

*Preface by  
Didier Guillaume*

Christian Huyghe  
Bruno Desprez  
Vincent Laudinat  
*Editors*

# SUGAR BEET

**A competitive  
innovation**

éditions  
**Quæ**



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Éditions Quæ

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## PREFACE

Sugar beet is an essential contributor to the French agriculture and agri-food industry. Located in the fertile plains where it contributes to maintaining a diversity of production, the sugar sector is being transformed to meet economic and environmental challenges.

The industry has focused on innovation to optimize production and processing, by exploiting economies of scale to the maximum, with the objective of keeping factories at the heart of production areas.

The innovation is paying off. Over a decade, the significant yield improvements have been achieved while reducing environmental impact, with a sharp reduction in the use of nitrogen fertilisers.

Advances in agricultural equipment are also remarkable: not only harvesters, but also laser-guided mechanical weeding with weed recognition-technologies that may soon move into other crops.

But, most of all, the genetic progress has been truly remarkable in this recently-domesticated species.

The AKER programme, selected in 2012 as part of the French Biotechnologies-bioresources Investments for the Future Programmes, has made a major contribution to these innovations. The new varieties that emerge from this programme, more productive and more resistant to disease, offer great prospects for the whole sector. By harnessing high throughput genotyping and phenotyping technologies, and exploring genetic diversity, the AKER programme is recognizing the major role of biodiversity, which is at the heart of agroecology.



Innovation is one of the pillars of our country's agroecological transition. The AKER programme also reminds us that innovation and this transition are based on our capacity for collective action. It has brought together a particularly dynamic and creative French company, INRAE teams, universities and Grandes Écoles, and an agricultural technical institute, together constituting a French exception in innovation that accelerates progress and its take-up.

Thus, the AKER programme has been emblematic of what public-private research partnership makes it possible to produce in the service of an economically efficient and environment-friendly agriculture and agrifood industry.

*Didier Guillaume*  
*French Minister of Agriculture and Food*





**Context**





The first beet sugar refinery in Silesia built by Frédéric-Charles Achard.

## ■ A short history of sugar beet breeding

Sugar beet (*Beta vulgaris* L.) appeared in Silesia, at Cunern in 1802, thanks to the work of Frédéric-Charles Achard. It is the first plant to have been systematically selected because the beet has a biennial reproductive cycle (one year in vegetation, one year in seed production), which makes it difficult for the farmer to reproduce.

In 1806, during the Continental Blockade, the measures taken by the Emperor Napoleon 1<sup>st</sup> to stifle the maritime trade of the United Kingdom led to the first developments of beet cultivation on 30,000 ha in France, in order to find a source of supply in sugar other than cane. At that time, Benjamin Delessert industrialized the process of making beet sugar. This “ersatz” of imported cane sugar has experienced considerable growth since then, not only in France but in many regions of the world.

Selection methods have evolved over time. Long before Gregor Mendel's arrival, mass selection (best individuals are kept) had become genealogical or pedigree selection (progenies are separated) thanks to the Vilmorin family in the 19<sup>th</sup> century. In addition, at the request of Florimond Desprez, and under the impulse of Louis Pasteur and Charles Viollette (successive deans of the Faculty of Sciences of Lille), the quantification of sugar by density gave way to the chemical method by reduction of copper salts.



Napoleon 1<sup>st</sup>, initiator of sugar beet and protector of industry (Museum of Fine Arts of Lille).



The Florimond Desprez laboratory at its beginnings (watercolour painted in 1893).



The discovery by V.F. Savitsky in 1948 of monogerm beets on the one hand, and the description by F.V. Owen in 1952 of the cytoplasmic male sterility of the plant on the other hand, offered to the breeders the possibility of producing monogerm hybrids. The latter made it possible to carry out regular sowing and to mechanize the cultivation of beets by eliminating the labour which was required for the thinning, essential for multigerm beets.

### WHAT DOES AKER MEAN?

Aker is an Egyptian god represented by two lions back to back, one watching the sun rise (in our context, genetic resources) and the other watching the sun set (in our context, elite germplasm). Aker is the "Saint Christopher" of the Egyptian gods, carrying the sun from one world to another on his back, which, according to ancient beliefs, was sinking west into the Earth. The symbolism of the two lions can also be associated with the partnership between the Public and the Private, and the phonetics of the name AKER sounds like "Hacker", the one who breaks the (genetic) code. The beet visual is positioned between the two lions, whose leaves are entangled in the manes. It highlights that AKER is a research programme to improve the competitiveness of sugar beet, the deadline for which is included in the signature "Beet 2020".



Betterave2020



The genetic improvement in beet is a good reflection of the improvement in cultivated plants, to which the breeding company Florimond Desprez has contributed for five generations. Today, with high-throughput genotyping and phenotyping, with sequencing and the use of molecular markers, with bioinformatics and genomic selection, as well as systematic exploration of genetic diversity within cultivated beets and the *Beta* genera usable in crossing... the AKER programme brings its stone to the building of this construction that is always in evolution.

## ■ The objectives of the AKER programme

When it was launched in 2012, the AKER programme corresponded to the wish to put beets at the centre of the field of field crops; the desire to remake one of the scientific supports for agricultural research; the opportunity to enrol in the Investments for the Future Programmes and benefit from the dynamics of the “Grand Emprunt” launched by the French authorities at the time.

### Two strands

The first part of the AKER programme aims to identify genetic diversity, build an allelic reference collection and crossbreed with elite material (material with agronomic performance corresponding to market expectations), so as to broaden the diversity of exploitable genetics, accelerate genetic progress and obtain new varieties with high potential.

Concomitantly, the second part consists, in an original way, in valuing all the diversity acquired, in controlling it and in evaluating it by new phenotyping and genotyping methods based on high-throughput tools.

### Four steps

In terms of biological material, the AKER programme was deployed in four stages and is now approaching the production of improved varieties, which can be registered in the Official Catalogue of varieties and cultivable plants:

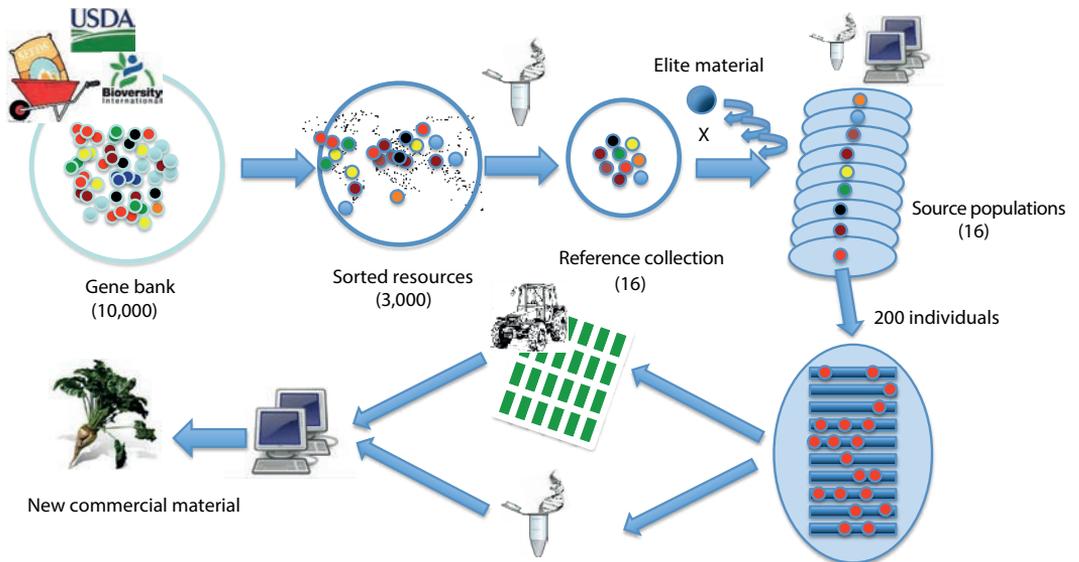
- the first step (2012 and 2013) consisted in identifying sixteen reference plants from 10,000 genetic resources collected that summarized the diversity available for all frequent alleles;
- the second stage (2013 to 2016) implemented crossing of the reference collection with elite germplasm to obtain variable source populations;

- the third stage (2017) focused on obtaining and multiplying these source populations;
- the fourth step (2018 and 2019) consisted in evaluating and analyzing the source populations, taking into account of the year and the place effects;
- finally, the AKER programme has resulted in the selection of new varieties and the associated new knowledge, from 2020.

### HOW EFFECTIVE ARE INVESTMENTS FOR THE FUTURE PROGRAMMES?

*“The action ‘Biotechnologies–bioresources’ of the French Investments for the Future Programme, initiated in 2011, funded nine major projects, on crops of major economic interest for our agriculture and our country. These projects have above all made it possible to improve knowledge of these species through the exploration of the diversity of the multiple varieties, drawing in particular on the achievements of the scientific interest group Genoplante. They have also made it possible to consolidate public–private partnerships, by encouraging business investment on the national territory, and to develop for the coming years new varieties better able to meet the needs of farmers, industrialists and consumers, as well as the major challenges of climate change. Finally, these long–term projects have fostered the training of a new generation of researchers, through the involvement of numerous doctoral and post–doctoral students.”*

Guillaume Boudy, General Secretary of the SGPI  
(General Secretariat for Investment)



General diagram of the AKER programme, as designed at the origin of the project.



## AKER in brief

AKER aims to improve the competitiveness of sugar beet by 2020 by doubling the annual growth rate of its sugar yield per hectare.

AKER is part of French Investments for the Future Programmes; it is supported by eleven public and private partners representing the entire French beet industry.

AKER is an original and innovative programme which concerns at the same time research, development and training; it confirms sugar beet as a reference plant and sector.

AKER is also:

- 16 reference plants;
- 40 million molecular data points;
- 8 km of hybridization cages;
- 3,000 selected hybrids;
- 63,000 phenotyping plots in the field;
- 11 partners;
- 100 collaborators (including 60 researchers);
- €18.5 million budget (including a €5 million in state grant).

## In addition...

The AKER programme has highlighted the entire genetic diversity of beet on the basis of sixteen reference plants.

It is developing a new approach to selection that places genotyping (analysis of genetic composition) before phenotyping (evaluation of traits).

It uses a new selection method (genomics) that relies on plant DNA and new selection techniques (labeling, genome sequencing).

It manages a large number of data (by bioinformatics) to make predictions.

It uses technologies (imaging, robotics) from other research sectors (physics, medicine).

It is interested in the sugar yield of beet, but also in all its components, including resistance to diseases and nitrogen recovery, while respecting the environment.

AKER is a research, development and training programme. It contributes to ensuring the generational renewal of researchers.

## WHAT IS THE INTEREST OF THE AKER PROGRAMME FOR INRAE?

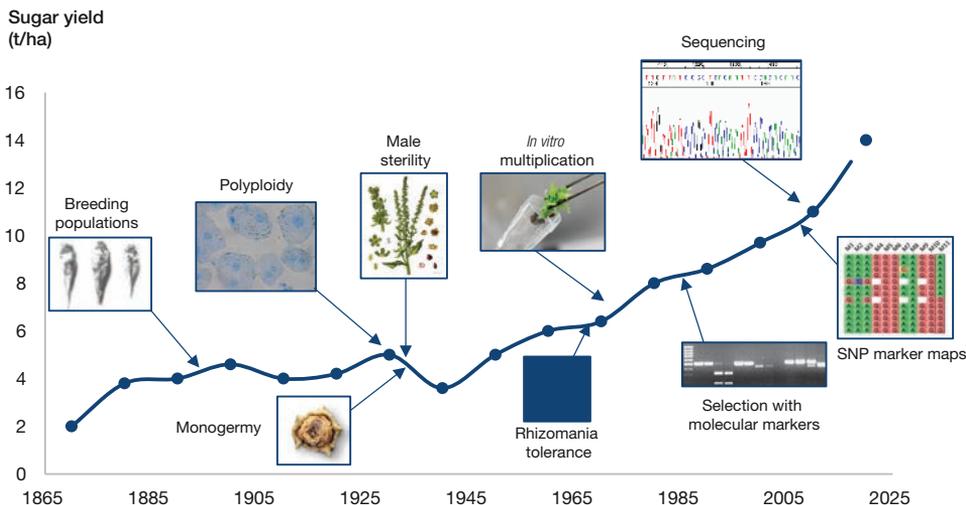
“At the time when the AKER programme was launched, Inra, now INRAE, did not have a dedicated sugar beet programme. And yet the contributions of laboratories and teams from our establishment were very important, whether it was studied in genomics and bioinformatics in Évry and Toulouse, developments in high-throughput phenotyping in Montpellier in interaction with the Capte mixed technology unit in Avignon, or studies on seeds and seedlings at Inra in Angers and Geves. Scientific knowledge has progressed, as shown by the numerous publications of these teams.

But, beyond science, the AKER programme demonstrated the capacity we had to implement, on a new species, all the resources, methods and knowledge developed within the Institute. This considerably widens the scope of the research carried out within INRAE and the scope of the partnerships. Cognitive advances, the quality of the genetic material created within the framework of AKER and partnerships with the Florimond Desprez company, the Technical Institute for Sugar Beet, the universities of Lille and Angers or Agrocampus Ouest are remarkable demonstrations.”

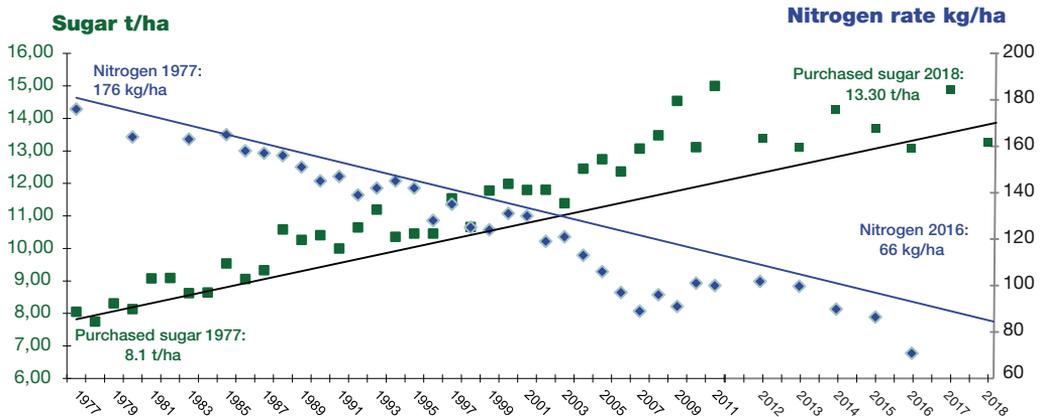
Philippe Mauguin, CEO of INRAE

## ■ Sugar beet today

Beet is a cultivated plant which appeared at the beginning of the 19<sup>th</sup> century, boosted by the Continental Blockade. Since then, yield and quality have improved steadily, while the various inputs required for cultivation decrease over time.



Evolution of beet yield regarding to the technical evolutions (tonnes of sugar/ha).



Evolution of mineral nitrogen inputs (kg/ha) and sugar yield (t/ha).  
Sources: CGB, 1977-1996; ITB, 1997-2018.

Sugar yields from beet have increased more than twenty-fold in France. They have gone from 700 kg/ha of raw sugar with a sugar content of 7% from the crop, to 14,800 kg/ha of white sugar with a sugar content of 18% in the best recent years. Mention should also be made of the improvements in the industrial process, which have ultimately enabled the extraction of much higher quality sugar. Finally, energy consumption per ton of beet has been halved in 40 years.

But genetic progress is not measured only considering the “simple” character of sugar productivity. Reduction of the environmental impacts of beets has become particularly remarkable. Since 1983, there has been a 50% drop in the quantities of phytosanitary products used (fungicides, insecticides, herbicides), part of this drop being linked to the development of active ingredients at the end of the 1990s. The trend continues with a strong investment by beet growers towards rational cultivation methods. Over the past 40 years, the amount of nitrogen supplied to beets has dropped by 68% per ton of sugar produced. All of this progress is the result of an interdependent improvement in cultivation techniques, agro-equipment and variety selection.

## ■ What has changed since the launch of AKER

Since the launch of the AKER programme in 2012, the beet industry has undergone major transformations by subscribing to a process of continuous progress both in terms of productivity and economic and environmental efficiency.

These changes affect the entire production chain, from the beet seed to the marketing of sugar, including cultivation and processing into sugar and alcohol. They have a direct impact on the economy of the sector and its agricultural practices.

### The end of European quotas

The first of these transformations, and certainly the most important for the organization of the sector, is the end of sugar quotas. On October 1, 2017, the system that regulated the European sugar sector ended. The quantities produced are no longer controlled and the liberalization of the market allows each actor in the sector to produce as much sugar as he or she wishes, with a view, among other things, to exporting it.

Thus, from the following marketing year, even as third countries continued to increase their volumes in unprecedented proportions,

Hoing beets as an alternative to chemical weeding.





Beet weeding robot.

European sugar production also increased. Very quickly, world sugar production and stocks were faced with surpluses causing prices to plummet. These then settled at their lowest level in ten years, causing a loss of profitability for the sector and resulting in reorganization decisions, restructuring, plant closings and an adjustment in the remuneration of planters.

### Greater societal demands

In addition to these economic issues, there are societal demands which have also been asserted in recent years. Consumers are demanding an agriculture that uses less or no phytosanitary products, and limits its impact on the quality of the environment and biodiversity as much as possible. These expectations are reflected in an increasingly marked regulatory control of agricultural production. A recent example is the use of neonicotinoids in France, insecticides which were used in coating seeds to fight against green aphids, which are vectors of virus yellows. In 2019, their use was totally prohibited on the territory even though the sector did not have alternative solutions as effective and preserving the auxiliary



fauna. It should be noted that this coating with neonicotinoids had to be replaced by aerial spraying with insecticides which may be less selective and much more expensive for the sector. Under this pressure and at the same time, the possibilities of improving productivity through new genomic editing techniques (NBT) have not been authorized in Europe, unlike the rest of the world.

In this unprecedented context, the AKER programme aims to provide farmers with sugar beet varieties that will reconcile societal and environmental expectations, productivity and profitability, by appropriating all of the current technological advances and sometimes not sufficiently known or investigated in 2012 (genomics, observation drones, image processing, rapid phenotyping, etc.).

### HOW TO SAVE SUGAR BEET IN FRANCE?

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*“The French beet industry has been under pressure for a few years. To adapt to its new environment, it must gain competitiveness while improving its ecological footprint. The challenges are multiple, the ITB puts its expertise at the service of the AKER programme. The aim is to increase the productivity of beet, but also to reduce its production costs and limit the use of inputs. Putting new varieties with high yield potential or resistant to pests on the market is one of the solutions that will make it possible to achieve these objectives.”*

Alexandre Quillet, President of the ITB  
(Technical Institute for Sugar Beet)

## ■ The international sugar economy

If sugar consumption is mature in Europe, world consumption increases by 1.2 to 1.5% per year. By 2030, the world will need an additional 30 Mt of sugar: the equivalent of current production in Brazil or India. France has a role to play on the European and international markets.

### Rise of sugar cane

The competition from cane sugar production has increased over time. Carried by emerging countries, notably Brazil, cane now supplies almost 78% of world sugar production compared to 74% in 2005–2006, even if this relative increase comes from the increase in absolute value of the market.



The French industry has been preparing for a long time to meet the challenges imposed by the rise of sugar cane. It has a very high production potential, which was restrained by the various successive European sugar regulations. In fact, the areas sown with beet could be increased, the industrial tools could be redeployed, which other European countries envy.

In reality, France produces as much sugar as in the past, but with an ever-decreasing area. It releases around 2% of surface area per year, the equivalent of increasing its sugar yield. Conversely, the emerging cane-producing countries need more and more land to produce, due to the lack of genetic progress in this species, which can encourage them to deforestation. They can be limited by logistical issues when production areas are increasingly distant from consumption areas or port infrastructure.

## France's capacity on the markets

With the end of quotas in 2017, France has regained its capacity to supply sugar-deficient basins in Europe, the Middle East and West Africa. The first quota-free campaign enabled the beet industry to generate a trade surplus of 1.2 to 1.4 billion euros.

France is thus the leading European supplier of sugar, significantly in Spain where 40% of the sugar consumed is of French origin, but also in Italy and the United Kingdom. In these territories, it must compete with sugar imported from third countries, in the form of white or raw for refining purposes.

However, in recent years, the methods of importing sugar into the territory of the European Union have been considerably relaxed: no limit for imports from the poorest countries (ACP/LDCs—Africa, Caribbean, Pacific / Least Developed Countries) and the introduction of quotas at reduced or zero duty, for more than 1.5 Mt of sugar per year. Thus 20% of the sugar consumed in Europe was imported during the last year under quota.

### WHY, AS AN INDUSTRIALIST, USE BEET SUGAR?

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*"At Ferrero, we have been using beet sugar for a very long time because of its technological characteristics, but above all because it is the ideal ingredient to satisfy the production of our delicacies. Nothing can replace it and the alternatives will not replace it if we want to maintain the quality and safety requirements of our recipes. Beet sugar has all its usefulness and is available near our factories. In fact, 100% of the sugar we use to make our two products, Nutella and Kinder Bueno, is French!"*

Joane Husson, Ferrero, Director of Public Affairs,  
Corporate Communication & CSR

Sugar beet benefits from a high production potential.



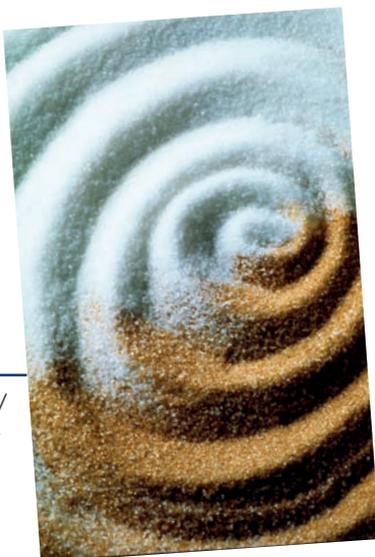
### Strengthening competitiveness

To preserve its market share in Europe, France must therefore be continuously more competitive. Similarly, when it exports to third countries, it has to face competitors whose production costs are often much lower, due to very different health, environmental or social standards, and further accentuated by monetary instability.

However, in this long-term buoyant environment, the imperatives aimed at adapting to climate change, reducing inputs or saving energy and water are urgent priorities of which the beet industry is fully aware.

The AKER programme thus aims to make the most of sugar beet in order to:

- allow France to take its share in the rise in world demand, and to contribute to the French agrofood trade surplus;
- allow the sector to support the demand for bioethanol (+ 11% in France in 2018 alone), cleaner and cheaper, acclaimed by the consumer;



White sugar / raw sugar, in the spiral of competition.

- face environmental challenges, reduction of inputs and adaptation to climate change.

The French beet sector cannot remain stationary, and the AKER programme will allow it to exceed its current progression by 2% of annual yield to compete without waiting for sugar cane and offer room for manoeuvre to the beet farmer as well as sugar manufacturer.

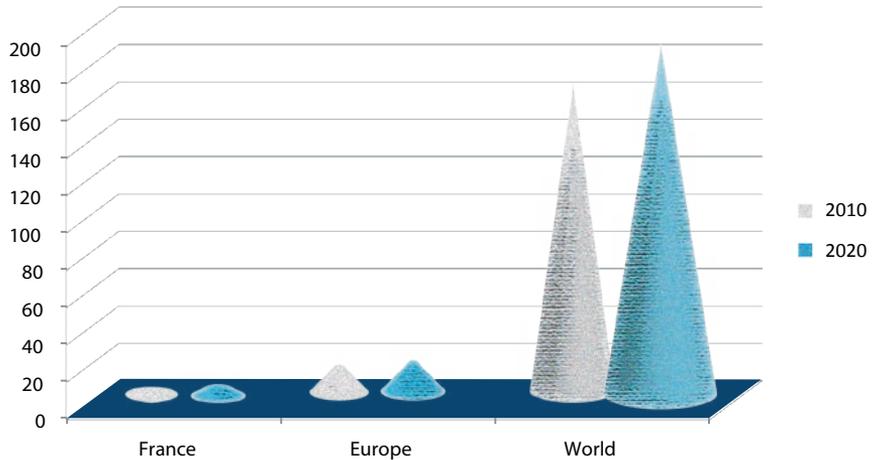
## BEET VERSUS CANE

David against Goliath? However, sugar beet has advantages over sugar cane. It consumes half the water (650/1,250 mm per year according to the FAO), occupy the soil half as long and contribute to biodiversity in the agronomic rotation. The rate of progress in sugar yield is 2% per year compared to 0.5% for cane.

