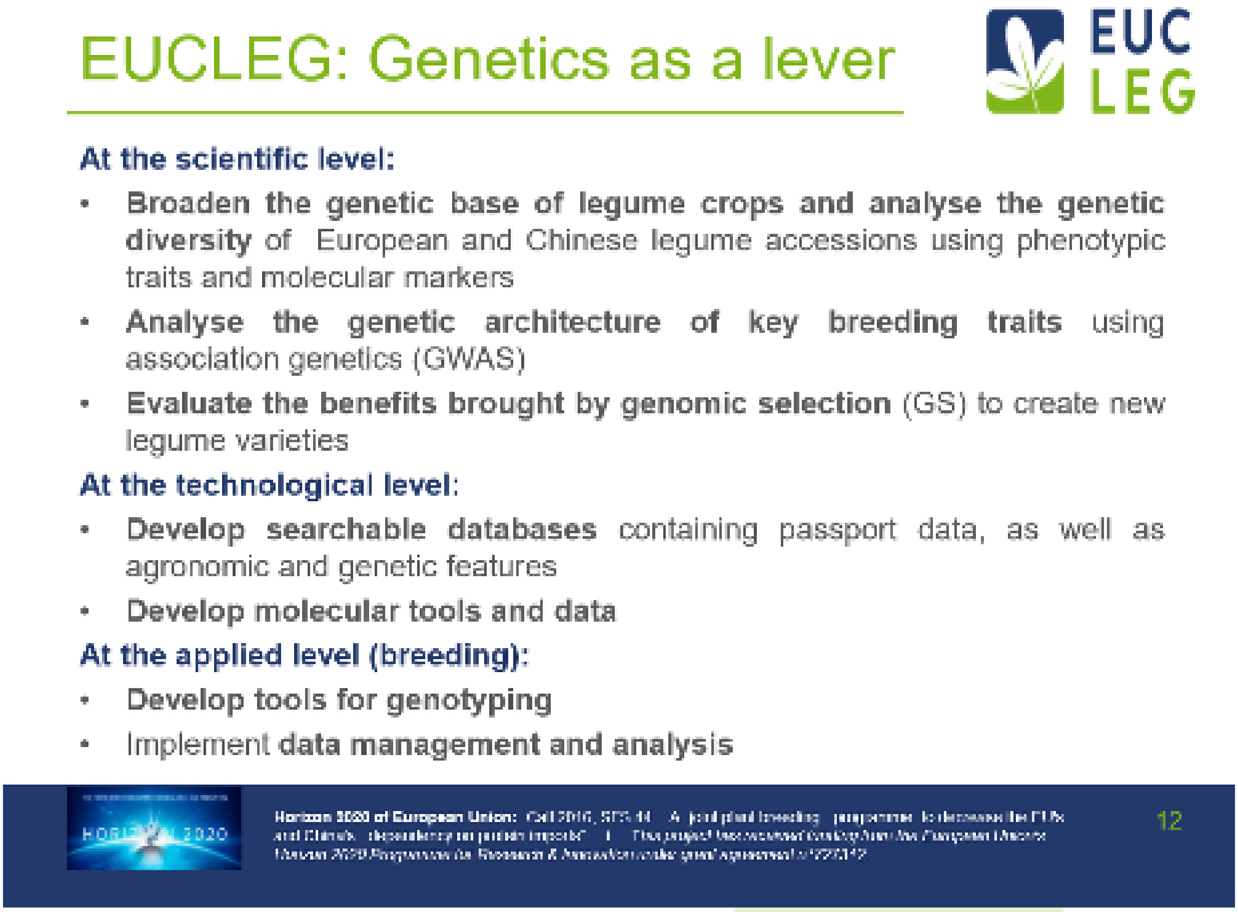

EUCLEG had ambitious objectives at different levels. At the scientific level, a first objective was to expand the genetic basis used by breeders in legume breeding programmes, after the analysis of the genetic diversity of European and Chinese legume accessions, using phenotypic traits and molecular markers. We also had an objective to analyse the genetic architecture of key breeding traits using genome wide association and anlaysis (GWAS) and to evaluate the benefits for genomic selection to create new legume varieties. At the technological level, we have developed searchable databases containing passport data as well as agronomic and genetic features, as well as developed molecular tools and data that will be available for future programmes. At the applied level, that is breeding in this case, the objectives were to develop tools for genotyping and also to implement data management and analysis that is now essential in genetic and breeding programmes.