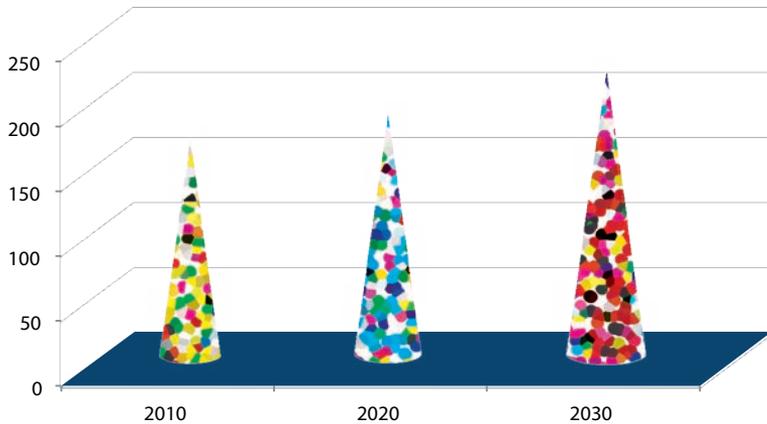
Beets are grown to ensure local production and consumption of sugar. Its sugar yield (around 14 tonnes/ha) is higher than that of cane; it can also produce 9,000 liters of bioethanol per hectare (compared to 5 to 8,000 liters for cane) — or 4.6 tonnes oil equivalent (toe). When brought to the surface and expressed in energy equivalent, the cumulative production of sugar and/or bioethanol (taking into account the bagasse of cane, which is also exploited for energy) is higher for beet. However, its cost of production is currently higher, mainly due to the cost of labour.

Sugar cane dominates today the world's sugar supply.





Evolution in sugar production, 2010-2020, in million tonnes. Source: FoLicht.



Evolution in world sugar consumption, 2010-2020-2030, in million tonnes. Source: FoLicht.

## HAVE AKER'S OBLIGATIONS TO THE ANR BEEN FULFILLED?

Following Tania Langon, and before Gaël Laporte, Jetta Keranen (Ayming company) was manager of the AKER programme. She draws up a report vis-à-vis the National Research Agency (ANR).



“The AKER programme fully meets the specific performance criteria and obligations established by the ANR for projects funded under the Investments for the Future Programmes (PIA). Thus, the budget of €18.5 million (including €5 million in public aid) was respected and controlled, with a tiny share of reallocation during the program. In accordance with the initial assumptions, 60% of the budget was devoted to phenotyping actions.

The assessment of human resources is very positive, the programme has mobilized a hundred collaborators from various backgrounds, laboratory directors, scientific researchers, industrialists, postdoctoral students, PhD students or apprentices. A real team spirit was established, especially during the annual coordination committees.

The AKER programme achieved the planned objectives with a few minor changes. 100% of important actions, tasks, milestones and deliverables have been completed. In reality, it made it possible to carry out more actions than planned, at constant resources, because high throughput offered the possibility of doubling field experimentation. As a result, it produced more robust results, proof of scientific solidity.

In total, over the duration of the programme, around 30 scientific publications and 150 communications were produced. Student training sessions have been scheduled each year in conjunction with Agrocampus Ouest and the University of Lille. Results have also been valued at the industrial level. The innovation developed thanks to the AKER programme is found not only in the results, but also in the methodology.

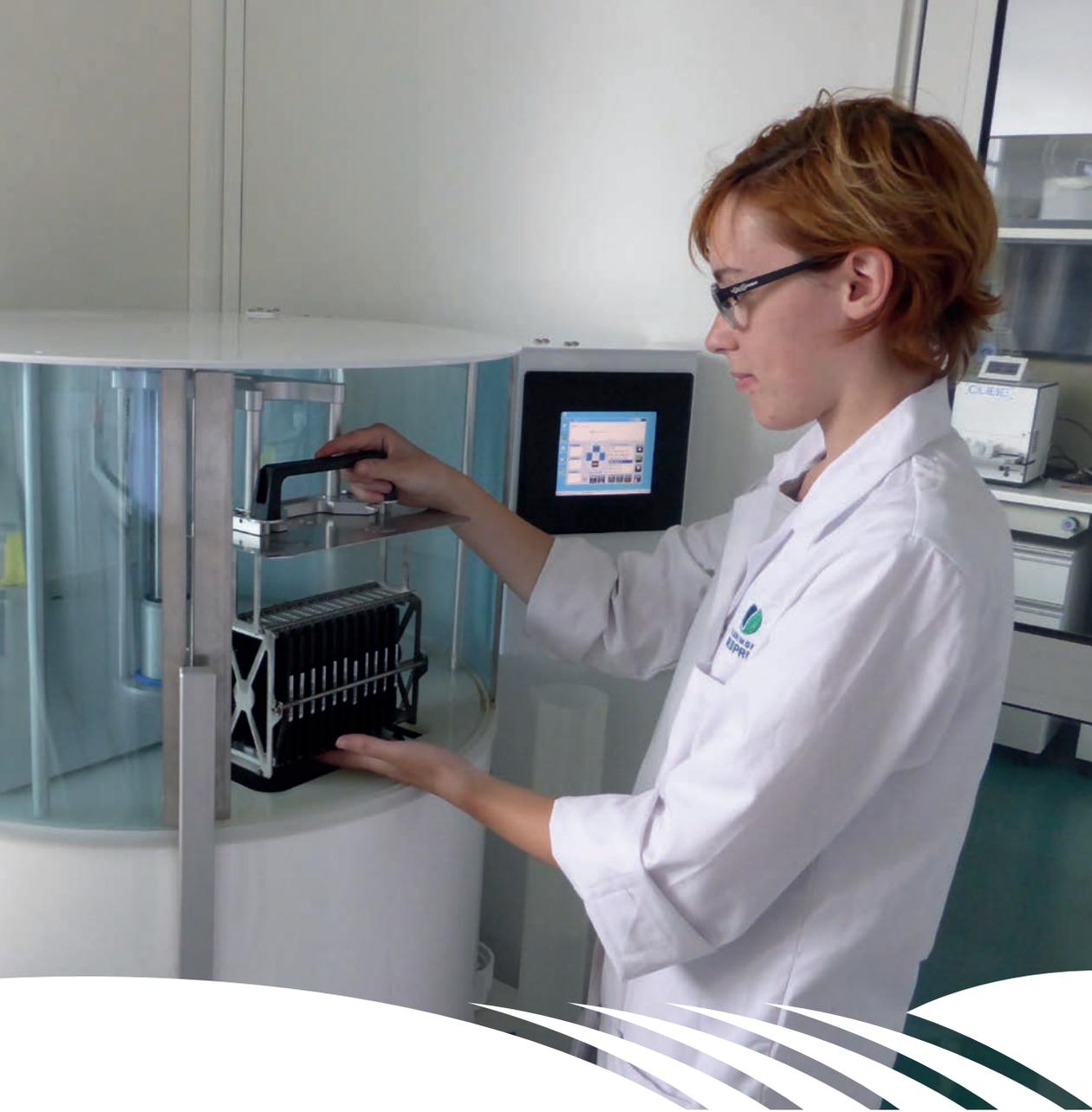
A collaboration has been established with the other PIAs via Scientific Interest Group ‘Green Biotechnologies’. Many areas of work may materialize in other collaborative projects, in the field of basic or applied research.

Finally, one of the real successes of the AKER programme lies in its ability to bring new elements to the community in terms of technological development, public/private partnership, long-term collaborations.”



A close-up photograph of a person wearing a white lab coat and gloves, using silver tweezers to hold a small, folded green leaf sample. The background is blurred, showing a laboratory environment. In the foreground, a blue tray containing several other similar green leaf samples is visible. A blue circular graphic is overlaid on the bottom right of the image, containing white text.

**Genotyping  
tools and  
methods**



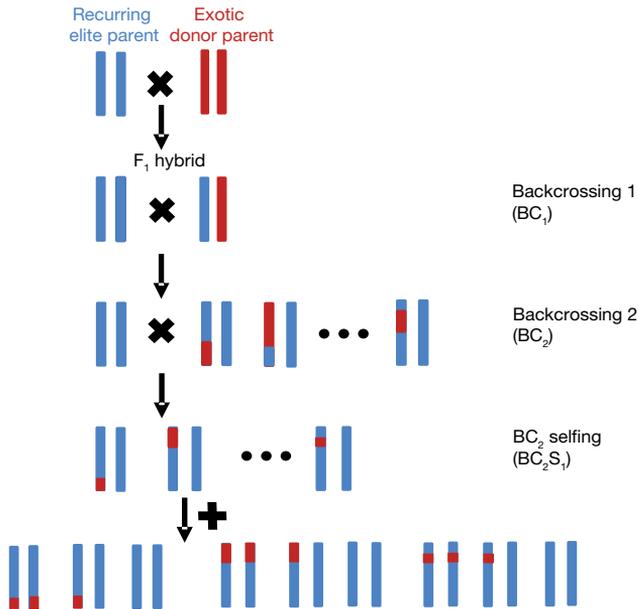


## ■ Sequencing and molecular markers

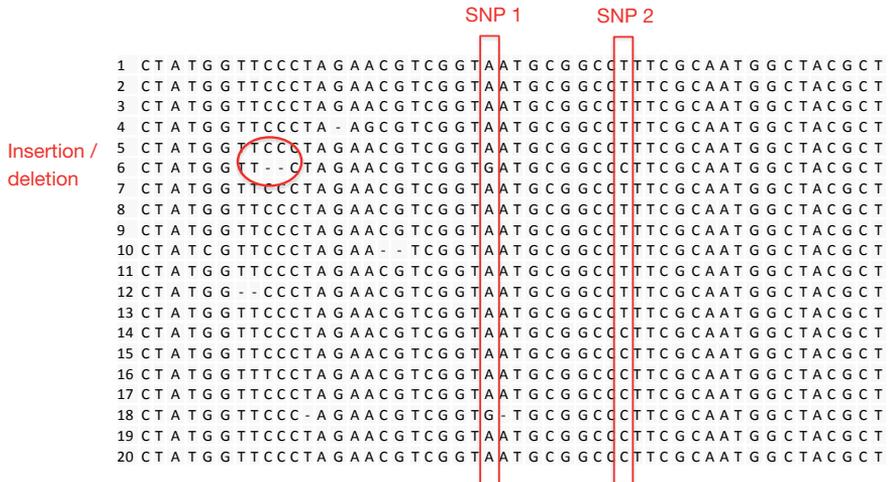
The objective of the AKER programme is to split the entire genome of each exotic accession into that of elite material. Molecular markers make it possible to identify fragments of chromosomes from each accession, but without knowing *a priori* what effects they will produce. The phenotyping carried out at the end of the programme made it possible to find out.

### Sequencing

The beet genome comprises approximately 760 million base pairs. During the AKER programme, those of the sixteen reference plants and elite material were sequenced, that is to say that the genetic code, made up of the four bases (A, T, G, C) of the DNA, of chromosomes has been read. Then the sixteen selected exotic plants and the elite line underwent different successive crosses:  $F_1$  hybrids – first generation (F = filial generation),  $BC_1$  and  $BC_2$  backcrosses (BC = backcross),  $BC_2$  self-fertilization ( $BC_2S_1$ ) or even  $BC_2S_1$  ( $BC_2S_2$ ) (S = selfing). All these plants were themselves genotyped with carefully chosen molecular markers.



Crosses made as part of the AKER programme.



Example of accession sequencing.

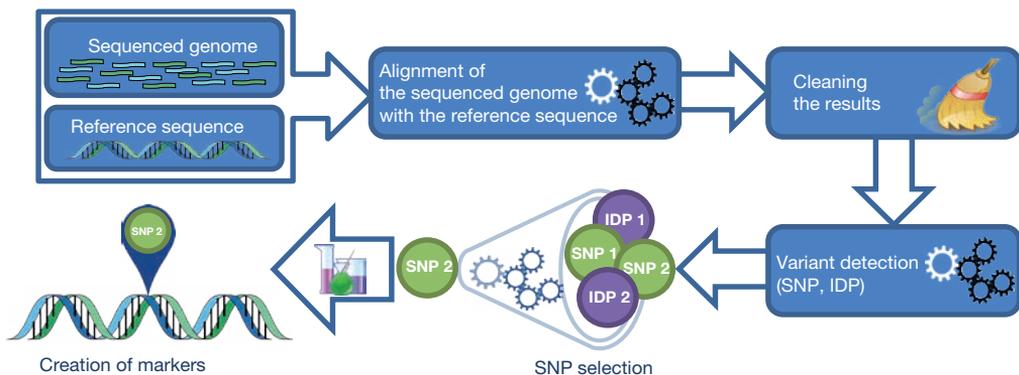
After cutting into small fragments of their DNA, millions of reads of 100 bases in length and in both directions of reading (forward and reverse) were performed for each of the sixteen plants, also called accessions. Thus, for each accession, a library of short sequences of its genome was obtained. We could compare them to bags containing the pieces of a puzzle that must be assembled to obtain the DNA sequence of these accessions. The background image of the puzzle which makes it possible to order them is a reference sequence which had previously been obtained in a project originating from the SESVanderhave company, a subsidiary of the Florimond Desprez group, and the German programme Gabi. So it was a question of starting from this reference sequence and aligning on it the readings of the elite and the sixteen accessions. We then identified differences between the sequence of the elite and that of the different accessions aligned with the reference and defined molecular markers between the elite and the accessions.

### Molecular markers

Most molecular markers consist of primers on the order of 20 bases complementary to the target DNA. This DNA is then amplified in a large

number of copies by the Polymerase Chain Reaction (PCR). In the AKER programme, the objective was to detect several thousand of them distributed homogeneously throughout the genome and polymorphic between exotic accessions and the elite line. Researchers have identified, by bioinformatics analysis, more than 40 million variations in the genetic code of the study material, including SNP (Single Nucleotide Polymorphism) and IDP (Insertion Deletion Polymorphism). The totality of these variations represents approximately one for 20 base pairs. In other words, the 19 out of 20 invariants represent the information that defines a beet.

During the AKER programme, SNP and DArT markers were used and combined to select the accessions whose DNA will have been sequenced. DArT (Diversity Arrays Technology) markers correspond to a different molecular marking technology, based on DNA–DNA hybridizations. These detect the presence or absence of individual DNA fragments in large genomic representations. In the context of AKER, DArTs have been used to capture specific genomic regions of wild accessions which escape, in part, from detection by SNP markers, these being designed from cultivated sugar beets. After sequencing, only the SNP markers, specific and predominant, were retained.

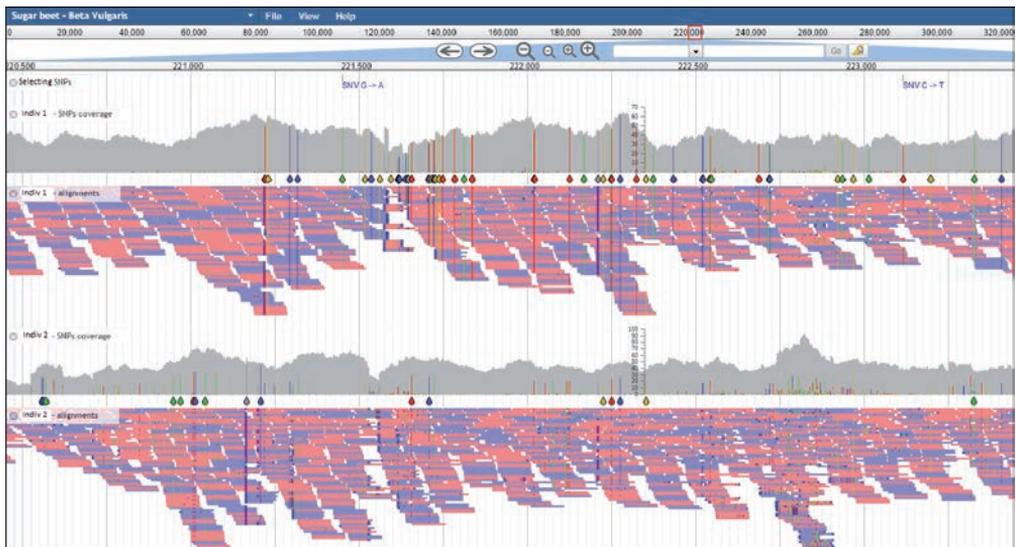


Detection and selection of polymorphisms.

## MOLECULAR POLYMORPHISM

Molecular polymorphism expresses a variation between individuals in the sequence of their genomes. These variations may be responsible for the differences observed between individuals in a population, such as the shape of the root. The polymorphisms correspond either to SNP (Single Nucleotide Polymorphism), or to insertions or deletions IDPs (Insertion Deletion Polymorphism). It should be noted, however, that other types of variation can also cause phenotypic differences.

Not all of these SNPs can become usable molecular markers. There are indeed biological constraints which prevent, in certain cases, their detection: for example, their too great proximity. In order to avoid this type of problem, significant bioinformatics work has been carried out. This selection requires going through several stages and using appropriate software. In order to automate the procedure, Florimond Desprez created a software automation chain in an appropriate environment (Galaxy!).



Genome browser (JBrowse).

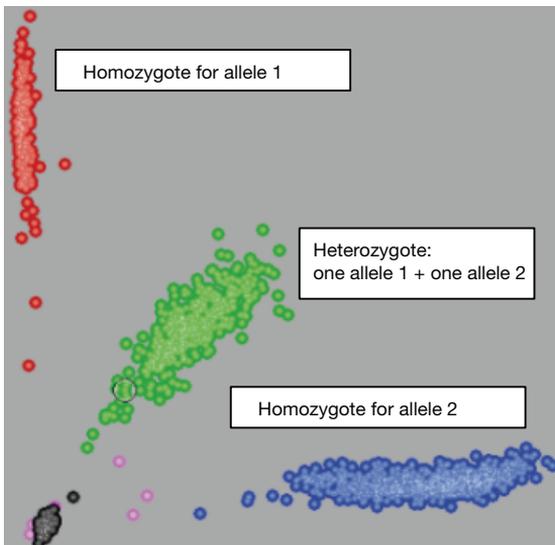
[www.galaxyproject.org](http://www.galaxyproject.org)

In the end, 200,000 reliable, robust and reproducible SNPs among the 40 million crossed all the selection stages.

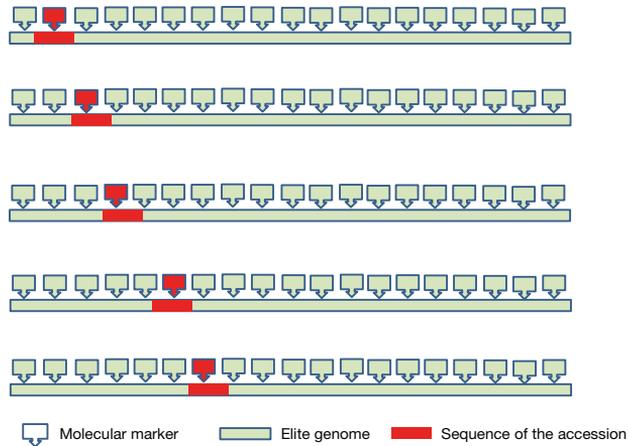
One of the objectives of the AKER programme has been to generate by successive crossings a set of individuals genetically almost identical to the elite line. Their differences played on the integration of different fragments of sixteen exotic accessions (covering the whole of their genome) and the beets obtained were called quasi-elite.

Following the sequencing of the sixteen exotic accessions and the elite line, the search for SNP molecular markers was initiated. Once chosen, these molecular markers make it possible to follow the result of the crosses (backcrosses or self-fertilizations) so as to detect the desired plants. The plants must not be redundant but complementary to cover the entire exotic genome. The DNA primers constituting each marker were synthesized by a specialized service provider. In total, sixteen populations represented by 6,500 individuals each were followed with more than a hundred molecular markers, which represents more than 10 million marking points per generation.

By identifying molecular markers that can be used in the descendants of exotic/elite parent crosses, we can visualize the different parts of the

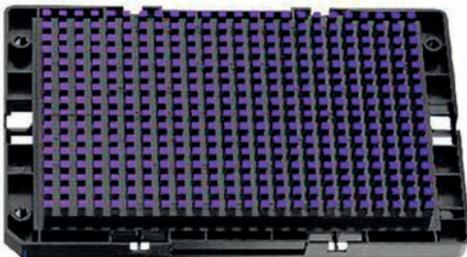


SNP (Single Nucleotide Polymorphism) marker profile. SNP genotype: the points correspond to individuals; colours with different genotypes.



Explosion of accessions in elite germplasm.

parents' genome present in each individual. The analysis of variations in their phenotype (sugar yield, resistance to disease, etc.) and their genome allows us to discover the effect of these exotic chromosomal fragments on the phenotype of quasi-elite beets, to identify chromosomal regions carrying genes of interest and finally to generate original basic material for breeding programmes.



Florimond Desprez, in collaboration with its subsidiary SESVanderHave, has developed a DNA chip for beets called Bv15FDSV. This small plate of a few square millimeters was used in the AKER programme. It allows 30,000 SNP molecular markers to be processed per DNA sample at a time. It makes the work of genotyping and analysis of individuals more precise and faster.

## A STORY OF SCRIBES

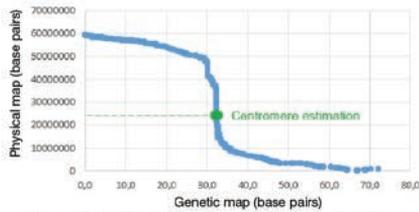
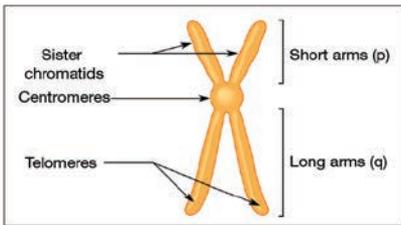
The genomes of two different beets are like two copies of the same book made by two different scribes. Anyone can introduce small errors, synonyms, additions or omissions of varying sizes into their copy. These differences can have a more or less significant impact on the story described in these books. They are identified by text comparison software (bioinformatics software) and will serve as specific reference points (molecular markers) in order to distinguish each of the copies of the book without having to read all the books.



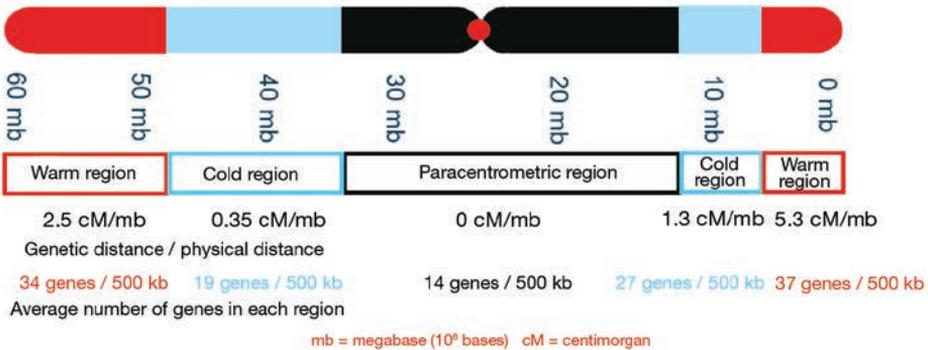
## PHYSICAL MAP AND GENETIC MAP

The physical map is a representation of the genome based on a distance in number of bases. The genetic map is a representation of the genome based on a genetic distance (expressed in centimorgans, a measure of recombination between them). This reflects the probability that two genes or two markers will be transmitted together or not in an offspring. The closer these genes are physically, the more they will be transmitted together; the more distant they are, the more possibilities there will be for recombination and therefore for a separate transmission. This probability of recombining depends only partially on the physical distance, because there are regions with frequent recombinations, others with few or none. Recombination events are generally more frequent at the telomeres (extremities) than at the centromeres of the chromosome. Thus, the correspondence between the physical distance and the genetic distance of the two markers is variable along the chromosomes.

These genetic maps are essential tools for relating phenotypic characters to genes identified by markers. This supposes that the markers of this map are well distributed over the genome and thus make it possible to follow the transmission in the offspring of genes coding for visible or measurable phenotypic characters.



Recombination according to the physical map



Physical map / genetic map.



Pierre Devaux supported the various stages of the AKER programme selection scheme, starting with the study of allelic variability, then sequencing and finally using molecular markers.

## PIERRE DEVAUX, BIOTECHNOLOGY CONTRIBUTOR

Polytech-Lille engineer and holder of a State doctorate thesis in natural sciences, member of the French Academy of Agriculture, Pierre Devaux heads the Biotechnology Laboratory at Florimond Desprez. “This laboratory is a biotechnological platform”, explains Pierre. He believes that he has provided technologies, tools and methods for the AKER programme.

“We have invested a lot in high-performance tools, including robots for extracting, quantifying and transferring DNA, genotyping and high-speed reading chains, software for the design of molecular markers and analysis, etc., so as to use a quantity of data which is far in excess of our routine research programmes.”

From the start, genetic biologists characterized the accessions harvested around the world from a molecular point of view, based on knowledge obtained from tomatoes, rice and peppers. “For this, we use molecular markers,” explains Pierre. “We looked for exotic accessions, sixteen in number, representing all the allelic variability available and different from that present in the elite.”

The Biotechnology Laboratory has sequenced the genome of the sixteen selected accessions. “We have aligned the sequences of the accessions with the reference sequence, so as to highlight the differences and, from these, to define and develop new molecular markers.” And Pierre to conclude: “Breeders and biologists are linked like fingers of the hand in the AKER programme.”

## ■ Processing of genomic information

The AKER programme has made it possible to collect a large number of genomic data using molecular markers. These data were used and valued throughout the genotype selection process.

### Linkage disequilibrium

Linkage disequilibrium allows us to study the link between causal genes – that is, those that explain the differences between individuals – and genetic markers. If a gene has a linkage disequilibrium with a marker, it means that the information in the marker will retrieve information from the linked gene.

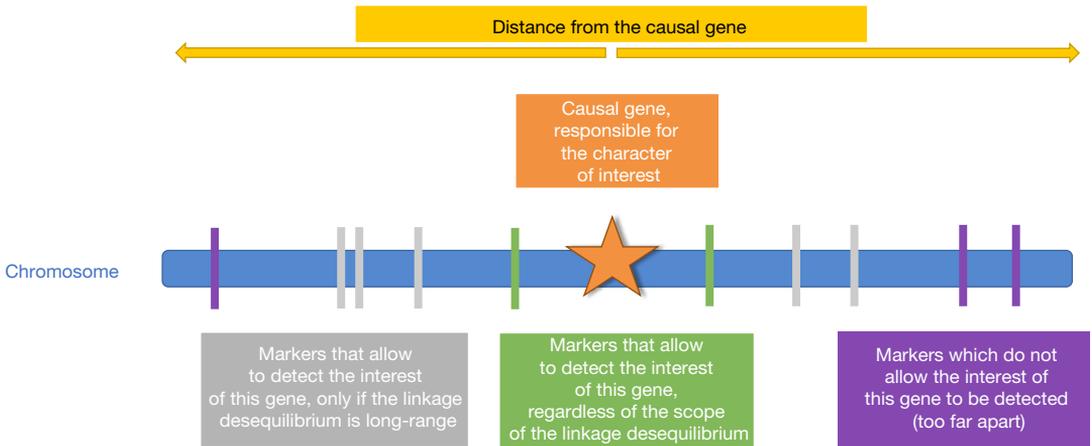
The linkage disequilibrium is linked to the physical proximity of the markers to the genome. If two markers are physically close, the



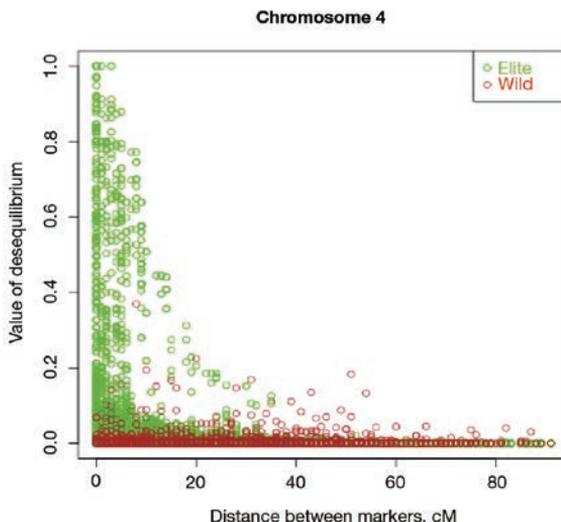
desequilibrium between them is significant; if they are far apart, the disequilibrium is small. These phenomena (connection and physical proximity) combine. Consequently, if the linkage disequilibrium is short-range, the gene/marker links detected will be precise, but it will take a lot of markers to be able to detect them (high marking density). On the other hand, if the linkage disequilibrium is long-range, fewer markers will be needed, but the detected links will be less precise.

Thus, the study of the linkage disequilibrium can make it possible to determine the optimal marker density making possible analyzes for which all the causal genes must be in linkage disequilibrium with at least one marker in order to be able to capture all the information. We determine for this the maximum distance between two markers to obtain the linkage disequilibrium between them and deduce the number of markers necessary for the correct analysis of the genome. Determining the optimal labeling density makes it possible to plan the genotyping (if the analysis is carried out *a priori*) or to verify the veracity of the results obtained (if the analysis is carried out *a posteriori*).

By comparing the linkage disequilibrium between elite populations and wild accessions, we can also identify traces of selection. Indeed, in certain areas of the genome corresponding to regions highly subject to selection, the linkage disequilibrium is more extensive (long range) in elite populations than in wild populations. It's sort of the signature of the



Concept of linkage disequilibrium.



Representation of the linkage disequilibrium in the elite and wild populations.

breeder. In particular, we were able to highlight a very strong selection pressure in terms of monogerm, male sterility or resistance to *rhizomania* genes (gene *Rz1* on chromosome 3).

The linkage disequilibrium is therefore at the service of new selection methods based on marker / causal gene links, in particular association genetics and genomic selection.

### Association genetics

Association genetics or GWAS (Genome-Wide Association Study) does not have an *a priori*: it will carry out a statistical test with each marker of the beet genome to examine the relationship between the allele to the marker and the characters of interest studied. These relate to the yield of white sugar per hectare (resulting from the root weight, the sugar content and the impurities), the seed growth and behaviour in the face of diseases (powdery mildew and *Cercospora*). This will ultimately allow the detection of genomic regions associated with the variation of a phenotype.

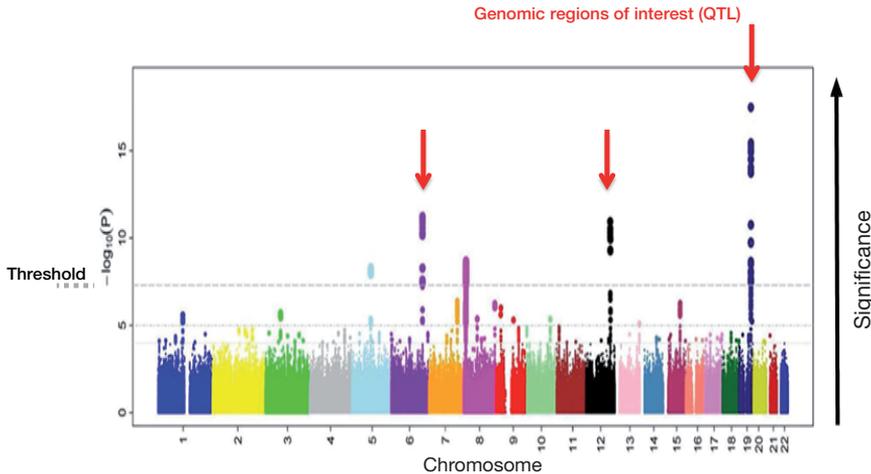
Once the statistical association tests have been carried out, Manhattan plots (graphs with shapes evoking the skyscrapers of New York) will be able to be drawn to detect the genomic areas of interest. When the association between a marker and a character exceeds a certain threshold value, the association can be considered statistically significant.



## RED BEAK AND BLUE PLUMAGE?

In these association analyses, it is essential to take into account the structure of the population studied, because it can generate false marker/character associations. For example, imagine two types of birds belonging to the same species. One from South America has a red beak and green plumage while the other from a small island in Indonesia has a yellow beak and blue plumage. In addition, a bird with a red beak and blue plumage has never been found. If we make a test of association between the two characters "colour of the beak" and "colour of the feathers", a very strong association is detected since knowing the colour of one makes it possible to determine 100% the colour of the other character. And yet, in reality, these two characters are not physically linked on the genome. This is a false association caused by the geographic isolation of the two types of bird. If the two types of bird had been able to meet in the past, mixtures would have taken place and we would have found in nature birds with red beaks and blue feathers. This example demonstrates the advantage of considering the structure of a population (here, in two distinct groups) before doing an association analysis.

Once the genomic regions of interest have been detected, they will be able to be introgressed (introduced) directly into material adapted to different market segments (material tolerant to rhizomania, high rhizomania pressure, cyst nematodes, etc.) thanks to linked genetic markers.



An example of a representation of the Manhattan plots type.

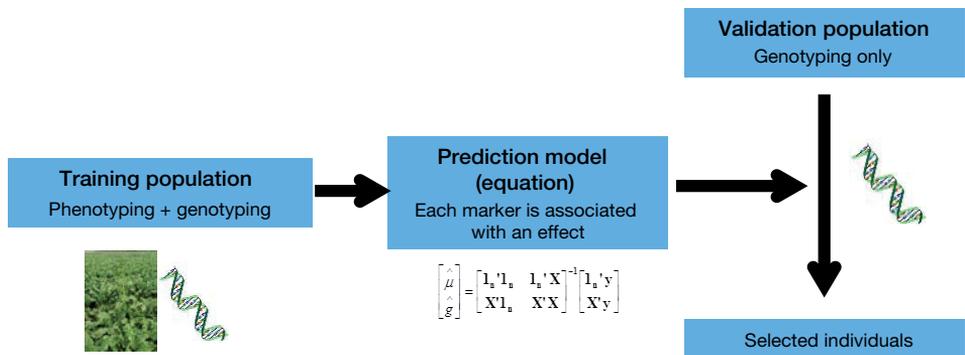
## Genomic selection

The AKER programme was inspired by a method of selection which has proved its worth in dairy cattle and which it has proposed to apply to the selection of sugar beet. Its interest lies in the time savings it offers in selection (acceleration of selection cycles), as well as in the increase in selection precision (selection directly on plant DNA).

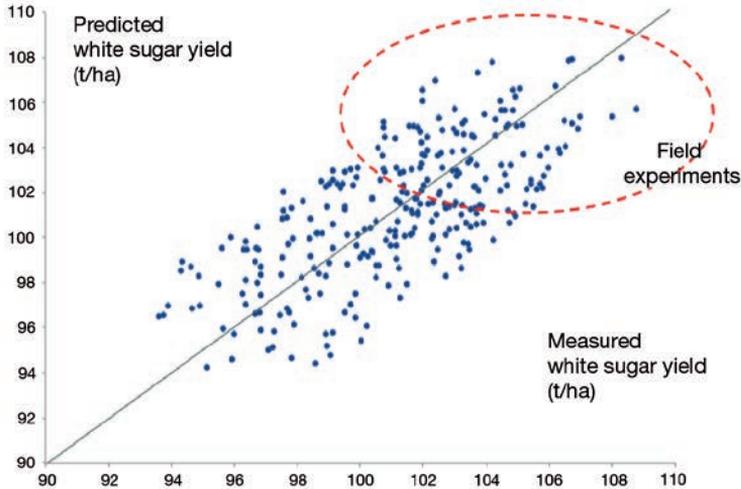
Indeed, for farm animals, the rapid evolution of sequencing and genotyping techniques has raised new challenges in the development of selection methods: the concept of genomic selection was thus introduced in 2001. It proposes to predict simultaneously all the effects of the marked regions of the genome, then to build a genomic index by summing the effects of each region and finally to select the best animals based on this index. Genomic evaluations allow more precise evaluations of individuals and have the ability to rapidly increase the genetic gain of traits of interest.

As part of the AKER programme, this new selection method was implemented on elite populations. First, we wanted to prove that this method was also applicable to the selection of sugar beet by making a proof of concept by cross-validation.

For this, we first took an elite global population for which we had genotyping and phenotyping (real performance of individuals in the fields). From this global population, we defined, on the one hand, a training



Implementation of a genomic selection scheme.



Ability to predict white sugar yield by genomic selection.

population which would allow the model to be calibrated thanks to the parallelization of its phenotyping and its genotyping, and, on the other hand, a validation population of which only genotyping would be used. The training population was used to predict the performance of the validation population, which was then compared to the actual performance available (phenotyping).

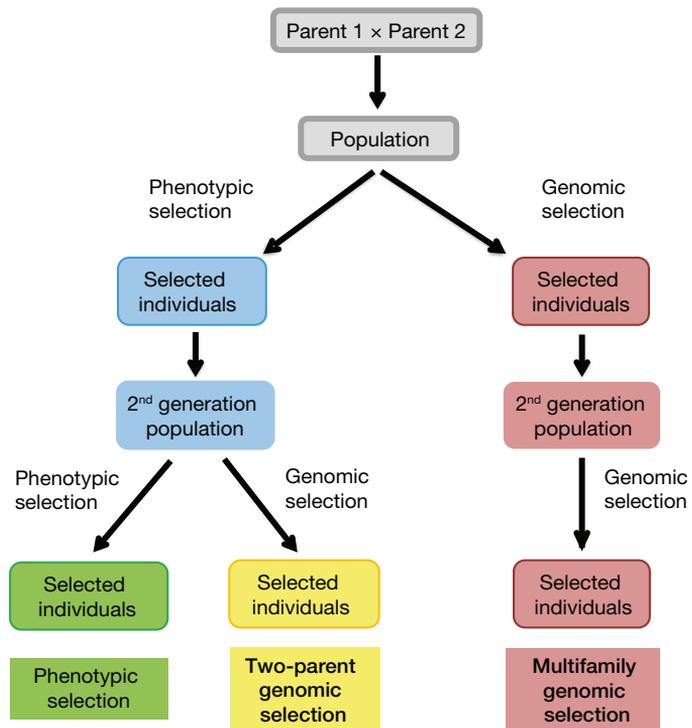
The *in silico* feasibility demonstration (using the computer tool) shows that the genomic selection of beets works with predictive precision of 75–80%. For example, a training population of 1,300 lines of beet provides an average predictive capacity of 0.775 for the “white sugar yield” trait.

This model should then be applied to populations in the process of selection, whose phenotype is still unknown and originating from crosses between two elite parents. In the AKER programme, three main schemes were compared: a phenotypic selection scheme and two genomic selection schemes, one biparental and the other multifamily.

The phenotypic selection scheme proposes two consecutive cycles of selection based on the evaluation of the performance of individuals in the field under multi-environmental conditions. The two-parent genomic selection scheme intends to train the prediction model on the first generation population for which genotyping and phenotyping data in the

field will have been collected. This scheme is called biparental, because the training population of the model comes from the same initial crossover as the population to be selected, but from different generations. As for the second genomic selection scheme, known as multifamily, it proposes to carry out two consecutive cycles of genomic selection using a prediction model created on a historical population from all the selection programs. The prediction model will therefore have been trained on a set of populations, related or not to the population to be selected.

At the end of the AKER programme, the best *in vivo* selection scheme will have been defined by comparing the performance of the selected individuals using the different schemes. The AKER population from crosses between an elite parent and wild accessions can then be taken as a training population, and we can apply genomic selection for the prediction and selection of individuals from post-AKER crosses.



Comparison of classical and genomic selection schemes.

## ELLEN GOUEMAND-DUGUÉ, AT THE SERVICE OF BREEDERS

Ellen Goudemand-Dugué specializes in experimental statistics, genetic mapping, detection of regions of the genome linked to traits of interest... But her main concern is genomic selection on many species. “The value of this method is that it saves time and increases the selection precision it allows,” says Ellen.

The Genetics and Biometrics Laboratory that she heads has the mission of providing tools (bioinformatics, databases, genetics, statistics) in support of breeders to facilitate their daily work. “Our engine is innovation, which feeds on direct contact with public research organizations. Our job is to internalize international public research to appropriate it.” The laboratory’s multi-species activity corresponds to Florimond Desprez’s field of investigation (cereals, beets, potatoes) and promotes transversality.

Ellen Goudemand-Dugué accompanied young researchers as part of the AKER programme. She has often been asked to present it, both in France and at international scientific congresses.



Agricultural engineer, graduate of Agrocampus Ouest, Ellen Goudemand-Dugué is director of the Genetics and Biometrics Laboratory at Florimond Desprez, and specialist in genomic selection.

## ■ Implementation of the information system

Florimond Desprez contributed to the creation of Genoplante, at the origin of the GnpIS information system, transformed for the AKER programme into an open computer tool called AIS.

### GnpIS (Genoplante Information System)

GnpIS is an integrated information system, developed by Urgi (Genomics-Info Research Unit), research unit of Inra (today INRAE). It allows to manage Inra genetics and genomics data for plants and their pathogens. In constant evolution for more than fifteen years, it has been used in all Investments for the Future Programmes – AKER (beet), Amaizing (maize), Biomass for the future, Breedwheat (wheat), PeaMust (peas) and Rapsodyn (rapeseed) – where the version maintained at Urgi serves as an integrated data warehouse and the provision of this data in the long term<sup>2</sup>.

<sup>2</sup> <http://urgi.versailles.inra.fr/gnpis>.

## AIS (AKER Information System)

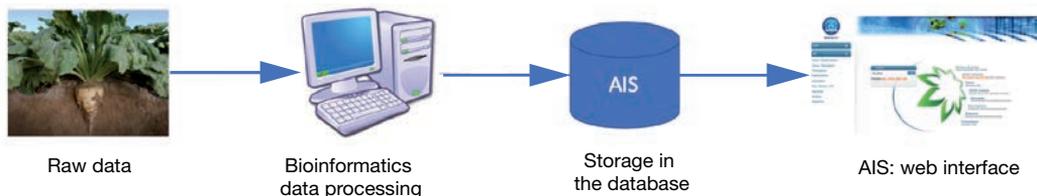
AKER is the very first programme to have developed and maintained a version of the GnpIS information system specialized for beet. To this end, the architecture of GnpIS has been duplicated under the title AIS (AKER Information System) and a physical infrastructure allowing it to be accommodated has been put in place by the Genetics and Biometrics Laboratory of Florimond Desprez. The database structure, as well as the GnpIS web interfaces, were then installed.

AIS integrated from the outset the 10,000 beet accessions listed worldwide, by recording their original contact details, correcting their passport data, so as to match the multiple gene banks into a single bank available to the scientific community. This system also makes it possible to centralize the data produced (genetic resources, genotyping, phenotyping, sequencing, etc.) for the 3,000 accessions initially studied as part of the AKER programme and thus to ensure their sustainability.

All the data useful or generated by the AKER programme has been deposited in AIS:

- the raw data, knowing that the images are not loaded in AIS but that it is possible to make them accessible via a URI (Uniform Resource Identifier). This system guarantees their uniqueness and their durability;
- the data produced from an analysis process, statistical calculations or image analysis software.

In terms of throughput, the AIS structure is suitable: out of the 3,000 accessions studied, no particular problem with loading the results



AKER information processing chain.

## BIOINFORMATICS AND BIG DATA

Bioinformatics is a science at the interface of digital disciplines (computer science and mathematics) and life sciences (biochemistry, biology, microbiology, ecology, epidemiology). As scientists generate an increasing number of new data on genomes, biomolecules, organisms, their interactions and evolution, there is a growing need for computerized approaches to manipulation, storage, visualization and analysis of this often very complex data.

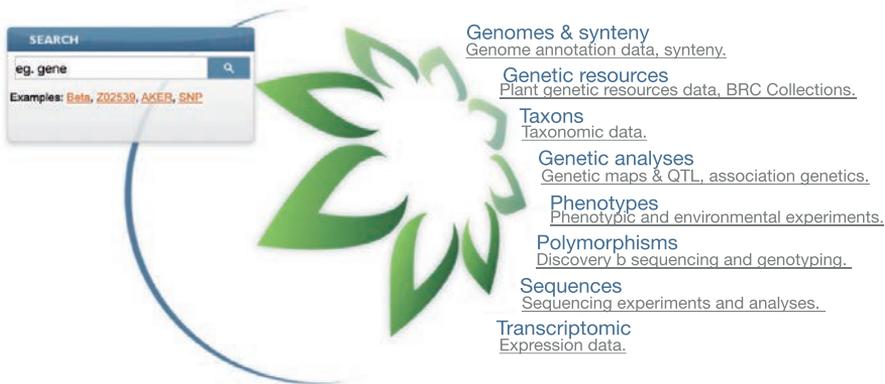
The concept of big data covers a combination of technological advances linked to the massification of data and the use of this data. In fact, a set of technological innovations has transformed the way in which data is generated (in particular with exponential volumes and speeds) in recent years, transmitted, stored and used, and this in an increasingly distributed way (via the cloud).

In addition, these data are very heterogeneous (video, text, sound, image) and the exchanges also follow this tendency to massification. We also observe revolutions in their structuring, their visualization and their search engines (for example Google). These new capabilities to make large volumes of very heterogeneous data talk constitute the third Internet revolution and are based on a “semantization” of this data.

However, the rise of big data is not just about technology. The generation and sharing of information are key elements of the richness and volume of data, and must be compared with cultural developments.

of the experiments was noted. In addition, public data from the AKER programme has also been transferred to Urgi's information system, GnpIS, so as to give them visibility and bring them to the attention of the international community of plant biology researchers.

This information system made it possible to aggregate the data and make it accessible in a user-friendly manner. For this, it helps to manage very large amounts of data. For example, those for sequencing weigh a total of 4 terabytes: 2 for raw data, 2 for generated data. The weight of the information to be stored therefore depends on the filters in place to sift this data. AIS also offers the possibility of viewing essential information at key moments in the programme and of sustaining important results after the analyzes.



Availability of AKER information on the Web.



A geneticist by training, Anne-Françoise Adam-Blondon hopes that information technology will serve genetics and biology in the bioinformatics couple.

### ANNE-FRANÇOISE ADAM-BLONDON: INFORMATION TECHNOLOGY MUST MEET THE NEEDS OF BIOLOGY AND GENETICS

A nod to history, it was during a student visit to Florimond Desprez in Cappelle-en-Pévèle (France), supervised by her professor André Gallais (who would then become a member of the scientific committee of the AKER programme), that Anne-Françoise Adam-Blondon confirmed her vocation for genetics. An agricultural engineer from Montpellier, she then did a thesis in genetics at Orsay, then became interested in the vine after her recruitment at Inra.

In 2012, she joined the bioinformatics unit of Urgi (Genomics-info research unit) and in 2015 took over responsibility for the Information Systems team. "I am not a computer scientist, but rather a biologist and geneticist, says Anne-Françoise, I manage the team's strategy and projects within the Urgi, and then I let my employees do their daily work."

Anne-Françoise appreciates in the AKER community the relaxed and friendly atmosphere that she had already known in the vineyard sector. She was impressed with what she discovered, not only the amount of plant material created and the data generated, but also the development of phenotyping tools.

His wish? "Let the results, in particular in terms of innovative selection methods, be exploited, gradually and lastingly opening up to a wider scientific community."



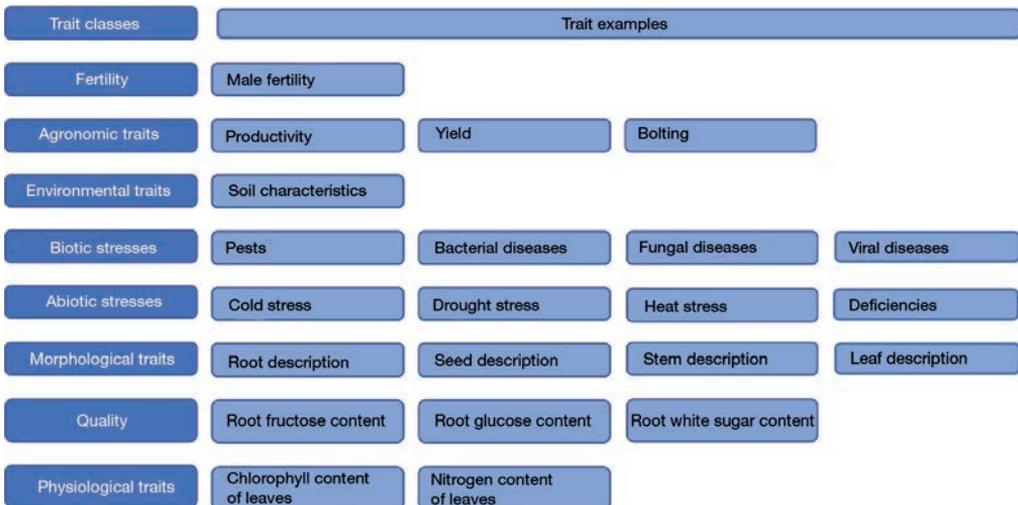
## AN ONTOLOGY FOR BEETS

The AKER programme was an opportunity to develop an ontology (in the computer sense of the term) for beets, which did not exist before\*. This work was carried out by all of the project partners (ITB, Geves, Inra, University of Angers and Florimond Desprez), under the supervision of the Genetics and Biometrics Laboratory of Florimond Desprez and with the contribution of the Urgi expertise at Inra.