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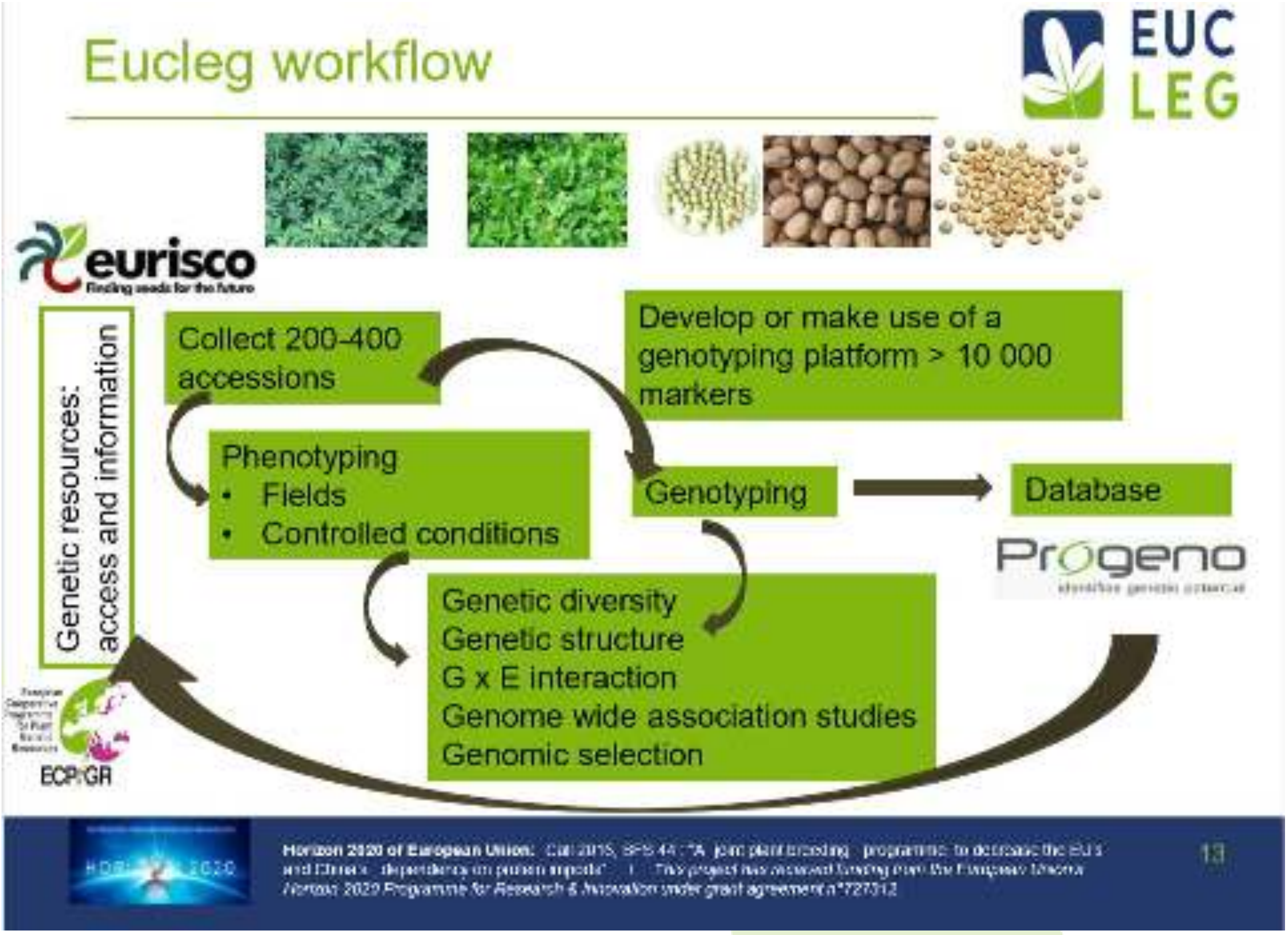

The EUCLEG workflow illustrated here is the basis of the presentations of this workshop amd booklet. For each of the 5 species, we have adopted the same general scheme. Based on collections of genetic resources we have collated between 200 and 400 accessions. We have developed or made use of existing genotyping platforms, depending on the species, with the objective was to obtain more than 10 000 markers. We have genotyped accessions with the chosen genotyping platform. Accessions were phenotyped in different conditions, either in multi-location field conditions, but also in controlled conditions, especially for diseases or drought stresses. All these results have been transferred to databases on Progeno and this programme is available for the analysis of genetic diversity, genetic structure and genetic control of traits.

The objective of this workshop and booklet was to disseminate the results obtained so far for both forage and grain legumes. We also wish to share general considerations about the design of the experiments, genotyping and breeding methodology. The idea is to talk and discuss with you, with legume breeders and scientists to imagine the future of breeding of legumes, what we could call post-EUCLEG breeding.

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About the author

Bernadette Julier

Dr Bernadette Julier is Research Director at INRAE, Unité de Recherche Pluridisciplinaire Prairies et Plantes Fourragères (URP3F), in Lusignan, France. Since her PhD, she has been continuously working on legume genetics and mostly on alfalfa or lucerne, the most famous, and protein producing forage species. Her main topic was first to evidence genetic variation for energy value and to combine it to forage production. She has been involved in projects on seed production and protein degradation too. More recently, her research is focused on the genetic bases of aerial morphogenesis, either in pure stand or in mixtures with forage grasses. The use of molecular markers to assist breeding is a major topic to promote genetic progress on this autotetraploid species. She is currently leading EUCLEG, an European project (H2020, 2017-2021) "Breeding forage and grain legumes to increase EU's and China's protein self-sufficiency" that aims to use more genetic resources and develop molecular tools to be able to create improved legume varieties (www.eucleg.eu) and thus promote protein production. She is a member of the Permanent Technical Committee of Selection (CTPS) in France, in the section "Forage and turf plants" since 1998, in charge of the variety registration.

This chapter is based on a presentation given to the EUCLEG online workshop on the application of cutting-edge genomic technologies in the breeding of legume species held on the 30th September and 1st October 2021

A recording of the presentation is available at https://youtu.be/z6AWKmKwXJ0

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