An ontology is both a universal vocabulary and structured information that makes it possible to better share data acquired by different researchers and above all to do automated searches. It is a dictionary, a lexicon, a tree structure, a map of relationships between terms that works with cascading logic. This medium, in English, fixes the terms with which the observations made on beet are described, so that the international research community speaks the same language. For example, he rules that we measure the leaf in centimeters and not in inches; we talk about the root and then talk about its length, shape, colour... Currently, around a hundred terms are in place and the first public version is launched.

This beet ontology, which is first intended to be applied to the phenotyping data generated in AKER, evolved during the programme. It has been made accessible by Urgi, and is currently continuing to evolve in order to constantly adapt to the international context of beet phenotyping, far beyond the AKER programme.

\* <https://data.inra.fr/dataset.xhtml?persistentId=doi:10.15454/0JIL23>.



Simplified structure of the beet ontology.



Both from Inra's Urgi laboratory, Daphné Verdelet (right) has now arrived in the North of France, while Dorothee Charruaud (left) has stayed in Versailles. In constant contact throughout the AKER programme, they worked on bioinformatics and the information system.



## **DAPHNÉ VERDELET AND DOROTHÉE CHARRUAUD, A DUO FOR BIOINFORMATICS**

A research engineer in bioinformatics, Daphné Verdelet obtained a research master's degree in bioinformatics and genomics at the University of Versailles. She started her career at Urgi (Genomics-Info Research Unit) working on data integration and development on GnpIS. In 2013, she joined the Genetics and Biometrics Laboratory at Florimond Desprez. She intervened, within AKER, on bioinformatics analyses, the establishment of the infrastructure and essential tools for this type of work, the hosting of the necessary data but also produced within the framework of the program. "Bioinformatics started at Florimond Desprez with the AKER programme," she underlines.

Application development and deployment engineer, Dorothee Charruaud followed a university course before arriving at Urgi: a master's degree in marine biology (fundamental and applied microbiology in the marine field) in Brest, University of Western Brittany, then a professional master's degree of bioinformatics at Talence, University of Bordeaux 1. "In AKER, I captured the needs of biologists to transcribe them into graphical interfaces within the information system," she explains. She helped develop the GnpIS (Genoplante Information System) upstream, then AIS (AKER Information System). To conclude, Dorothee underlines the pleasure she experienced in making AIS evolve.

## WHAT IS THE INTERNATIONAL IMPACT OF THE AKER PROGRAMME?

Mitchell Mc Grath, USDA (US Department of Agriculture) beet and soybean geneticist and chairman of the AKER Scientific Committee, highlights the originality of the project and commends the work accomplished.



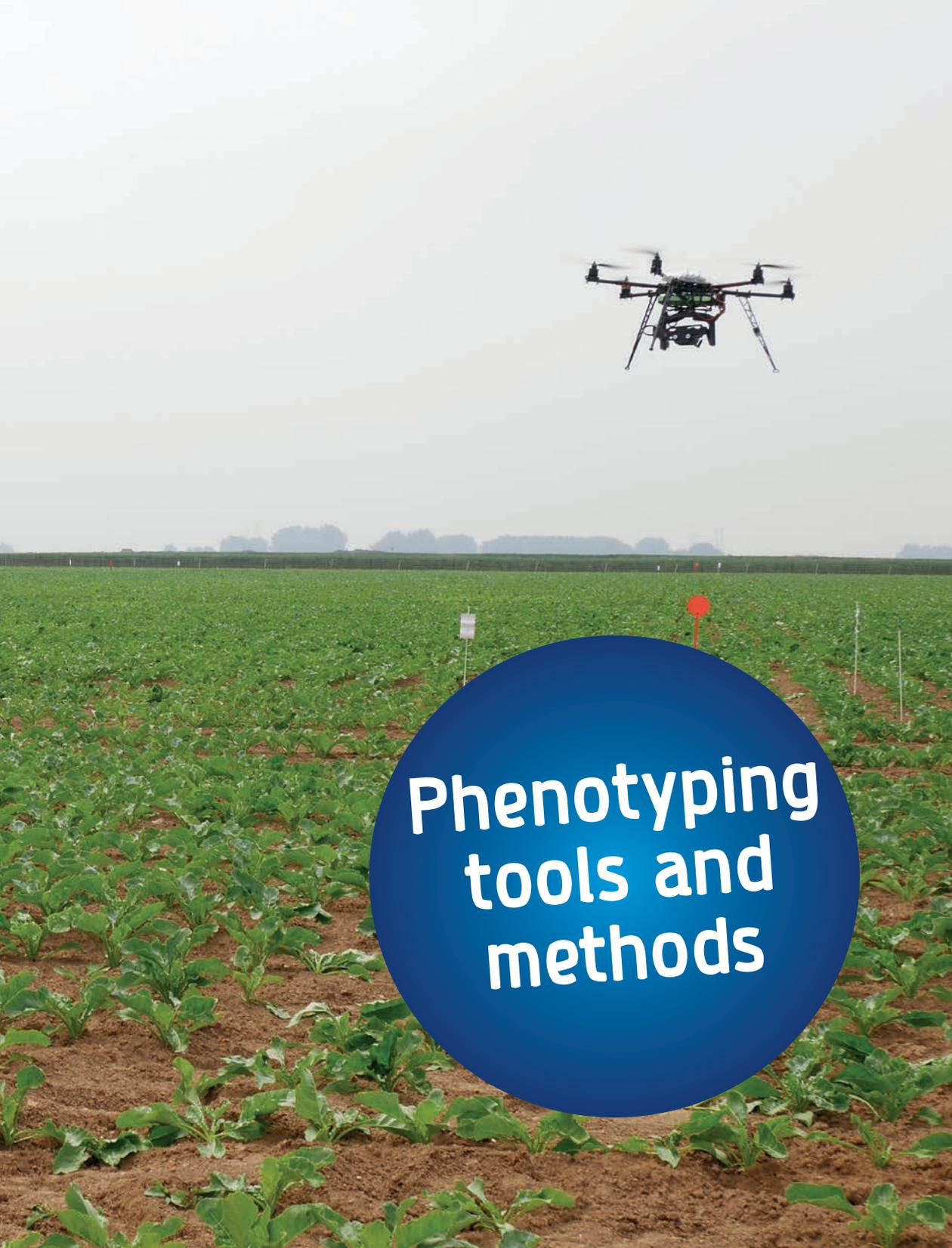
“Congratulations to the AKER Project for reaching this final milestone! This is not the end, but a new beginning. Plant breeding is a long-term endeavor, and the focus of the AKER Project has been to prepare for this new beginning. A solid scientific foundation for future improvements in sugar beet genetics will support the highly productive and storied history of the beet sugar industry, in France, and worldwide. A great deal of progress has been made, and as with any good scientific quest, many new questions are now available for inquiry.

This project could not have been successful without the dedicated efforts of many talented researchers and support staff. Their efforts have been essential. These folks deserve all the recognition and applause for their effort and insight in meeting the goals of AKER, and many of them will continue to be involved in sugar beet improvement for many years to come. After all, they are now the experts in the tools and resources they have worked to develop in bringing new the new tools, resources, and insights to the forefront of modern sugar beet breeding.

As a representative of the Scientific Committee, I can attest that the committee as a whole has been very supportive of the projects conducted under the AKER umbrella. I very much appreciate the time and consideration of both the committee and the AKER leadership in discussing and addressing the inevitable concerns that arise during the course of investigating the unknown, especially a complex biological system such as sugar beet breeding. The breadth and depth of scientific expertise represented by each of these highly trained and scientifically respected individuals has helped keep the project focused on the mission, and in some small way, helped lead to many successful outcomes. We are delighted to have the chance to participate.

Clearly, a large impact has been made by the AKER Project: in breeding and genetics, in agricultural technology development, and in an integrated system allowing connections to made between these essential aspects of sugar beet variety development. These impacts, and the efforts of personnel trained under the auspices of AKER, will be felt for years to come. Again, congratulations to the AKER team in seeing their diligent efforts come to fruition!”



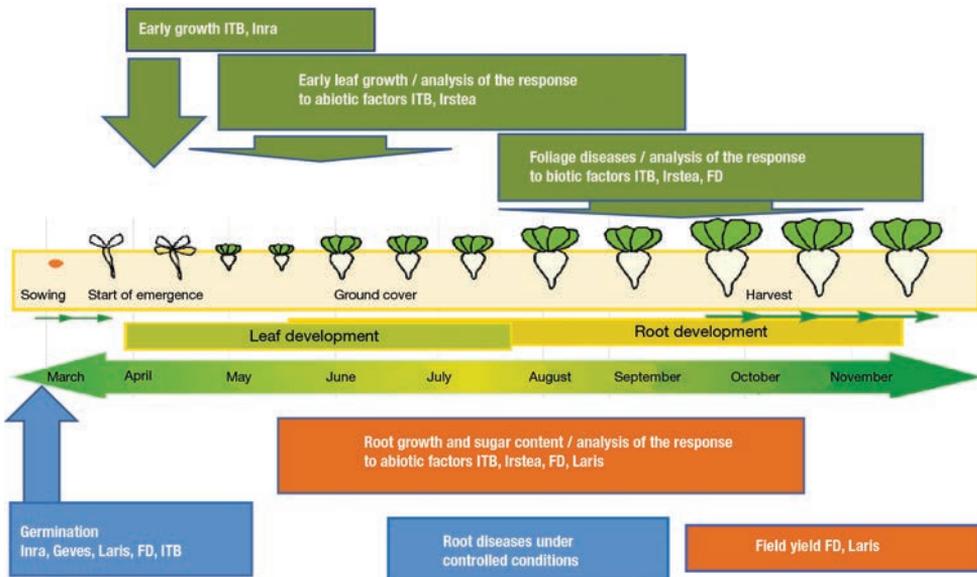


**Phenotyping  
tools and  
methods**



## ■ Phenotyping programme

Phenotyping, or character assessment, consists of studying observable characters (phenotypes). The phenotype is the result of the interaction of the genotype (genes) with the environment. Its accuracy depends on the quality of the tools and methods used. In addition, high throughput phenotyping makes it possible to analyze a large number of individuals directly in a given environment, but also indirectly under different environmental conditions. We talk about phenotyping at different scales: from the cell to the field, via the seed or the plot. In the AKER programme, phenotyping focuses on all the visible stages in cultivation, from seed to harvest.



Phenotyping programme implemented as part of AKER.

## ■ Phenotyping of seeds and seedlings

The phenotyping of seeds consists of evaluating their physical and physiological characteristics using instrumentation and imaging techniques. It has two parts: a descriptive part (structural, physical and chemical analysis of the seed), and a physiological one (germination of the seed and growth of the seedling under different controlled conditions). The object of the study is therefore the seed and the beginnings of the emergence in the field.

### Phenotyping objectives

Researchers involved in the AKER programme at Inra, Geves and the University of Angers are working to develop innovative methods for assessing the physical and germination quality of beet seeds. For this reason, they favour automated high-speed phenotyping driven by IT. At the end of the AKER programme, 3,000 genotypes were thus analysed.

The main objective is to check whether there is genetic variability in the structure of the seed, at the stage of germination and at the start of

### CONSEQUENCES OF SEED PHENOTYPING FOR THE SECTOR

Considering the importance of agronomy for beet growing, relevant phenotyping is based on good knowledge of yield development and its modelling. Indeed, the yield is a function of multiple factors such as the speed of soil cover by the plant, or the amount of water or nitrogen available. Hence the importance of studying the rapidity of the establishment in cold conditions linked to increasingly early sowing, the development of leaves in the face of diseases, resistance to water stress, nitrogen assimilation. In addition, faster soil cover limits weed development, resulting in less use of herbicide treatments.

For beet grower as well as for sugar manufacturer, the final objective consists in obtaining the best possible economic return, taking into account the optimization of the processes and their environmental impact. The consumer, meanwhile, is sensitive to the final cost of the product, the location of production and respect for the environment. Undeniably, for the advisors of the sector, the AKER programme makes it possible to better understand the needs of each of the players so as to provide them with the tools to meet them. The advice will take more and more importance in the dynamics of the beet-sugar sector.



seedling growth. The researchers are particularly interested in the early phases: the seed and its germination under favourable conditions, or on the contrary under stressful conditions (for example at low temperatures). All these measurements, carried out at an early stage, going as far as the germination and growth of a very young seedling, should make it possible to feed prediction algorithms for a set of characteristics linked to adult plants. Thus, the sugar level of an adult plant can be predicted by the sugar level of the seedling, and its rooting capacity by the speed of root elongation.

## Tools for phenotyping seeds and seedlings

Inra, the University of Angers (Laris) and the Geves jointly designed and developed imaging and digital processing tools, the performance of which was evaluated and improved throughout the programme. Different modern imaging methods, inspired by biomedical imaging, have been tested for their contribution to plant phenotyping. In terms of research, certain studies have appeared to be “first” in their application to beet phenotyping. In addition to these fundamental laboratory studies, the phenotyping techniques identified as the most relevant have been developed up to routine implementation, by dedicated automated systems, on large populations of seeds under various conditions. Digital image processing methods have also been widely developed. Specific techniques have been developed and implemented to reproduce by computer the criteria of human experts in the analysis of phenotypic traits. The great variability in behaviour of AKER genetic material also required numerous technical adaptations, such as pushing the limits of the conditions of observation.

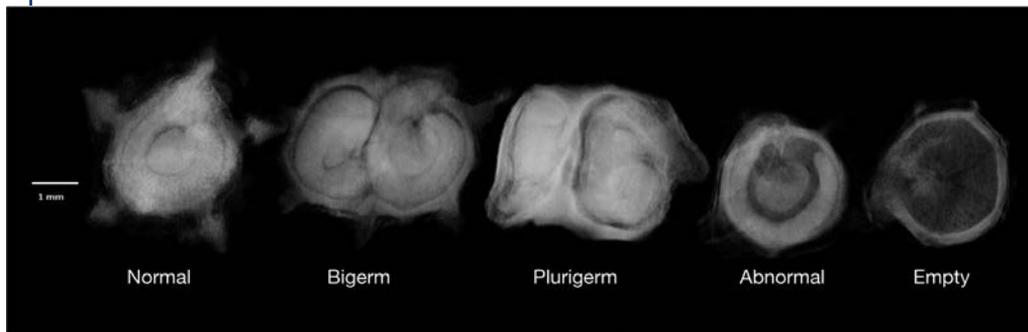
### *Radiography*

It is a method using X-rays to observe the interior of an object. It provides an image of its internal structures, the contrast of which depends on the thickness and the attenuation coefficient of the structures crossed. This technology is non-destructive and non-invasive. Applied to seeds, the X-ray makes it possible to observe the different internal components of the seed – embryo, perisperm (reserve) and seed coat (wall) – and to highlight characteristics such as empty seeds, abnormally formed seeds, damage insects or other damage.

### 2D radiography

It reproduces a two-dimensional (2D) image of a three-dimensional (3D) object. It has been applied routinely on seeds for several years. This technology has the advantage of being fast but, in most cases, 2D radiography has mainly a qualitative interest. As part of the AKER programme, it was mainly used to pre-sort seeds before 3D analyses (more precise but longer) and germination analyses. Thanks to its high throughput, this technique has made it possible to sort and eliminate abnormal, empty, bigerm and plurigerm beet seeds.

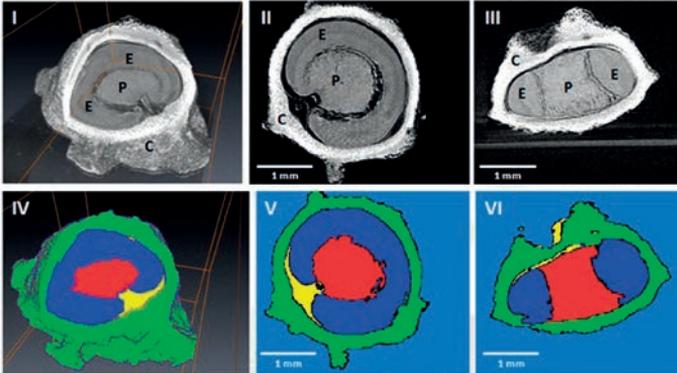
Beet seeds pictured in 2D radiography and presenting the pre-sorting carried out as part of the AKER programme.



### 3D X-ray tomography

This technology makes it possible to generate 3D and high-resolution images. The principle is as follows: the 3D image of the internal morphology of the object is obtained from a large series of 2D radiographic images taken around a single axis of rotation. These 3D images are then analyzed using image processing tools. Unlike 2D radiography, the analysis rate is slower but this technology will allow access to very precise quantitative measurements, of volume but also of contact surface of the seed compartments.

During the AKER programme, a new method for phenotyping the internal structures of beet seeds by 3D tomography was developed and the analytical throughputs gradually increased. This method allows a detailed characterization of seeds of different genotypes by extracting fourteen traits of interest. An automated image processing chain enables the segmentation and individualization of the three main components of the seed (embryo, perisperm and seed coat). In this way, the volumes, surfaces and shapes of these internal components as well as the



Segmentation of beet seeds images. 3D volume rendering showing the embryo (E), the perisperm (P) and the seed coat (C) with (I) a wedge section, (II) a cross section, and (III) a longitudinal section. The segmentation shows separate components with different labels (blue for E, red for P and green for C for each section view [IV, V, VI]). Yellow represents the void: the filling rate takes this into account.

filling rate of the seeds were evaluated. For the first time at the global level, this device has made it possible to highlight large differences in the internal structures of seeds within a population of beets from a single cross.

### SYLVIE DUCOURNAU, INNOVATIVE METHODS FOR SEED PHENOTYPING

“Thanks to its National Seed Testing Station (Snes), Geves is, with Inra, which has become INRAE, the biological side of imagery,” explains Sylvie Ducournau. The mission of the Snes germination laboratory is to assess the germination quality of seeds to determine their germination potential, and to provide results for the marketing of seed lots in France and abroad.

As part of the AKER programme, “it was a question of developing new innovative methods which made it possible to assess the germination quality, but also the physical quality of beet seeds, with phenotyping if possible automated”, continues Sylvie.

Thus, Snes has germination benches which operate within the framework of the Phenotic platform. It also includes the Laboratory for Physical Seed Analysis, which in 2013 acquired a tomograph, a new three-dimensional X-ray imaging tool. “The team fought alongside Bruno Desprez to acquire this equipment as part of the AKER programme, because they believed in its potential,” recalls Sylvie.

She summarises the seed phenotyping approach implemented in Angers: “We had to develop new methods, adapt them to beet, extrapolate them at high speed and produce results.”

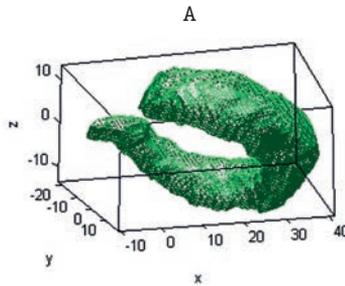


Head of the germination laboratory at Snes, within Geves, Sylvie Ducournau coordinated work on the phenotyping of seeds for the AKER programme.

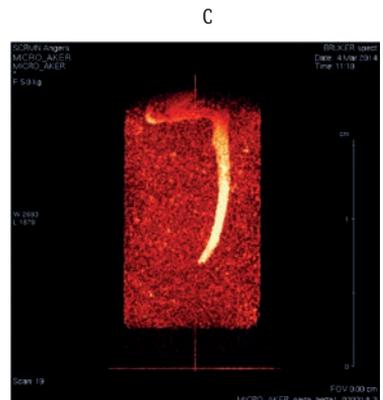
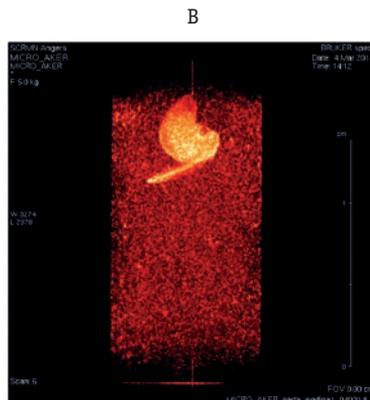
### MRI (magnetic resonance imaging)

It allows non-destructive and non-invasive 3D mapping of essential biochemical constituents of the seed and seedling. At the start of the AKER programme, very few studies existed regarding the application of MRI to beets. MRI has been shown to locate and quantify the lipids that characterize the embryo, an essential organ for the early stage of plant development.

MRI has also been used to characterize in 3D the spatiotemporal dynamics of the water entering the seed during the imbibition phase which triggers germination. The functional information provided here by MRI is usefully coupled with the anatomical information from X-ray tomography which identifies networks of microchannels distributed in the semen. Here we access non-invasive imagery of an essential phase of the process, from an inert dry seed to a growing living plant.



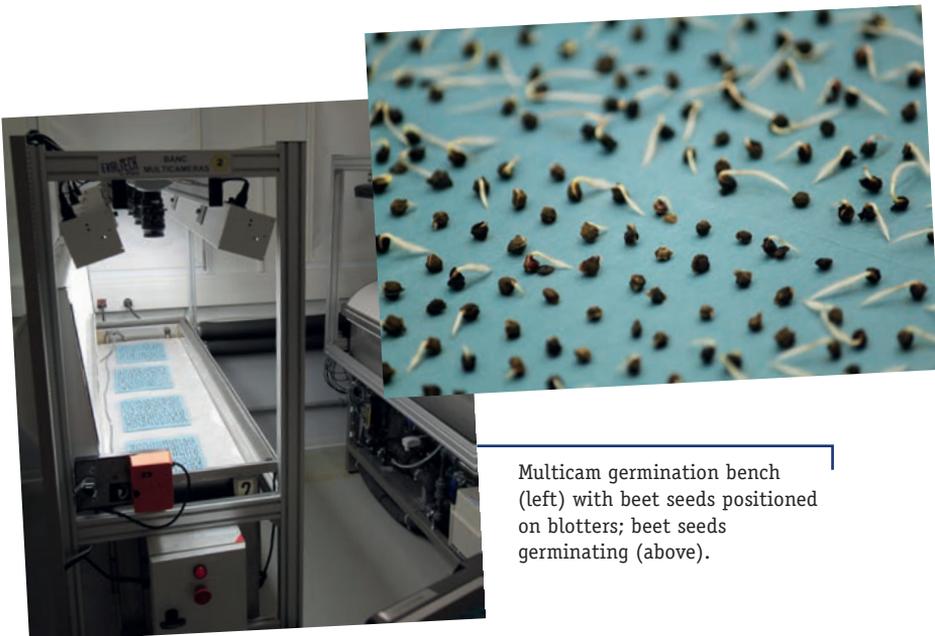
Typical images of 2D sections and 3D MRI of beet seeds.  
 A: visualization of lipids allowing that of the embryo.  
 B and C: visualization and quantification of the dynamics of imbibition triggering the germination and growth of the plant.



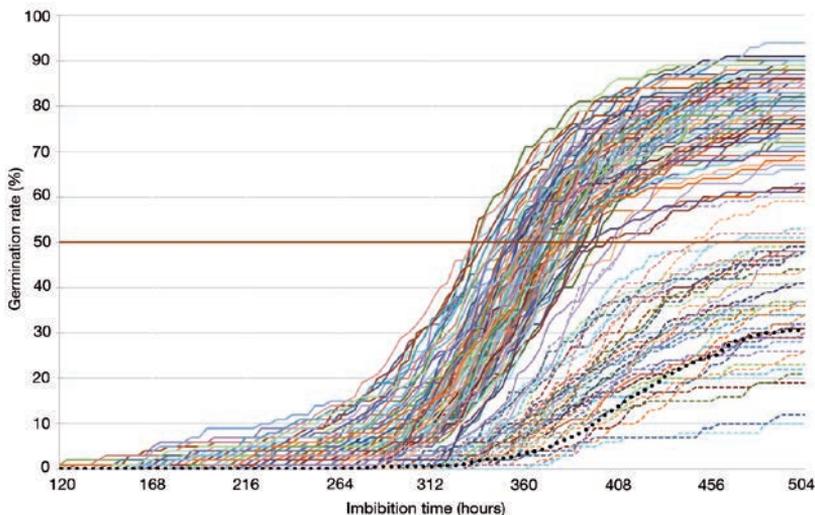
### ***Multicam automated germination benches***

These benches were used to germinate beet seeds on blotter supplied with water continuously, in order to study the kinetics of germination at different temperatures. This equipment was developed in the early 2000s and has been continuously improved during the AKER programme for beet seeds, on the phenotyping rate and on the widening of the temperature range. Thus, we can now phenotype the germination of 2,400 beet seeds simultaneously on the same bench and widen the germination temperature ranges between 5 and 20°C. Automation of image processing allows data and curves to be produced to assess the speed of germination.

Once the seeds are placed on the blotter, images are taken automatically at time intervals chosen according to the duration of germination: every two hours when germination is carried out at 10 or 20°C and every four hours when germination is slower at 5°C. The detection of the germination time on the scale of the individual seed is carried out thanks to an automated processing of images based on the movement of the seed during its germination and on the appearance of its radicle. The cumulation of individual measurements on all the seeds and of the images taken over



Multicam germination bench (left) with beet seeds positioned on blotters; beet seeds germinating (above).



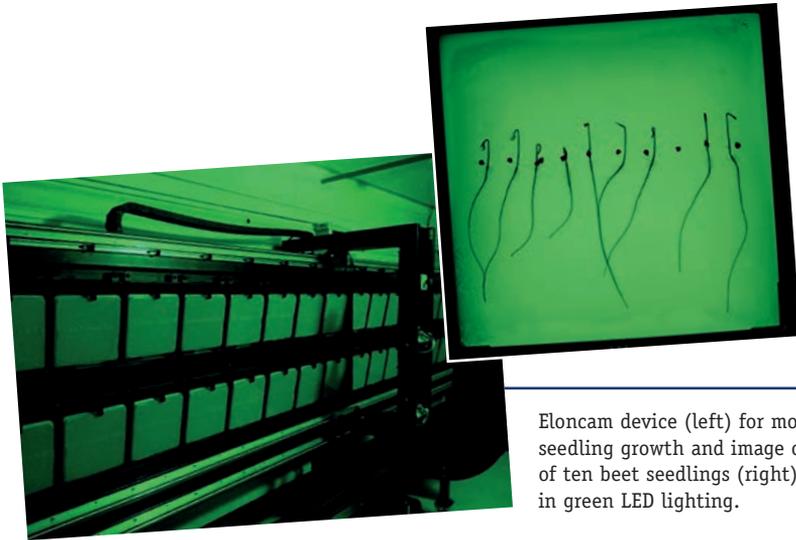
Germination kinetics of 136 beet genotypes, produced during the AKER programme, exhibiting contrasting behaviors at a temperature of 5°C.

the duration of the germination process makes it possible to produce kinetics of germination and to assess the speed of germination (for example, time required to reach 50% of germination or percentage of germination after x hours or days).

Four germination benches are available for these trials, within Geves, partner of the Phenotic platform. This high throughput has made it possible to phenotype, during the last two years of the AKER programme, 2,744 genotypes for their germination at 5°C. Then, on the basis of these results, the 136 most extreme genotypes were selected and phenotyped more completely for their germination at 10 and 20°C. Finally, their growth speed was determined using the Eloncam device (see below) and their internal morphology thanks to 3D X-ray tomography (see above).

### ***The Eloncam automated bench***

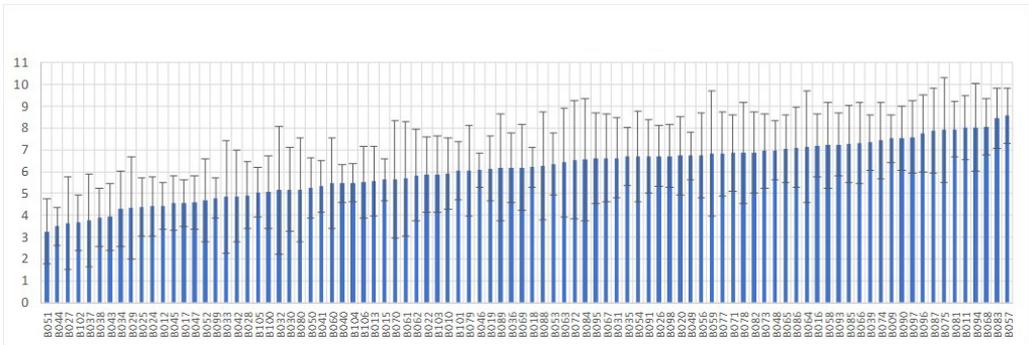
The principle of this bench is to use a camera under green light inactinic to acquire images during the growth of seedlings. This device models the growth after germination and underground of the seedling, which is a decisive phase. In addition, certain traits of root growth are likely to be correlated with those of growth at more advanced stages of the plant.



Eloncam device (left) for monitoring seedling growth and image of a box of ten beet seedlings (right), taken in green LED lighting.

The images are analyzed digitally to measure the elongation of the seedling and its various organs of interest (radicle, hypocotyl, cotyledons) during the first stages of growth. The platform allows 400 growing seedlings to be filmed simultaneously.

During the AKER programme, the Eloncam device was used for greater capacity, for relatively low temperatures (10°C) and without any problem of malfunction over long periods. Jointly, computer programmes have been developed for the analysis of images measuring the evolution of the



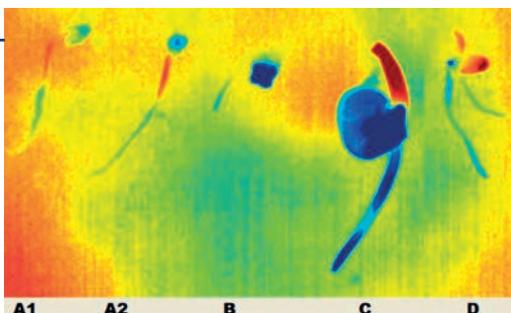
Elongation rate in mm/day measured at 10°C on 20 seedlings from 93 beet genotypes.

lengthening of the seedlings and their three organs of interest. The automated measurements were compared to the measurements carried out manually. It is thus possible to obtain, for the selected beet genotypes, comparative characterization elements.

### *Thermographic imagery*

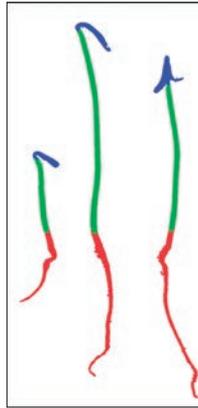
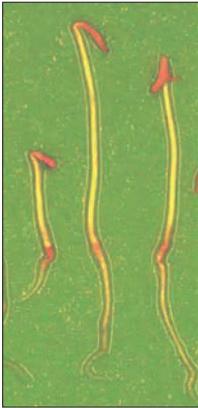
This allows to map temperature contrasts finely by translating them into colors. This imagery does not require lighting. It has notably been used to follow the growth of the essential organs of the seedling under real conditions of darkness. It has also been tested to non-destructively dose sugars in the seedlings and their internal organs.

Thermography images of developing seeds and seedlings, with temperature contrasts allowing in particular to distinguish and follow the growth of the three essential organs of the seedling. In the image, the root part appears (in blue) colder than the aerial part (in red).



### *Hyperspectral imagery*

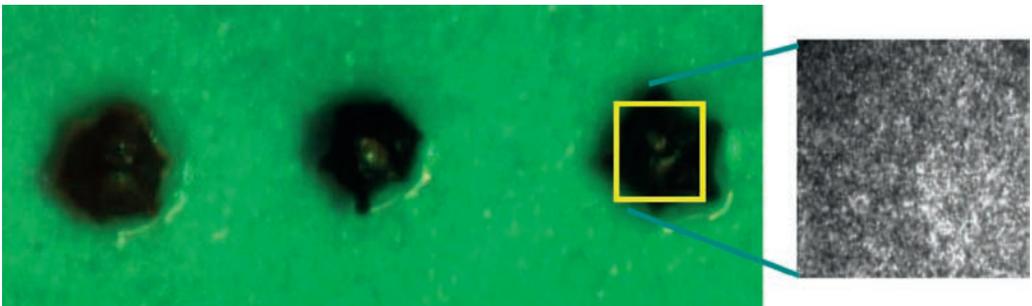
This type of imagery allows images to be produced in a very large number of spectral bands (160 for AKER studies), when standard colour imagery is limited to three spectral bands around the three primary colours red, green and blue. The hyperspectral also delivers images that are no longer limited to the visible spectrum, but extended in the infrared. It is thus possible to obtain much richer information to observe and characterize plant structures and especially their chemical composition. As part of the AKER programme, we were able to achieve, for example, automatic segmentation of the internal organs of the seedlings. This technique, however, requires lighting in a broad spectral range; it is therefore not directly applicable to darkness.



Hyperspectral images of seedlings where the exploitation of the best adapted spectral bands makes it possible to distinguish, detect and measure invisible or barely visible characteristics in standard imagery.

### ***Biospeckle imagery***

This imagery uses coherent laser illumination to obtain images at a submicron spatial resolution corresponding to the wavelength of the incident light. This non-destructive technique makes it possible to follow the surface conditions and their evolution on plant samples. On seeds, for example, the technique can be applied for the detection and monitoring of the development of pathogens. We can also tackle the characterization of the seed imbibing process, by monitoring peripheral water flows, their establishment, intensity, decrease, stopping. While MRI gives a volume view of water flows in the seed, but using a sophisticated and expensive instrument, the biospeckle gives a 2D surface view of water flows using much less expensive instrumentation and therefore more easily accessible.



Typical beet seeds biospeckle images allowing the characterization of surface conditions, microroughness, micromovements, at the submicron scale of the wavelength of an incident laser beam.

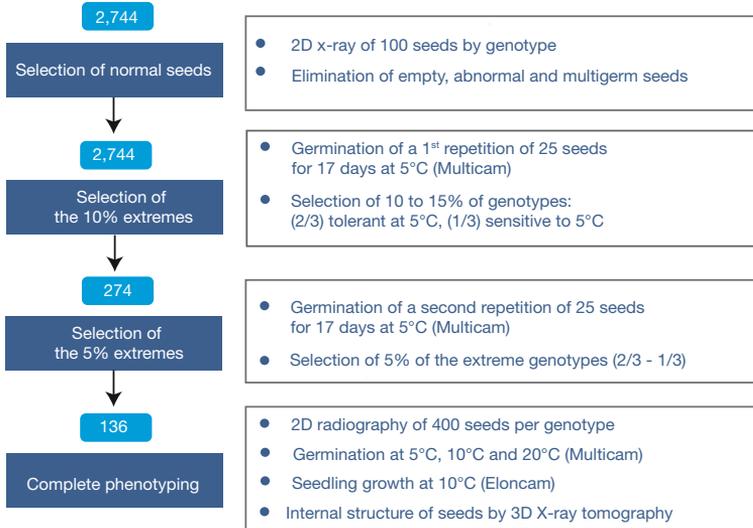
## PROSPECTS OFFERED BY THE DEPLOYMENT OF PHENOTYPING TOOLS

Besides prospective imaging techniques, whose acquisition capacities are still relatively reduced, other equipment already developed on other species and other projects have been adapted and optimized for the phenotyping of beet seeds: that is Multicam and Eloncam equipment. RX 3D tomography has been implemented and developed specifically for the AKER programme. All of these three pieces of equipment, along with automated image processing methods, enabled the phenotyping of the approximately 3,000 beet hybrids produced during the programme. Over its duration, AKER has supported and contributed to the deployment of information science and technology in the service of plant phenotyping. We now have a set of imaging instruments and techniques that can be used for many other studies, on a wide variety of cultures, to progress both in basic knowledge and in practical mastery of phenotyping.

The most advanced computer processing at the end of the programme exploits the most recent methods of artificial intelligence and machine learning. Rather than being based on an explicit statement of the criteria to be programmed by computer for the extraction of phenotypic characters, these new approaches are based on machine learning by computer. From a set of images previously classified and annotated by the expert, the computer system is able to adapt in order to infer by itself operating rules integrating the approach of the human expert. All these advances in instrumentation and data processing for phenotyping open up broad prospects for progress in better knowledge and mastery of plants.

### Synthesis of the seed phenotyping protocol

Thanks to the developments carried out throughout the AKER programme, with the objective of multiplying the tools and increasing the phenotyping throughputs, it was possible to phenotype in a very short time a very large number of beet genotypes, within the framework a sequential phenotyping protocol on seed numbers reduced at the start to 2,744 genotypes. The objective of this protocol was to select 5% of all genotypes based on their extreme germination behaviors at 5°C, then to complete their phenotyping with germinative characteristics (at 5, 10 and 20°C), growth at 10°C and internal structure. The quality characteristics of the seeds of these 136 extreme genotypes must be compared, on the one hand, to their behavior in the field (at the early and more advanced stages) and, on the other hand, to the genetic structure of the material produced, so as to identify any characters of interest.



Successive stages of phenotyping seeds from 2,744 beet genotypes during the AKER programme.

## FRANÇOIS CHAPEAU-BLONDEAU: AKER BRINGS TOGETHER THE “HARD” SCIENCES AND THE LIFE SCIENCES

François Chapeau-Blondeau is part of the Stic (Information and Communication Sciences and Technologies) community, focused on the “hard” sciences. He is a member of Laris, which brings together 50 teacher-researchers, doctoral students and trainees from the University of Angers, ten of whom are involved in work on plants.

“The interface with the life sciences is very rich in terms of scientific questioning, says François, this creates a real dynamic. Indeed, the reputation of the Angers region in the field of plants and seeds constitutes a strong pole of structuring. “Of course, plants are not our nominal area of competence, he continues, but there is a real synergy between the disciplines. Biologists need to combine the skills of physicists, and Stic need concrete impact and applications for their work. In addition to the plant sector, that of the medical sector has indeed benefited from the collaboration of the members of Laris for a long time.

This partnership has been beneficial for the AKER programme, which François Chapeau-Blondeau confirms: “AKER has made it possible to develop the dynamics of the Phenotic phenotyping platform, to bring the ‘hard’ sciences closer to the life sciences, in a more formalized, more conceptualized way,” with the prospect of using these tools for other plant species.



A professor at the Faculty of Science at the University of Angers, François Chapeau-Blondeau is attached to the Department of Physics and participated in the development of imaging methods for phenotyping seeds.



## ■ Phenotyping in vegetation, in the field

The solution combining multispectral on-board drone imagery and vegetation index estimation methods seems to satisfy all the operational constraints required for high-speed phenotyping of beet canopies in the field. It was implemented in 2018 and 2019 to phenotype the 3,000 AKER genotypes.

### Phenotyping of aerial parts

Alongside the classic phenotyping variables (yield, visual disease ratings, etc.), methods have been developed as part of the AKER programme to characterize the development and growth of cover throughout the crop. Among these variables, the structure of plants (green fraction, leaf index, etc.) provides information on their ability to intercept incident photosynthetic radiation, while the content of leaf chlorophyll provides information on their ability to absorb this radiation intercepted for photosynthesis. These structural and biochemical variables advance knowledge of the behaviour of varieties in their interaction with their environment. They are also valuable indicators of possible stress.

To access these measurements, optical remote sensing is an essential tool for estimating variables non-destructively over time. Indeed, most of them affect the spectral response of plant cover between 400 and 2,500 nm, and are therefore theoretically measurable via multi- or hyperspectral sensors on board a satellite, plane, drone or terrestrial platform.

### The requirements of phenotyping in the field

The specific case of field phenotyping imposes a certain number of operational constraints on the use of remote sensing. The characterization of test plots of a few square meters requires observations with high spatial resolution (typically, of the order of a few centimetres, or even less). In addition, these measurements must be carried out with relatively short revisit times in order to be able to follow the critical stages in the development of the culture. They must also have sufficient throughput in order to be able to characterize the approximately 3,000 genotypes created in AKER. Finally, the estimates must be precise enough to properly discriminate the genotypes. During the programme, various phenotyping solutions were considered, from optical measurements carried out on the ground to the development of methods dedicated to multispectral imaging on board a drone, a technique ultimately used for phenotyping.

## GILLES RABATEL, HYPERSPECTRAL IMAGING IN THE FIELD

“Our work on beets has made it possible to supplement our knowledge and expertise in the field of measurement in the field by conventional and hyperspectral imagery, by encountering new difficulties compared to our previous experiences on perennial crops and on wheat. Beet taught me humility!” says Gilles Rabatel. It is part of the “Multi- and hyperspectral imaging” division within the COMiC laboratory (Optical sensors for complex environments) in Montpellier.

“In 2015, Irstea and the ITB started their reflections on a global prototype for phenotyping in the field,” recalls Gilles. “We have evaluated and developed various phenotyping tools and methods for measuring the height of plants and leaf area, the nitrogen and / or chlorophyll content of leaves, in comparison with reference measurements.” These are photogrammetry, VNIR and SWIR hyperspectral imaging, radiometry. Irstea researchers also looked at the possibility of switching from a hyperspectral image to a multispectral image.

In the process, “Irstea recommended a solution installed on a drone with different alternatives depending on the number and importance of the measurements to be made,” summarizes Gilles Rabatel. ITB and Hiphen have continued to investigate this.



Research director of Irstea, now INRAE, in Montpellier, Gilles Rabatel has a long experience of imaging in many applications, notably for the phenotyping of the beet leaf within the framework of the AKER programme.

## First exploratory work in proxidetection

### *Photogrammetry*

During the first three years of the AKER programme, several proxidetection imaging techniques were explored to estimate the structure and biochemistry of the beet leaf cover. First, a study was carried out to examine the value of photogrammetry to characterize the structure of the vegetation. The principle of this technique consists in constructing a 3D image from partially superimposed RGB images, acquired from different viewing angles. This allows direct access to the plant architecture via 3D models. These models not only make it possible to very estimate the height of the vegetation precisely, but also to reconstruct the leaf area for the early stages. On the other hand, the reconstruction process can be considerably disturbed in the presence of wind, and overlaps between leaves lead to underestimations of the leaf area for advanced stages.

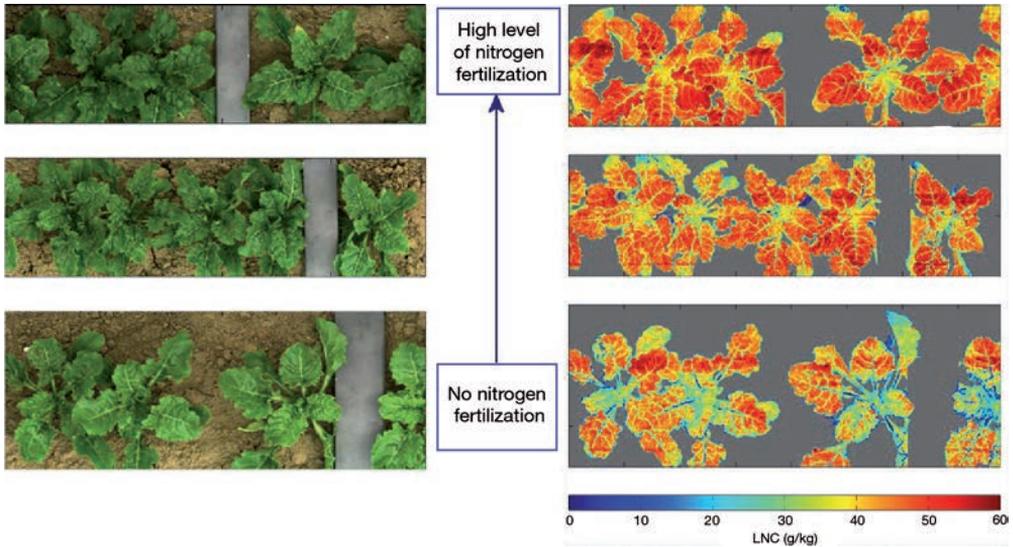
### *Hyperspectral imagery*

The AKER programme then studied the potential of hyperspectral proxidetection imaging to estimate the nitrogen content of beet greens. In 2013 and 2014, the experimenters collected a large amount of data including several sites, stages of development, varieties and levels of fertilization. They acquired many hyperspectral images in the VNIR (Visible and Near Infrared Range, 400–1,000 nm) and SWIR (ShortWave Infrared Range, 1,000–2,500 nm) areas. The shots were taken 1.50 m above the ground, achieving a spatial resolution on the order of a millimetre.

In parallel, reference measurements of chlorophyll, nitrogen and leaf index contents were carried out. The calibration was first done by PLS (Partial Least Squares) regression, which showed acceptable results in the VNIR. On the other hand, disappointing results were obtained in the wavelength range of SWIR, the effect of nitrogen being masked by that of water present not only in the leaves but also in the atmosphere.

Robotic bench used for the acquisition of hyperspectral images in the field.





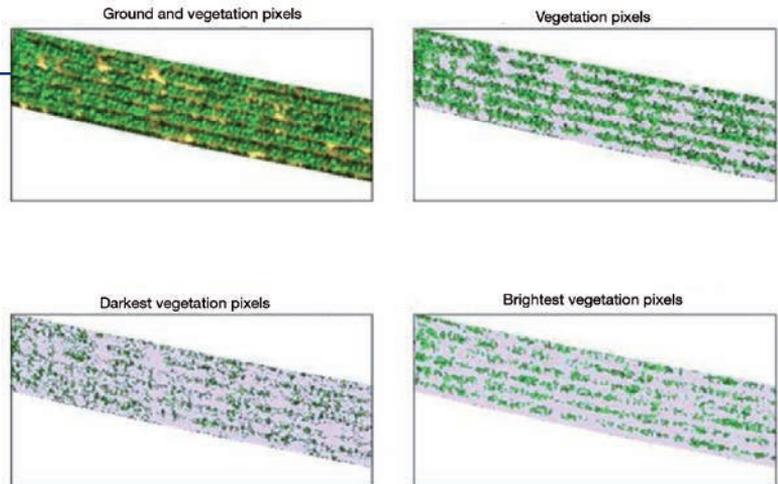
Despite the correct results obtained in VNIR, the relevance of using multivariate statistical methods (PLS) for phenotyping was questioned at the end of 2014. In the particular case of nitrogen content for example, the correlation with the chlorophyll content is too dependent on years, stages of development, soil-climatic conditions or even varieties. This situation is not compatible with the phenotyping of thousands of varieties that have not been integrated into the calibration database.

From 2015, the researchers studied the possibility of simplifying the acquisition of images by judiciously choosing a few representative bands in the spectrum, for the sake of economy and speed of execution. Indeed, acquiring a multispectral image is instantaneous, while acquiring a hyperspectral image requires from a few seconds to a few tens of seconds depending on the number of bands. New spectral processing methods have therefore been explored, based on the calculation of vegetation indices or on the inversion of the Prosail radiative transfer model. Vegetation indices are obtained from a combination of two to three wavelengths.

From hyperspectral images in the VNIR domain, different vegetation indices were thus tested by varying the spatial resolution and the choice of pixels used to calculate the indices. These included indices published in the scientific literature, but also optimized indices thanks to the numerous spectral bands available with the VNIR camera. In order to estimate

Nitrogen mapping performed by VNIR hyperspectral imaging.

Choice of pixels allowed by milli- to centimeter resolution.



chlorophyll, and for sufficiently fine spatial resolutions, the use of a reference band located at the level of the main absorption peak of chlorophyll (around 440 nm) is generally preferable to a reference band conventional use located in the near infrared. In particular, the vegetation index mNDblue, constructed from the wavelengths at 440, 728 and 850 nm and calculated on the brightest vegetation pixels, is significantly better at estimating the chlorophyll content of the leaves (errors relative to less than 10%) compared to all the other vegetation indices tested.

By this method, the exploitation of the milli- to centimetre resolution makes it possible to exclude the ground pixels for the calculation of the vegetation indices, while the use of the brightest green pixels makes it possible to minimize the effects linked to the plant architecture. Through this work a single multispectral, centimetre image acquired vertically is sufficient to characterize each test plot.

### ***Spectroradiometry***

To enrich the experimental database, the experimenters also used the Fieldspec spectroradiometer (ASD) from 2015. Even if this tool does not allow access to images, it has the advantage of being quick to set up work in the field to validate the results obtained with the hyperspectral camera. The measurements were made at different inclinations of the device.

The information obtained in oblique sight, therefore without apparent ground, makes it possible to obtain results close to those obtained with the hyperspectral camera on the pixels of vegetation.

Spectrum processing methods were based on calculating vegetation indices or inverting the Prosail radiative transfer model. The optical measurements were made 1.50 m above the row, providing a spatial resolution of around 70 cm, and in three directions (one aimed vertically and two obliquely aimed).

Vegetation indices have shown satisfactory results. Indeed, good performances (relative errors less than 10%) were obtained for the estimation of the leaf area and the nitrogen and chlorophyll content of the leaves. These results thus solve the problem of underestimating the leaf area encountered with photogrammetry for the most developed canopies.

Fieldspec spectroradiometer (ASD) used for carrying out field measurements at different inclinations.



In all cases, the performances obtained with the vegetation indices are better than with the inversion method of the Prosail model.

In conclusion of this exploratory work, the researchers showed that a single multispectral sensor is sufficient to characterize the structure and biochemistry of beet leaves through the use of vegetation indices. At the end of 2015, high-speed phenotyping by multispectral imaging could then be considered.

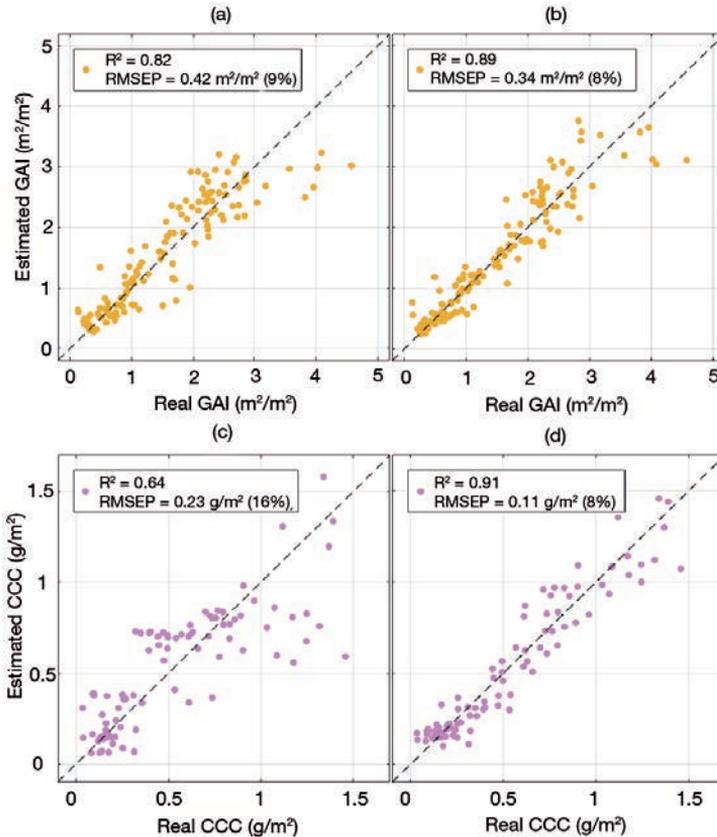
### Towards high speed phenotyping

To phenotype 6,000 test plots in one day, very high speed was essential. So in full swing, the use of RGB and multispectral cameras on board a drone appeared to be the best solution, making it possible to acquire enough spectral information in a reasonable time. The Airphen multispectral camera (Hiphen) was chosen for the relevance of its six spectral bands between 450 and 850 nm.

Hexacopter drone used for phenotyping as part of the AKER programme.



As of 2016, methods based on the use of vegetation indices were applied to images acquired by a multispectral camera on board a drone. Again, numerous experimental data were collected between 2016 and 2017 to validate that the measurements acquired by drone made it possible to obtain results similar to those obtained from ground measurements. Exploiting the centimetric resolution of multispectral images via the calculation of vegetation indices makes it possible to correctly predict the amount of chlorophyll and nitrogen in the leaves.



Results of estimation of agronomic variables from multispectral imagery on board a drone. GAI: leaf area; CCC: amount of chlorophyll at the canopy scale. The vegetation indices are either calculated on the average reflectances of the microplots (a, c), or from a subset of pixels (b, d).



An engineer at ITB, Ghislain Malatesta is responsible for the Experimentation and regional expertise department. He contributed to the acquisition of data for phenotyping, notably by drone, within the framework of the AKER programme.

### **GHISLAIN MALATESTA, DRONE PILOT**

Ghislain Malatesta was born, so to speak, among beets, more precisely on the family farm located in Rouvres-en-Plaine (Côte-d’Or), near the former Aiserey sugar refinery. He obtained a BTS in Plant Production at the Lycée Agricole in Dijon-Quétigny and started his career in 1993 at ITB on the theme of low sugar syndrome.

Ghislain contributed to phenotyping by acquiring measurements and setting up experiments. These have enabled the development of the Scio non-destructive root probe for measuring sugar content, and the calibration of RGB and hyperspectral cameras on board a drone for leaf coverage measurements. As a result, he passed his drone pilot license, accompanied by a ULM theoretical exam, and has a perfect command of the tool. “Traveling with my drone over the entire beet area allows me to meet colleagues and create a transversal link,” he said.

According to him, the AKER programme has helped to intensify relations between partners in the sector, notably Irstea and the ITB. “It is an opportunity to meet in order to understand each other between Public and Private.” His wish: “Continue to test innovative techniques to improve the sugar yield per hectare, and adapt to the plot each new variety that will come from the AKER programme.”

For all the agronomic variables, the methods developed in AKER, based on the exploitation of the fineness of the spatial resolution, make it possible to significantly improve the performance of the estimates compared to the conventional remote sensing methods applied to the reflectances averaged over all the pixels of each plot. They thus confirm the good results obtained on the ground with the hyper-spectral camera.

### **Measuring the sugar content of the growing taproot**

Near-infrared spectrometry is a recent discovery with great potential in the agriculture and food industries, particularly for its non-destructive, rapid and inexpensive characteristics.

As part of the AKER programme, a model was developed to predict sugar content using a spectrometer placed on the emerging part of beet roots. This development results from a collaboration with Irstea and more particularly the COMiC laboratory (Optical sensors for complex



Plant probe (ASD) used for the exploratory tests of the sugar content measurement.

environments) which brought its know-how in chemometry. To develop such a system, the researchers linked the measured spectrum and the sugar content of the whole beet.

### *First exploratory work*

Initially, Irstea developed a probe based on near infrared spectrometry. From 2012 to 2015, thousands of spectra were acquired on different varieties. Various probe configurations were tested: optical fibres used in endoscopy in the medical sector, or non-invasive contact probes. The researchers analysed 1,000 beets from 10 different varieties. Results were better with a contact probe than with an invasive probe. The commercial Plant probe (ASD) was used in testing with the Fieldspec spectroradiometer, which scans a wide range of wavelengths from 400 to 2,500 nm.

The results also show that the relevant wavelength range for estimating the sugar content is between 740 and 1,070 nm.

### *Towards a pocket spectrometer*

The Scio spectrometer sold by Consumer Physics fulfils the spectral range requirements and has the advantage of being light for easy use in the field. It is coupled with a smartphone application allowing the spectral measurements of beets to be read directly on site.

Starting in 2017, a large number of samples were analyzed to calibrate the tool. To do this, the ITB sampled beets on different dates, from June to November, on several varieties subjected to contrasting nitrogen regimes in order to have the widest range of variations possible in the calibration data set.

For each beet, a measurement was made with the spectrometer, and the sugar content reference of the whole beet was measured in the laboratory by the conventional saccharimetry technique. The ITB thus obtained 474 data in 2017, 290 in 2018 and 150 in 2019. The database dissociated a set of calibration data and one of validation. A PLS (Partial Least Squares) model was developed on the calibration data to link the spectrum obtained to the sugar content of each beet.

Scio spectrometer for the analysis of the beet sugar content.





The validation results show an error of 0.98 sugar content point for a measurement made on the surface of a single beet, knowing that the range of variation of the sugar content oscillates between 15 and 22% in the data set available. Accuracy improves as the number of beets measured increases. Thus, for 25 beets, the average sugar content error drops to 0.2 point, which corresponds to a significant difference between two varieties compared on the same site.

## AKER, an incubator for personalized advice

Technical advice is becoming more professional, reflecting the evolution of the medical sector. The general practitioner is no longer so much a therapist as a data analyst, a referral to specialists. Hence the importance of preventive medicine...

Thus, AKER is a programme that will benefit the improvement of beet performance by obtaining superior genetic material, but it will also provide

### FABIENNE MAUPAS: "AKER MADE ME EVOLVE"

Originally from Normandy, close to the agricultural world, Fabienne Maupas is an agricultural engineer from Agrocampus Ouest in Rennes. She has worked at ITB for fifteen years where, in her early days, she studied culture models and the characterization of abiotic stresses (water, nitrogen).

"We had get fully involved, Fabienne recalls, to lead the phenotyping work in the field of seeds and seedlings on the one hand, and the plant in the field on the other", which represented 60% of the AKER budget and mobilized around thirty researchers. She explains how much the programme has made her evolve: "AKER brings us new perspectives in our experimental work to understand the components of performance. It really changed our working methods, we innovate differently, it's exciting."

Fabienne continues: "During the programme, I felt that part of the researchers' work tools could be appropriated, and there was an increase in skills: the more researchers are interested, the more we can progress. A transfer of skills also took place, which Fabienne wanted to acquire during the programme in order to be able to then develop them. She continues on this idea to conclude: "There is a race for technological innovation. We don't want to stop here. We will continue beyond the AKER programme."



Implicated in phenotyping, Fabienne Maupas, agricultural engineer at ITB, believes that the AKER programme has made her evolve because it has changed working methods.



The AKER programme will offer farmers and their technicians personalised advice.

publications, dashboards, models to all advisors, ITB (Technical Institute for Sugar Beet) employees and technicians from the agronomic services of sugar factories. The diagnostic and evaluation tools and methods, as well as knowledge of the genes and underlying mechanisms, are all means of consolidating existing development structures, but also of providing their employees with continuous training and a possibility of strong evolution.

Beyond the sphere of advisors, the AKER programme will offer beet farmer users personalized advice and agronomic support so that the new genetic material offered can best express its potential. It will also provide them with decision support tools. For example, the miniature Scio root contact probe is already available for the farmer, who can easily and directly measure the sugar content of his or her beets non-destructively. In general, the phenotyping tools will be available to the beet industry, with a gradual ramp-up.

## WILL THE AKER PROGRAMME MEET THE EXPECTATIONS OF THE BEET INDUSTRY?

Vincent Laudinat, managing director of the ITB, chairman of the Interprofessional Committee of the AKER programme, takes stock of the adequacy between the expectations of the sector and the results obtained.



“The beet industry expects a lot from the AKER programme. This obviously involves improving the competitiveness of beets while respecting the environment, but also developing the use of technologies with new agronomic solutions or even increasing the collaborative scope of the work undertaken. The mission is fulfilled on all these points!

Above all, AKER's genomic results promise the arrival of new genetic material, a source of significant progress in terms of yield and resistance to diseases. It will increase productivity while limiting the use of phytosanitary products.

In addition, this unique and detailed knowledge of genetic material will allow us to respond more quickly to the already anticipated challenges of the future (new diseases, regulatory changes, climate change, etc.). Creating varieties adapted to these changes will be faster!

AKER is also the integration of high technologies in the sector: genetics, digital, bioinformatics... These tools and methods allow the development of new agronomic solutions for faster responses thanks to high throughput and more precisely thanks to increase in the number of indicators: decision support tools (OAD), phenotyping tools, non-destructive field measurements, etc.

Nearly a hundred collaborators, from all of the French sugar beet industry and from public and private research, joined forces to bring the AKER programme to fruition. It contributes, directly or through the spinoffs from its many other research projects, to the strengthening of the industry and ensuring its sustainability. Its collaboration model will be used to set up other large-scale projects in the long term; its research model will also help other sectors of agriculture to advance.”



A close-up photograph of a person's hands wearing white nitrile gloves, holding a small, green, spiky plant stem. The background is a blurred greenhouse with wooden panels and metal frames. A blue circular graphic is overlaid on the right side of the image, containing white text.

**New genetic  
material**





## ■ Double the rate of annual yield growth

As early as 2012, the AKER programme announced its objective: “to improve the competitiveness of sugar beet by 2020 by doubling the annual growth rate of its sugar yield per hectare”.

### A paradigm shift

The AKER programme corresponds to a paradigm shift in the field of beet research and selection, underpinned by two statements: AKER makes predictions; and it manages genetic variability.

We no longer do *a posteriori* selection, but *a priori*. In the course of the programme, genotyping actually happened before phenotyping. Previously, plants were assessed in the field and then genetics was used. Now, we start by using genetics, and we evaluate its results *a posteriori*. This leads to prediction by working on models. Hence the importance of bioinformatics, an essential discipline in AKER.

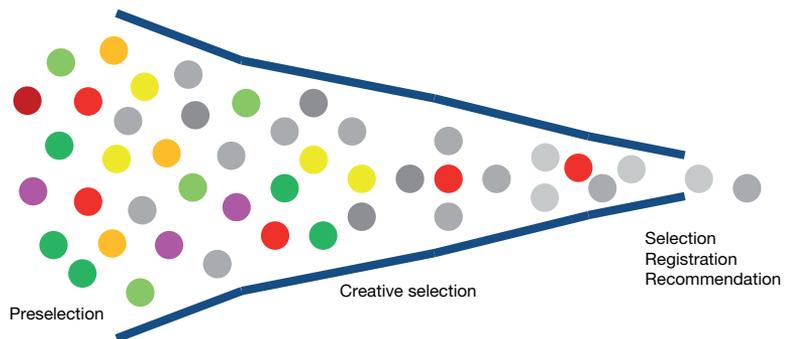
The programme manages variability by seeking diversity through the 10,000 genetic resources available, to arrive at the choice of sixteen reference plants representative of this diversity. The approach consists in researching, incorporating, organizing, valuing variability, in ensuring that it is always available to react and face new constraints.

In reality, it is a question of maintaining diversity in a dynamic and sustainable vision of its use. This is done by deploying all the modern techniques made available to researchers. The AKER programme thus offers the potential for diversity: those that are useful now and those that are neutral, even with a metabolic cost, but which may be useful later in order to answer new questions that are sure to arise, such as resistance to certain pests.

## Reduce selection time by two

In the future, it will be necessary to respond to new objectives (appearance of a new disease, new regulatory framework, new outlets, etc.) in the least possible time. The objective of the AKER programme is to halve this “reactivity” time. On the one hand, AKER benefited from techniques allowing to go faster in the selection. Thus, the “seed to seed” technique, advocating a short cycle in controlled above-ground conditions in a greenhouse (vernalization, induction, crossing), made it possible to deploy the selection programme in five years instead of ten years. On the other hand, AKER itself offers the means to shorten the selection schemes, in particular with genomic selection which should make it possible to reduce the selection cycle by one year out of the five currently required, i.e. 20%, to obtain a new variety.

AKER manages variability: widen the funnel and then shorten it.



### WHAT IS THE CONTRIBUTION OF THE AKER PROGRAMME TO THE PROFESSION OF BREEDER?

*“AKER is an example of an original methodology for the use of genetic resources, from the choice of accessions by molecular tools, their evaluation by innovative phenotyping protocols, to their use in genomic selection. New sources of variation are thus identified and used, which should help increase genetic progress. The results of this programme will have consequences far beyond the improvement of sugar beet.”*

André Gallais, honorary professor of genetics and plant breeding at AgroParisTech, member of the French Academy of Agriculture



## Relevant factors

The doubling of the annual growth rate of sugar yield per hectare is the result of a number of factors. We can cite the variability which makes it possible to detect interesting new genes, the speed of selection improved thanks to genomic selection, and the optimization of phenotyping.

We can formulate a certain number of quantified hypotheses regarding to the contribution of these factors which are leading to double the annual growth rate of the sugar yield per hectare, chematically from 2 to 4%:

- genomic selection makes it possible to reduce the selection cycle by one year in five, or 20%, increasing annual yield growth from 2 to 2.4%;
- genomic selection increases the “firepower” of the selection: the prediction at 75% makes it possible to increase the amount of material to be selected for the same efficiency. For example, with 50% more material screened, which results in efficiency increased by 30%, annual yield growth increases by 0.6%. This cumulative gain to the previous one brings the cumulative gain to 3%;
- finally, genetic diversity is globally doubled (16/40). In fact, the total diversity of beets is concentrated in 40 reference plants, which in genetics is designated by the term of effective size, while that is not used (useful or not) and not included in the cultivated material is concentrated in sixteen plants. We can therefore estimate we will now have a diversity reservoir equal to twice that used and thus gain 50%, then 1% more progress compared to the initial 2%.

We therefore reach a 4% annual growth in yield, not counting the indirect contribution of management tools such as genomic knowledge or high throughput phenotyping which make it possible to consolidate progress by making it more predictable, less risky, safer in the face new selection targets. It is also without counting on the progress that phenotyping can bring to production techniques. But this is another subject that may be reflected in other projects...

## ■ General outline of the AKER programme

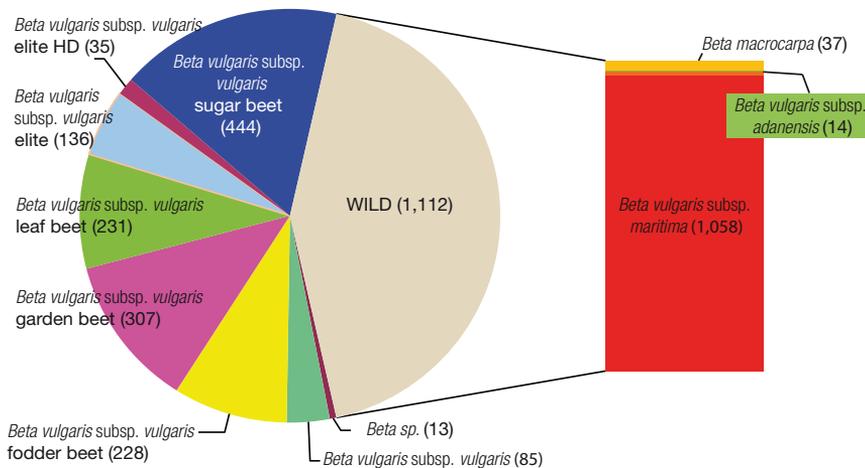
The AKER programme initially identified 46 gene banks with genetic material for the beet species around the world. We analyzed 10,000 accessions, 3,000 made up the reference collection and 16 plants were retained in the end.

## Reference collection

The allelic variability of beets is mainly preserved through two international networks: Grin-Ars and Eurisco<sup>3</sup>. The first, Grins-Ars, located in the United States, collects and multiplies genetic resources; it is available to the user via the Internet. The second, Eurisco, established in Europe, lists the collections of all species of the genus *Beta*; it redirects the user to the holder organization.

### AKER TOOK INTO ACCOUNT GROUP 1

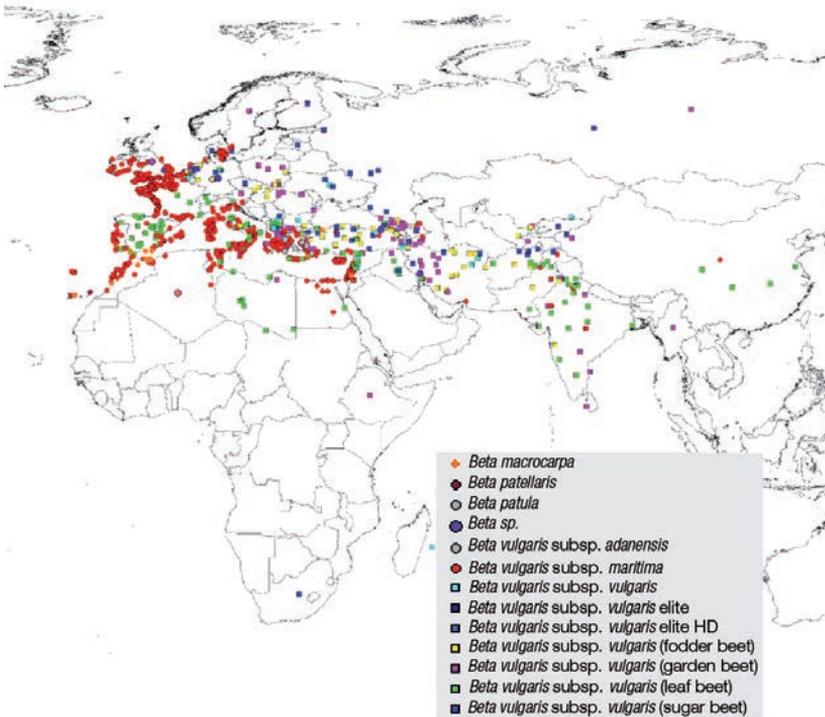
Beet belongs to the *Beta* genus, which has four sections, three of which are exclusively composed of wild/exotic beets. The fourth section includes cultivated beets (sugar, fodder, vegetable) and wild/exotic beets. The latter are mainly maritime beets (*Beta maritima*) located on the European coasts, around the Mediterranean basin and going back to the Baltic countries and England. To a lesser extent, there are beets *Beta adanensis* and *Beta macrocarpa*. This section constitutes the group 1 in which the AKER programme is interested because of the crossbreeding ability of these beets.



Distribution by type of the 10,000 plants available in group 1.

<sup>3</sup> [www.ars-grin.gov](http://www.ars-grin.gov) et [www.eurisco.ecpgr.org](http://www.eurisco.ecpgr.org).

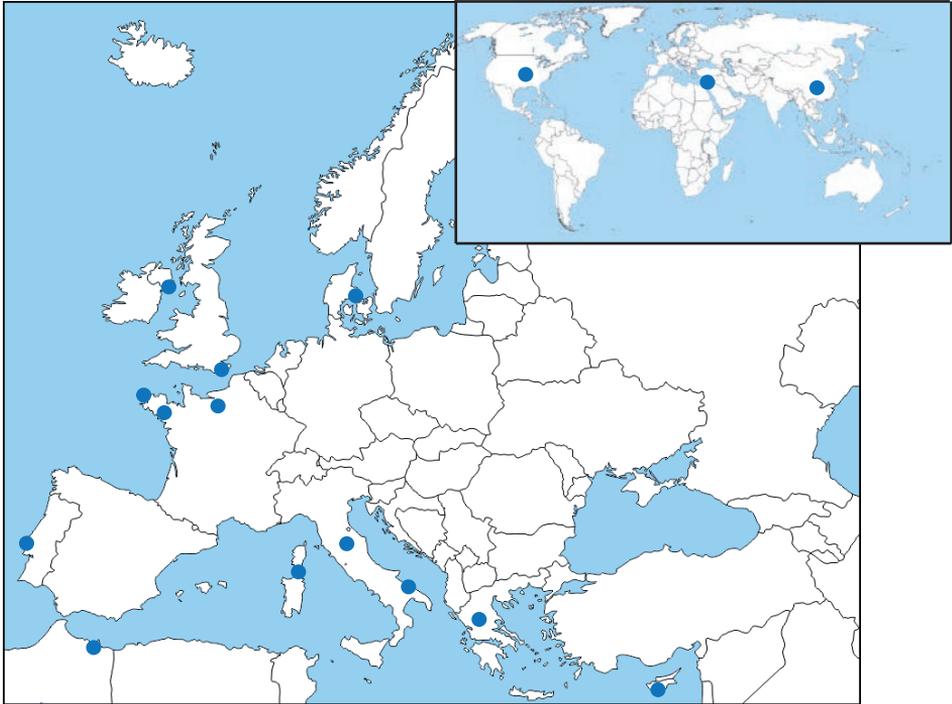
Geographical distribution of the 3,000 plants selected.



An estimated 20,000 accessions (sachets of different seeds with a satisfactory germination capacity) are potentially usable resources. The first task of the AKER programme was to identify these accessions available around the world, to note their passport data and their germination quality in order to characterize the natural diversity that was potentially available. In the end, 10,000 accessions were selected.

The inventory and analysis of the 10,000 available accessions made it possible to select 3,000 on the basis of a sampling carried out by geographical representation for wild/exotic beets, and by proportional representation for cultivated beets (sugar, fodder, vegetable). These 3,000 selected accessions made up the reference collection.

Finally, after a genetic analysis carried out in this reference collection, the AKER programme selected sixteen reference plants. In accordance with the hypotheses formulated at the start, these sixteen reference plants cover 100% of the allelic variability available within the genus *Beta* and complementary to the genetic resources already used in breeding. The set of reference plants therefore constitutes a representative sample of the total genetic wealth.



Geographical distribution of the sixteen reference plants.



Collection of the sixteen reference plants.

## Creation of genetic material

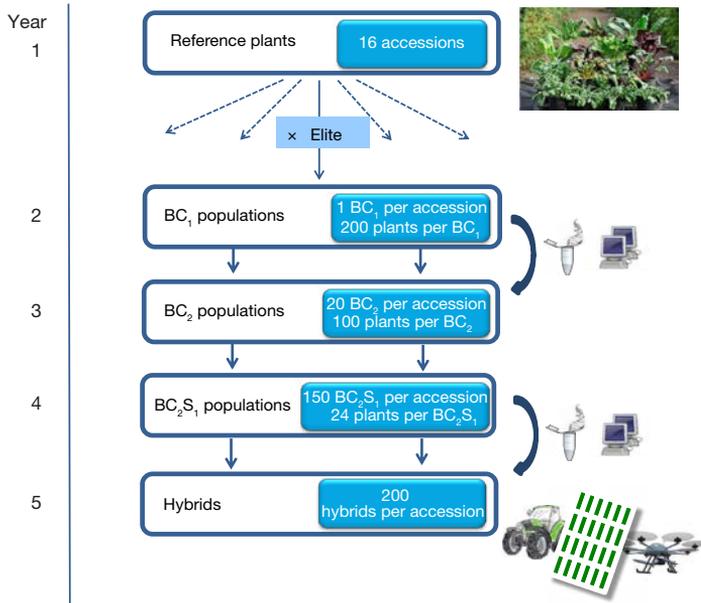
The objective of the AKER genetic material creation scheme is to “pulverize” pieces of exotic DNA carrying variability by means of successive crossings in the elite material.

Beginning in 2013, the sixteen reference plants were crossed with elite material in order to introgress, that is to say to introduce naturally by crossing, new alleles of exotic origin.

In 2014 and 2015, the  $F_1$  crosses ( $F$  = filial generation) obtained (elite  $\times$  exotic) were successively backcrossed by the elite. The goal of backcrossing was to increase the share of the elite, while keeping pieces of the exotic plant. In each generation, 100,000 plants were produced. Molecular analysis allowed us to choose 450 that would be used for crosses for the next generation.

In 2016, the fixation of plants from crosses by self-fertilization took place. 250,000 plants were thus sown in the greenhouse to select 3,000 for the rest of the programme.

Finally, in 2017, hybridizations were carried out in the field on the same genetically female plant so as to represent the variability of the 3,000 male genotypes. The hybrids obtained were then phenotyped in 2018 and 2019.



Scheme for the creation of AKER genetic material.



Aware of her implication in the success of the AKER programme, Karine Henry collected accessions from all over the world, selected sixteen and crossed them with elite material.

## KARINE HENRY: IN THE BEGINNING... THERE WERE 16 REFERENCE PLANTS

AgroParisTech engineer (INA-PG), with a specialization in plant improvement in her pocket, Karine Henry started her career at Florimond Desprez where she is a beet and chicory breeder. She explains: “We were certain from the start that with sixteen accessions, or reference plants, we would cover 100% of the allelic variability available within the genus *Beta*, complementary to the germplasm (genetic material) currently used.”

Scientific director of the AKER programme, Karine herself made the F<sub>1</sub> from the sixteen accessions in 2013 with elite germplasm, helped by her technicians. “It is a stressful work, you can’t miss it!”, she remembers. Subsequently, the breeding programme was busy until the hybrids were produced, not to mention the 350,000 DNA samples taken during the programme.

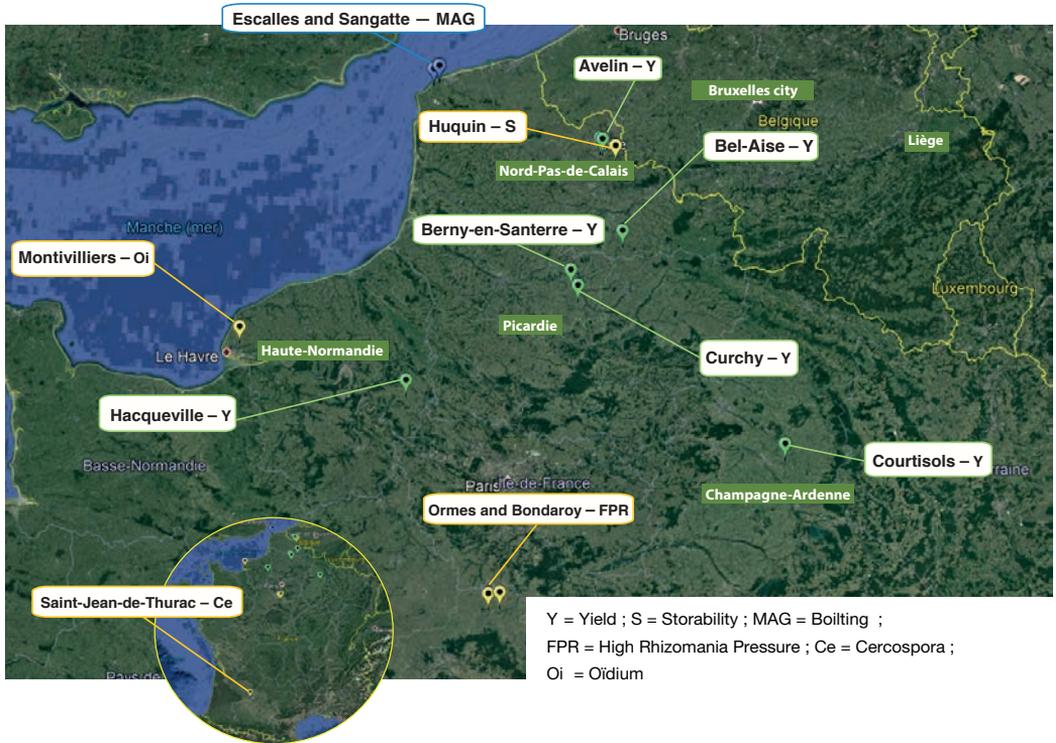
Karine Henry does not hesitate to affirm: “The choice of the sixteen reference plants is at the heart of the AKER programme, and this is what will allow the creation of new efficient genetic material.” A bet on the way to being won!

## ■ Evaluation criteria for the 3,000 hybrids

The phenotyping of the 3,000 hybrids from the AKER selection programme – crossing of the sixteen reference plants with elite germplasm, backcrossing in two generations with elite material, self-fertilization then hybridization – was carried out in 2018 and 2019 on the basis of a number of evaluation criteria.

### White sugar yield per hectare

The white sugar yield per hectare of beet is the result of the weight of its root (root yield) and its white sugar content. These yield tests were conducted by Florimond Desprez on six platforms distributed in the North – Paris basin. They were planted in two repetitions on 34,000 plots in 2018 and 22,600 plots in 2019. Each of them has an area of 10 m<sup>2</sup> and includes around 100 beets. The ABC harvester created for and dedicated to the AKER programme makes it possible to measure the weight of the roots directly in the field. A representative grating sample of the roots of each plot is frozen in the ABC, then stored at – 25°C for further analysis in the laboratory.



Location of phenotyping field trials conducted as part of the AKER programme.

## RAW SUGAR, WHITE SUGAR

White sugar comes from the raw sugar contained in the beet after extraction during the manufacturing process in the sugar refinery. In fact, beets contain melassogenic elements (sodium, potassium and alpha-amino nitrogen), impurities which have to be removed. The operation is carried out in a sugar refinery by calcocarbonic purification: a successive addition of lime milk (based on limestone), then carbon dioxide, forms a precipitate which fixes the impurities. Thus, the yield of white sugar is the result of the work of the beet farmer and the sugar manufacturer. Genetics are at the service of each other with a concern for productivity and quality.

Sowing in the field of phenotyping trials.



## The growth dynamic

Two yield test platforms were followed by the ITB with a drone to estimate the kinetics of the following canopy variables: coverage rate, leaf area, amount of chlorophyll and amount of nitrogen in the leaves. Four measurement sequences were carried out from May to July.

## The bolting susceptibility

Beets are a biennial plant. It reproduces “normally” during the second year of vegetation. The rise to seed is caused by vernalization (period of cold undergone by a plant necessary to make it pass from the vegetative stage to the reproductive stage). Very early sowing makes it possible to express this rise to seed, and therefore to characterize the genotypes and to eliminate the most susceptible to this characteristic.

These observatories were set up and monitored by Florimond Desprez on two platforms located on the Côte d'Opale. Dense sowing took place in mid-February on 5,700 plots in 2018 and 2,100 plots in 2019. An initial count of populations, then three counts of mounted plants (end of June,

July, August) made it possible to define the percentage of bolters and thus characterize the resistance of the genotypes studied.

## Leaf diseases

Tolerance/resistance to *Cercospora* and powdery mildew, the two main leaf diseases in beets, is a focus for obtaining new, efficient genetic material that can lead to the reduction/elimination of phytosanitary products, and fungicides in particular.

The observations were carried out in test plots and the behaviour of the genotypes of interest was later confirmed on a larger scale:

- for *Cercospora*, in the Southwest, after artificial inoculation on 2,850 plots, with phenotyping by multispectral imaging on board a drone, developed and carried out in partnership with the Hiphen company;
- for powdery mildew, in Normandy, on 2,850 plots in natural conditions, with an integrative rating.

Furthermore, tolerance to high rhizomania pressure has become a challenge for breeders since the monogenic resistance to rhizomania

Phenotyping platform followed by drone.



## FROM PERFORMANCE TO COMPETITIVENESS

The white sugar yield per hectare is the result of a number of selection criteria. It is therefore natural that the AKER programme is interested in all the components of this return. This concerns both the earliness of emergence permitted by the cold resistance of the seed, the adaptation of the plant to dry conditions during vegetative growth, as well as resistance to diseases through tolerance genes, nitrogen absorption and optimization in the growing cycle, etc. The goal is to be more productive, but also to be adaptable to a maximum of different environments and with the minimum of inputs.

Traditional selection has long focused on the criterion of productivity per hectare. We now need to go further with the AKER programme by selecting a beet that meets the concern for robust productivity through all the criteria that contribute directly or indirectly. Thus, yield is one of the criteria of productivity which, itself, is at the service of competitiveness, which has become the watchword of the programme: “competitive innovation”.

Harvesting of phenotyping trials with the ABC harvester.





conferred by the *Rz* gene has been circumvented in certain situations. The behaviour of AKER hybrids was also evaluated in central France, on 6,900 plots in 2018 and 2,300 plots in 2019, under mortality/survival observation in order to identify new sources of resistance to rhizomania.

## The storability

The extension of the length of the beet campaign – a consequence of the abolition of quotas and the improvement of the productivity of industrial processing tools – has implications for the need for quality of storability of the roots after uprooting and before transformation into sugar refinery. Florimond Desprez has developed a predictive methodology for this quality of preservation by physical resistance analyses. To this end, 5,700 plots in 2018 and 3,000 plots in 2019 were established in the North.

### NICOLAS HENRY: AKER, A TECHNICAL FEAT

Nicolas Henry has always been interested in plant biology. Originally from Anjou, he holds a master's degree in science at the University of Angers in Biophysiology applied to crop production, before joining Agro-ParisTech (INA-PG) where he obtained an advanced agronomy diploma in Plant Production Engineering. He then arrived at Florimond Desprez. "About 20% of the budget of the Beets and Chicory Laboratory was spent on the AKER programme in a normal year, more than a third in 2018 during the first year of phenotyping!" explains Nicolas. "Our service has contributed significantly to the programme by having researched, introduced, developed and tested the new genetic material." He will have managed 63,000 test plots in 2018 and 32,000 in 2019.

The pressure was high. "The fear of failure was real given the expectations of the sugar beet sector, and I had to encourage my employees," says Nicolas. According to him, "AKER is a technical feat that involves motivated collaborators, first of all Christophe Descamps, responsible for the agronomic service, doing a superb and enormous job, and through substantial investments".

For Nicolas, AKER's interest lies in the original approach of the programme, in themes that have never been dealt with, and in meetings that he would never have had the opportunity to have. "We hope to contribute to improving the competitiveness of the beet sector vis-à-vis the cane, and that of Florimond Desprez who is at the service of the sector."



For Nicolas Henry, director of the Beets and Chicory Laboratory at Florimond Desprez, the AKER programme was a challenge, a real human adventure.

## ■ Results achieved

The genetic results of the AKER programme, analysed in 2018 and being confirmed in 2019, bring significant progress on the criteria of yield and sugar content, but even more on diseases and seed production. These advances have been verified by QTL (Quantitative Trait Loci) analysis. The results obtained are a source of stability for the beet crop and for the sector as a whole.

### Analysis of genetic value

The results of the phenotyping tests of the 3,000 hybrids from the selection programme, carried out in 2018, were analysed during the winter of that same year. A classic statistical analysis was carried out with the help of different software on 13 reference populations, each made up of 200 genotypes. It was a spatial analysis of the average value of the sugar yield per hectare (root weight, sugar content, impurities). In parallel, the susceptibility of these populations to different diseases was observed – *Cercospora*, powdery mildew, high rhizomania pressure – or even the bolting. The data were compared to the genetic control, because it is this genetic material, the result of backcrossing on an elite variety, that serves as a control. The exotic fragment(s) thus modulate the genetic expression of these genetic controls and give phenotypes revealing these effects (positive, negative or neutral) on the measured character. We can thus highlight useful, beneficial or toxic genetic resources.

In parallel, breeders also make comparisons with commercial controls, that is to say the current most cultivated varieties (2018–2019). The latter are the result of the genetic progress made by traditional selection whereas, for the AKER programme, the backcrosses were made with elite or commercial material from 2012. The difference is therefore significant (1% of genetic progress by year on average).

The spatialization takes into account the position of the plots in the test (values corrected with respect to the neighbourhood but also to the residual heterogeneity of the experimental field) or between tests. This makes it possible to distinguish place effects but also, as the experiment is conducted over two years, year effects (climate effect). The whole participating in the environmental effect (E effect).

The genetic value (G) will therefore be evaluated by its phenotypic value (P), that is to say its expression in a given environment (E) (or group of environments). This can be summed up in the following formula:

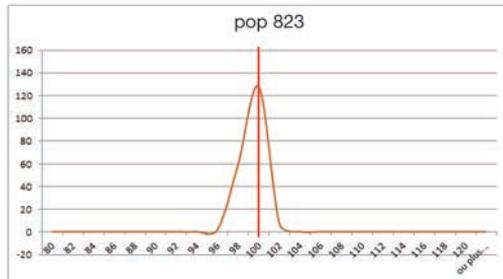
$$P = G + E + (G \times E)$$

(G × E) being the genetic–environment interaction.

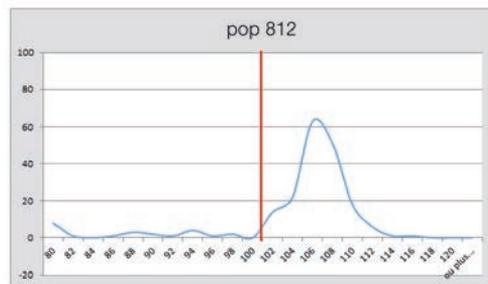
## Phenotyping results

The first work consists in observing the distribution of the phenotypic values of the individuals of the thirteen reference populations compared to the genetic controls. The data are represented by criteria. We look at quantitative criteria, like the yield, a Gauss curve centred on an average, with a minimum value and a maximum value. The profiles obtained are different according to the criteria, but also according to the value of the elite starting material which serves as a control. The elite material, for example, demonstrated a very good sugar content, result of the selection. This content is close to a physiological threshold: the sugar content cannot in fact exceed certain values, the cells not supporting even higher contents (defined by osmotic pressure on the cell membrane due to the concentration of solutes).

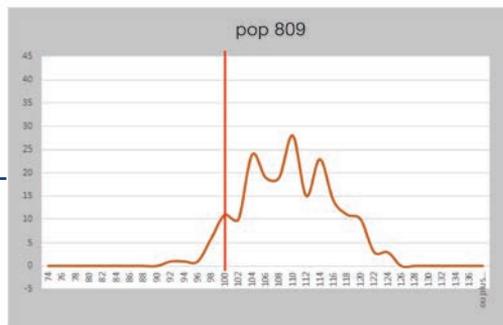
Profile A



Profile B



Profile C



Example of the different profiles observed according to the phenotyping criterion.

A: sugar content;

B: sugar yield/ha;

C: resistance to *Cercospora*.



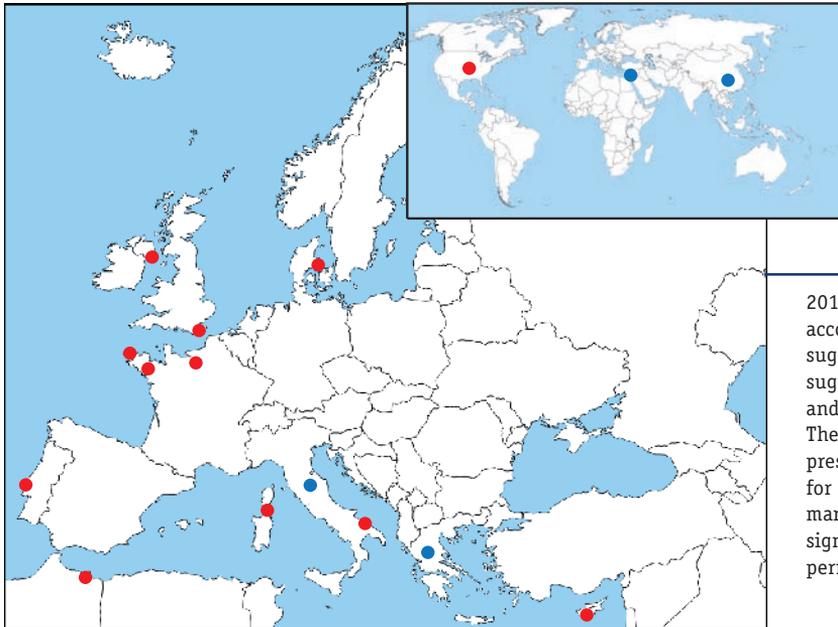
If we observe the variability curve for this characteristic, the expected effect is negative (the average is below the elite controls) and the maxima are very slightly higher than the elites. In other words, most exotic resources decrease the value of cultivated sugar beets but rare fragments of exotic chromosome produce an improvement, which remains, in any case, relatively low because limited by the physiological threshold. As far as performance is concerned, we are apparently still well below a ceiling. The variability is high and we can observe individuals with strong gains. The distribution is wide and very close to a normal distribution (Gauss curve) demonstrating the existence of very many genomic regions involved. The potential of the AKER programme is clearly evident here.

A third type of profile can also be observed: bimodal distributions (with two peaks). This is particularly the case for the distribution of disease notations. These variations reveal exotic fragments with stronger effects and a discontinuity in the expression of variability. The trait is influenced by only a few genomic regions.

If all these results give a first picture of useful variability, we must, however, look more precisely to distinguish which will be in the short

	Name	Year	Pop.	WSY com.	WSY génét.	%S com.	THA com.	ADPC_NDVIZ	ADPC_ERI	BOLT_CBN	BOLT_SAN
A	AKER1	2018	pop 807	105.21	110.52	98.94	106.19	98.50	8.14	0.14	0.09
	AKER2	2018	pop 812	105.06	110.37	96.09	109.67	106.17	8.56	0.24	0.19
	AKER3	2018	pop 815	104.42	109.70	99.89	104.41	100.22	6.86	0.12	0.04
	AKER4	2018	pop 815	104.17	109.44	99.99	104.09	101.19	8.60	0.20	0.08
B	AKER5	2018	pop 808	104.07	109.33	97.44	106.61	108.05	7.85	0.07	0.10
	AKER6	2018	pop 815	104.02	109.27	99.68	104.29	107.96	6.85	0.07	0.10
	AKER7	2018	pop 812	103.98	109.23	95.33	109.31	102.90	8.61	0.11	0.18
	AKER8	2018	pop 815	103.92	109.17	99.39	104.59	106.98	8.40	0.09	0.02
	AKER9	2018	pop 808	103.83	109.08	97.55	106.52	107.95	6.31	0.09	0.05
	AKER10	2018	pop 815	103.79	109.03	99.90	103.72	107.73	7.87	0.07	0.07
	AKER11	2018	pop 815	103.75	108.99	99.62	104.09	97.14	6.36	0.11	0.07
	AKER12	2018	pop 808	103.74	108.98	97.09	107.05	105.87	7.98	0.15	0.13
	AKER13	2018	pop 808	103.72	108.96	98.04	105.77	104.88	6.83	0.06	0.04
B	AKER14	2018	pop 808	103.71	108.95	97.48	106.56	108.35	8.08	0.10	0.05
	AKER15	2018	pop 808	103.69	108.93	97.10	106.89	108.03	7.79	0.22	0.11
	AKER16	2018	pop 808	103.68	108.91	96.69	107.36	103.99	5.75	0.09	0.03
	AKER17	2018	pop 808	103.58	108.81	97.41	106.52	110.37	6.93	0.15	0.22

Focus on the best AKER hybrids that can be used directly — combination of different factors: yield, bolting and disease. A: particularly efficient but a little less good in tolerance to leaf diseases; B: a little less efficient but clearly better in tolerance to leaf diseases.



2018 results spatialized according to the white sugar yield criteria (WSY), sugar content (% S) and root yield (t/ha). The 16 accessions all present sources of progress for these criteria and those marked in blue present significantly superior performance.

term or in the long term. The short term is evaluated as one of the best hybrids observed and therefore directly exploitable if it exceeds commercial controls. The long term is characterized by new breeding programmes to accumulate the newly detected effects.

To measure genetic progress, we have to look at all of the traits measured. A given individual can bring, for example, a yield gain but also susceptibility to a disease. This is taken into account in selection by the concept of an index. A selection index is a formula of choice applying a weighting for each character, the coefficient being relative to the importance attributed to the character. This will often be linked to economic criteria, but could also be a threshold below which the candidate cannot be retained despite remarkable performance on other factors.

The combination of these choices gives an index. For each market, type of farming route, importance of the economic/environmental criterion, etc., indices are generated and allow the right candidates to be listed. It is also important, by zooming in on the best potential candidates, to note the compatibility or incompatibility between criteria.



At this stage, we do not build genotypes by successive crosses, we see a diversity of performances united or not in the same genotypes.

When we look at the classification of results by criteria, we discover that they belong to certain populations rather than others. For yield, for example, four of the thirteen populations observed so far show their ability to provide genotypes with significantly superior performance. For another criterion, other populations will be highlighted.

As a result, the most promising populations among the thirteen were completely phenotyped again in 2019, for the corresponding criteria. For yield, for example, all the genotypes of the 4 best populations, i.e. 800 hybrids, as well as the 300 best among the 9 remaining populations (thresholds higher than commercial controls), were phenotyped again.

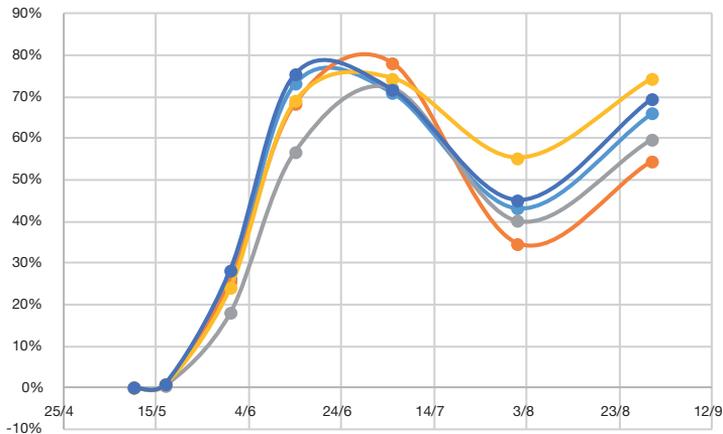
In addition, 3 new populations, not available in 2018, were in turn fully tested in 2019. Some genotypes carrying missing fragments, for which seeds were produced again in 2018, came to complete the system. By doing so, the 2019 experiment has been optimized.

## **Relationship between phenotyping and genotyping**

If we take the sugar content criterion, although we may finally see the potential for improvement constrained by the physiological impact and the already high content of the current material, new areas of the genome were detected and will contribute to the development and maintenance of high sugar content. Conversely, areas of the genome have been distinguished by their negative effects on this sugar content.

It remains to be verified, but we can already consider the accumulation of genomic zones favouring the high sugar content and/or the characterization of these zones for their interaction with the environment. Favourable genes, for example in oceanic or on the contrary continental conditions, can contribute to a better capacity and especially to a better stability of expression of this characteristic when these genomic zones are accumulated in the same genotype.

This reasoning can, of course, extend to all characters: the root yield, without a doubt, but also resistance to diseases, for example when there are different strains of pathogens or an aggressiveness/virulence dependent on the environment. The annotation of genomic areas is therefore essential work. The multi-local and multi-annual results will make it possible to estimate the assignment values. This knowledge is essential for better exploitation of genetic effects.



Contrasting varietal behaviours with different leaf cover profiles in situation of significant water stress.

Traits evaluated dynamically are also indicators of the development of yield and adaptive capacity of plants. Phenotyping tools such as those for measuring leaf cover can provide answers regarding the ability of certain accessions to perform well in untested environmental zones. The right prediction can feed on this dynamic. The same goes for the root yield: some accessions grow quickly but level off, while others, slower at start-up, continue to progress when the growth period is longer. The weather conditions obviously determine the duration of this race, but we can simulate many situations. This is the whole point of indicators and their dynamic measurement.

This reasoning can also apply to diseases: some accessions can exhibit good behaviours which unfortunately deteriorate in the middle/end of vegetation while others, markedly less good, without going to too great susceptibility, will show good consistency. Finally, to have a complete agronomic characterization, it is necessary for these genotypes with high agronomic potential, to analyse the quality of seeds and the dynamics of germination, this trait constituting a major factor of adaptation, particularly at low temperatures.



## QTL detection

Association genetics (GWAS) makes it possible to link phenotyping and genotyping data from the 3,000 selected hybrids, so as to detect QTL (Quantitative Trait Loci), i.e. genomic regions associated with the variation of a phenotype.

QTLs were detected on all nine beet chromosomes for the thirteen populations studied. Their number varies from one population to another. Where they differ between populations, the QTLs often seem to be specific to the particular reference exotic accessions. Thus only fourteen QTLs were detected in two populations and only one in three populations.

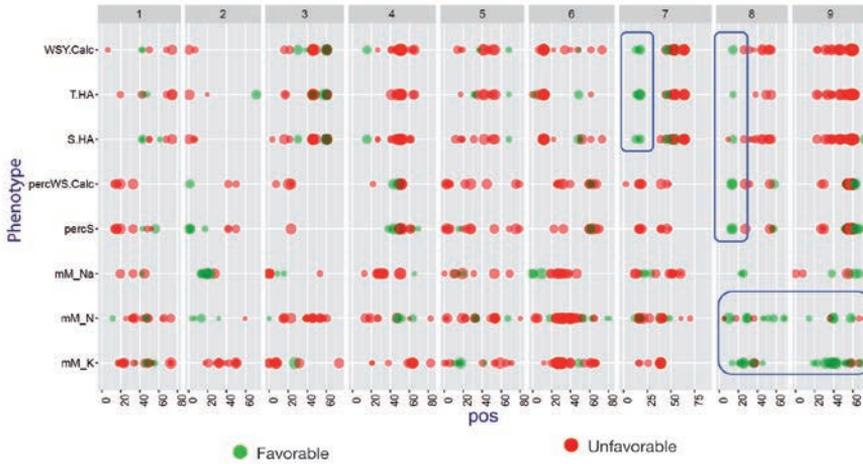
Traits	Variables	801	802	803	807	808	809	810	811	812	813	815	823	830	total
Purity	ratL_K	25	11	11	6	19	14	12	8	21	9	11	25	18	190
Purity	ratM_N	6	10	34	6	9	12	6	29	12	7	11	22	16	180
Purity	ratM_No	10	4	2	5	8	11	11	0	7	24	8	12	5	100
%S	peru_S	8	9	4	20	12	5	4	4	12	15	7	2	16	120
%s	peruWs_tale	8	12	2	19	2	4	9	5	19	2	7	2	6	96
Productivity	S_HA	3	32	12	24	8	2	16	10	17	10	10	2	21	167
Productivity	L_HA	5	20	15	30	14	4	2	18	14	14	10	2	15	162
Productivity	WSSY_Calc	3	27	11	30	11	3	9	11	15	11	13	2	19	165
Powdery mildew	ADPC_LH11	1	2	0	0	0	1	1	2	0	0	0	0	1	14
Cercospora	ADPC_FCOVE11	0	2	0	0	0	0	1	2	0	2	0	2	3	13
Cercospora	ADPC_NWH11	0	2	0	0	0	0	0	0	0	0	0	0	2	12
Cercospora	ADPC_NVD12	0	0	0	0	0	0	0	2	0	0	0	0	2	6
Moisture	MCII	6	1	5	4	2	1	2	9	5	4	8	5	5	64
Starch	PI1	1	1	5	0	10	2	0	4	3	2	8	0	4	41
Storage	K2	1	4	5	0	10	2	0	4	11	2	8	0	4	50
	total	77	140	107	144	106	61	77	109	136	106	96	84	137	1380

Number of QTLs detected for each of the populations.

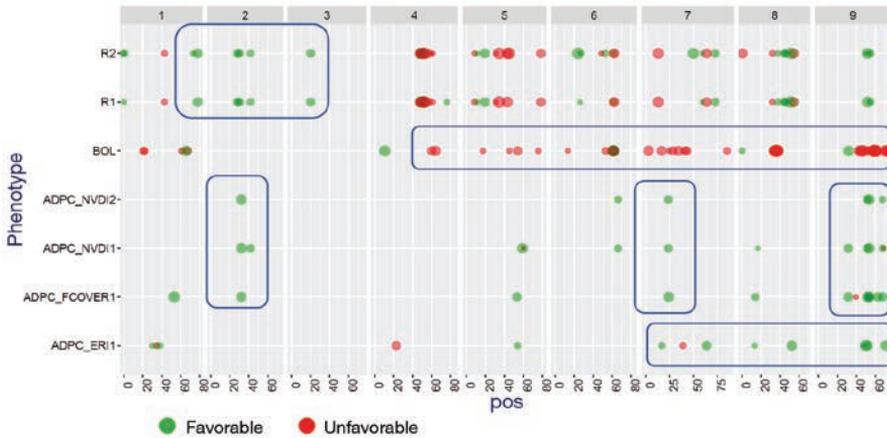
Of the 1,380 QTLs detected, all measured characteristics combined, 322 express a positive effect of the exotic allele. If we take into account criteria other than those linked to the white sugar yield, such as resistance to diseases (*Cercospora* and powdery mildew) and seed growth, 200 QTLs were detected of which 89 have a favourable effect.

If we now look at the results for all populations combined by phenotypic/genotypic criterion, for sugar yield and its components, very many QTLs have been detected but ultimately very few are favourable (therefore useful in the strict sense). They are distributed over almost all chromosomes, with more favourable areas on chromosomes 7, 8 and 9 for melassogenic elements, and less favourable on chromosome 2.

For diseases, there is many fewer QTLs, although these are largely favourable and therefore very promising for both *Cercospora* and powdery mildew. These QTLs are mainly detected in four populations: 802, 811, 823



Effect of the exotic allele on the variation of QTLs linked to yield.



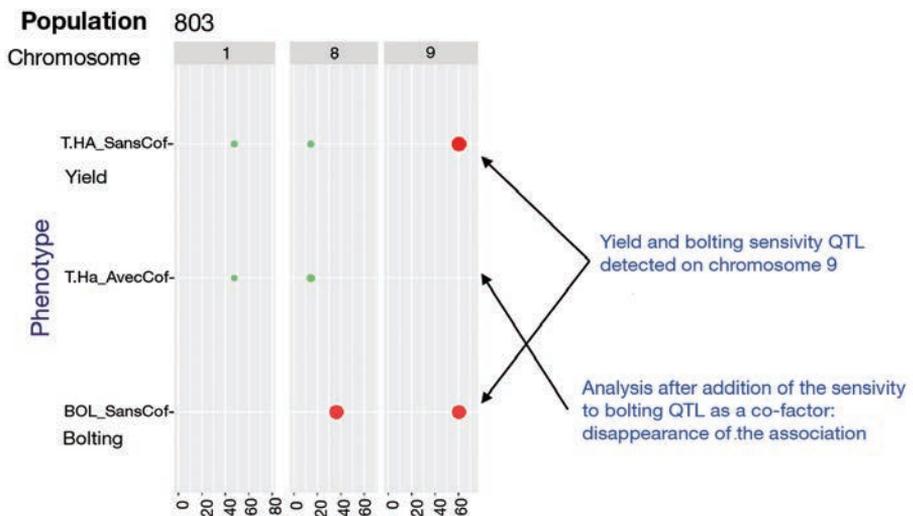
Effect of the exotic allele on the variation of QTLs linked to diseases resistance.

and 830, and some seem to be common between these two diseases. Numerous negative genomic zones have been identified for seed growth. These areas are different from the known gene for annuality and should therefore improve the resistance of seed growth of elite material, regardless of the annual / biennial aspect.

These resistance/tolerance resources have been compared to the resources already exploited in current sugar beet improvement programmes and their originality has been highlighted.

The results of the QTLs analysis by trait show a sugar content and a strong genomic distribution. However, two somewhat special cases must be distinguished: that of bolting and that of susceptibility to *Cercospora*. Exotic sources clearly bring susceptibility to bolting, which is to be expected as this trait has been strongly counter-selected throughout the improvement of sugar beet. But on the other hand, they also bring resistance to diseases like *Cercospora*. It is also surprising that, for the latter, unlike powdery mildew, there are almost no exotic unfavorable alleles.

Multiple analysis also brings new light with, for example, the reanalysis of the yield in combination with the bolting susceptibility. Negative yield



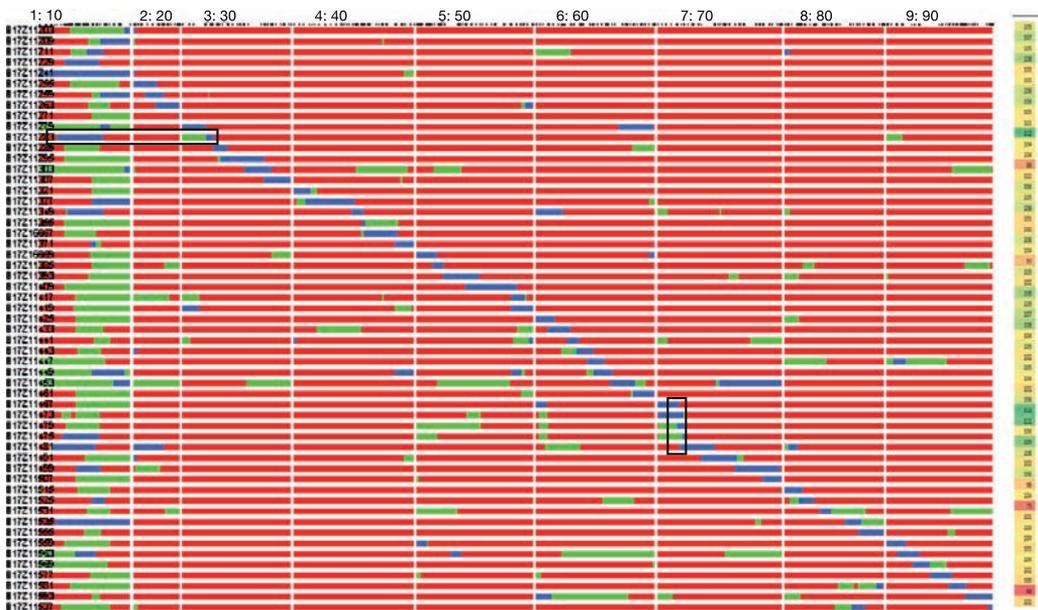
Example of the role of bolting as a factor for the detection of yield QTLs.

QTLs initially detected may be false negatives. They disappear if we introduce the bolting as a cofactor. Bolting susceptibility obviously decreases the yield, whatever the genetic background.

## Prospect

The first results of 2019, under detailed analysis, demonstrate a consistency of performance of the main accessions chosen from among the best (and worst) “performers” of 2018. This multi-year confirmation in the detection of the best accessions clearly supports the robustness of the experiment in the face of multi-year variation, in particular with climatic indicators that have demonstrated the very marked contrast between the years 2018 and 2019. The experimental design is therefore robust and also makes it possible to identify genotypes with a strong adaptation to a wide variety of environments. So we are clearly recovering fitness or adaptability from the genomes of exotic beets.

Faced with the challenges of global warming and especially of its greater instability, in addition to strong potential in terms of performance,



Consistency of interesting QTLs (in green) with the value of their results.



it is important to note that we can generate resources of stability, better able to absorb the inevitable variations of the environment. The elite varieties of tomorrow will be able, by their higher fitness, to ensure a more predictable income, both for the beet grower and for the sugar manufacturer, to reassure the agronomic advice by a stronger guideline, and finally meet higher environmental requirements.

## **AKER WILL DELIVER NEW GENETIC MATERIAL FOR A LONG TIME**

The AKER programme is a stimulator of innovation, responding to the aspirations of the sector which wishes to continue to benefit from genetic progress to improve its competitiveness. It will bring remarkable genetic material that will irrigate future varieties, and this for a long time.

### **New varieties to come**

In the short term, the breeders of the programme are able to detect a group of hybrids with confirmed performances that could be deposited for registration with the CTPS (Permanent Technical Committee for Selection) in 2022. It will still be necessary to count from there two years of registration and one year of recommendation, making three years before marketing.

In the medium and long terms, the AKER programme will make it possible to produce high-performance varieties by introgressing interesting fragments of the genome of reference plants in existing varieties. This dynamic was already underway, even before the end of the AKER programme.

### **No GMOs in the AKER programme!**

GMOs crops are banned in Europe, and therefore in France. The AKER programme is precisely an alternative programme compared to GMOs. It makes it possible to work on the entire beet genome, and not on simple genes from other specific species, which do not belong to beet, as can be the case with GMOs. AKER's strategy is fully based on knowledge of genomics and genes, and its ambition goes far beyond that of GMOs, which only focus on specific characters. In addition, while the GMO strategy also takes advantage of the variability between species, even between reigns (genes of animal origin, but also bacterial, viral...), the AKER programme focuses on the only variability available in species of the genus *Beta*. This variability, which remains to be exploited, is still very large, even infinite in its combinations.



## DOES THE AKER PROGRAMME SATISFY BEET GROWERS?

*“The AKER programme is identified as an essential lever to accelerate varietal selection, reduce inputs and improve the competitiveness of French beet growers. For this reason, the CGB wanted to be an actor. The tangible results of AKER will translate into the progressive appearance of multitolerant varieties, better able to respond to current challenges: greater pressure from pests and increased use of mechanical weeding. They will contribute, in the optimism, to revitalize the sector and comfort the beet growers.”*

Franck Sander, President of the CGB  
(General Confederation of Beet Growers)

## Selkit program: genomic selection kit

Selkit refers to a thesis started in 2017 for a period of three years, intended to prepare the end and the extension of the AKER programme. The objective of this thesis is to promote the integration of the interesting genetic variability, discovered from the sixteen reference populations, in a selection program. It is a question of comparing pre-breeding schemes which make it possible to use this new genetic variability and to quickly introduce it for the creation of new varieties. The optimal scheme will allow the maximum variability to be preserved (avoid its erosion) while producing the material necessary for the creation of elite lines, in a sustainable manner adapted to current and future market requirements.

This problem has already been studied in the past and the solution mainly proposed has proved its worth in real cases for the introgression of few genes or genomic regions into an elite genome. However, these introgression methods show certain limits when trying to integrate several genomic zones with weak into the same elite genotype. Indeed, the prior detection of these areas of interest is, for the most part, limiting since it identifies few or no regions with weak effects. However, in beet as in other species, the characters which most interest breeders (white sugar yield in beet, grain yield in cereals...) are polygenic characters governed by a multitude of fragments with weak effects. The classical methods presented above cannot therefore be used effectively to integrate a multitude of genomic fragments of interest.

Genomic selection offers an alternative method in which, on the one hand, favourable genetic variations can be selected across all genomes and, on the other hand, deleterious variations can be counter-selected,



without focusing on a finite number of genomic regions. This is therefore particularly interesting for genetically complex characters. In the context of a pre-breeding programme, genomic selection can then be used effectively to enrich a starting population with favourable polygenic variations and combinations. Such a population, enriched and close to the level of agronomic performance of elite populations, can then be used easily by breeders as a source for crossbreeding.



Prune Pegot-Espagnet defended a doctoral thesis on the Selkit project — genomic selection kit — complementary to the AKER programme.

### **PRUNE PEGOT-ESPAGNET: INCREASING GENETIC DIVERSITY**

Freshly graduated from Paul-Sabatier University, in Toulouse, a microbiology/agrobioscience degree as well as a master's degree in bioinformatics in hand, Prune Pegot-Espagnet joined the LIPM (Laboratory of Plant-Microorganism Interactions) at Inra from Toulouse with Brigitte Mangin. She worked on wheat, on sunflower in the Sunrise project, then on sugar beet as part of the AKER programme.

Prune explains: "The Selkit project consists in increasing the useful genetic diversity within the breeding material with interesting exotic genetic resources." The goal is to simulate different pre-breeding schemes with elite material and exotic resources, and then compare them to guide the design of the breeding schemes.

"At the start, I worked on the genetic architecture of the trait," she recalls. We looked for the genomic regions that are linked to a trait and determined those that have a positive or negative effect depending on the exotic accession. This allowed us to simulate the evolution of this character *in silico* (by the computer tool) according to different selection schemes, to see which would be the most interesting to carry out.

"The results confirm the initial hypothesis, that using exotic resources provides a new source of genetic diversity useful for yield," concludes Prune. "The process of introgressing exotic populations into the selection material is very interesting and brave to put in place. AKER is a programme needed to 'save' sugar beet."

## ARE THE ACHIEVEMENTS OF THE AKER PROGRAMME UP TO THE AMBITIONS OF DEPARTURE?

Bruno Desprez, president of Florimond Desprez Veuve & Fils and chairman of the Coordinating Committee of the AKER programme, considers that the achievements of the AKER programme go even beyond the initial ambitions.



“Let us recall what the ambitions of the AKER programme were in 2012. Political ambitions: to keep sugar beet in the top peloton of cultivated species. Scientific and technical ambitions: to be innovative and provide tangible results for sugar beet growers and sugar manufacturers.

The beet and sugar manufacturers trusted the AKER programme by providing it with human and financial resources through the ITB, an agricultural technical institute and a real research and development organization in the sector. Based on the results of the programme, we have good reason to believe that this trust has been earned.

AKER brings remarkable scientific production, concrete, measurable results, useful in the medium and long term. From this point of view, it has become unavoidable, equipped to be solicited, copied, certainly envied for other species. The programme has created a real scientific community around beets. It made it possible to mix engineers and doctors, researchers from the public and the private sector, technical and academic contributors, producers of knowledge and ‘disseminators’.

AKER brought in experts by giving them the opportunity to practice their discipline on a playing field, that of beet, which was not necessarily familiar to them. The programme took researchers to a higher level of knowledge, each of whom was able to increase their expertise thanks to the power of collective action. The programme has become part of everyday practical advice for farmers and technicians. It facilitates the transmission of knowledge to future generations of researchers, geneticists, breeders, agronomists, biologists, bioinformaticians, etc.

AKER has brought new approaches to the fore to better understand the effects of selection over time and space. The programme broadens the themes, brings out new ambitions and new dreams. In short, AKER has become implicit, natural; it has entered the daily life of research, selection, and advice serving the sector. We are already on board for the post-AKER.”



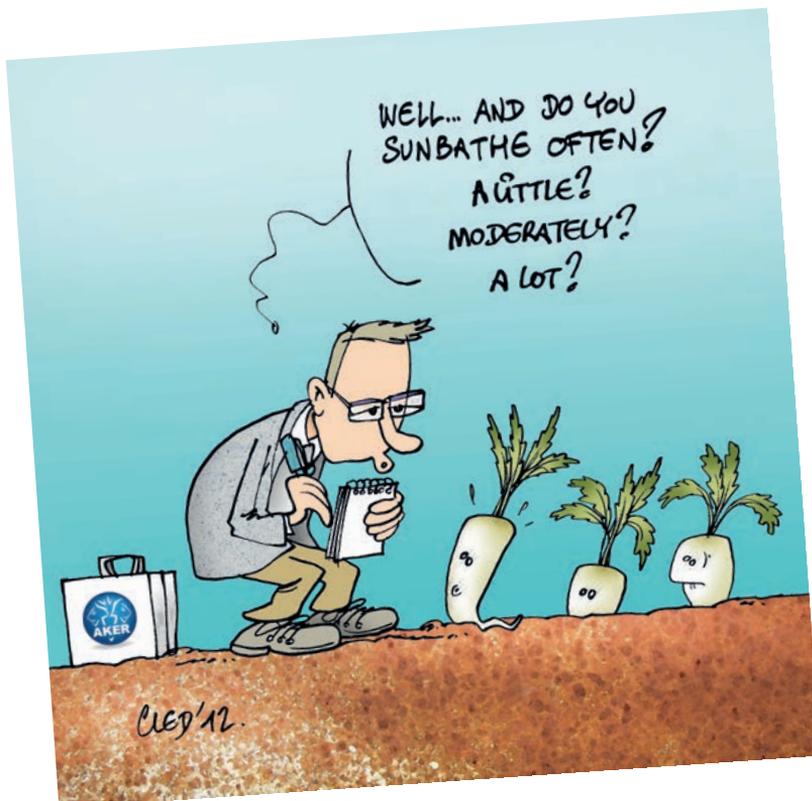


**An open  
programme**



## ■ Complementary programmes

Without waiting for the 2020 deadline, officials quickly considered the follow-up to the AKER programme. It is about valuing collateral genetic data either directly (Taker), or to feed new fields of scientific exploration with a spatiotemporal approach (Adapt, Be Domino).



## Taker programme: transversal use of data

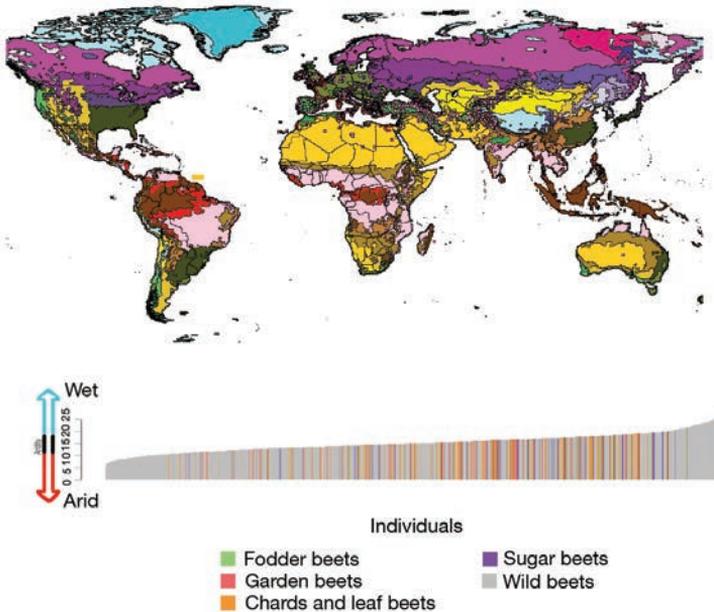
The AKER programme has accumulated a great deal of information on wild beets, but also on other cultivars (group of cultivars) than sugar beets, such as vegetable beets (red beets or other colours), economically important cultivars, that is why Taker programme (with the initial letter T for “table beet”) was launched.

Thus, among the reference plants sequenced under the AKER programme, there was also a red beet. In 2015, officials contacted the USDA (US Department of Agriculture) teams to send them all of this data so that American researchers could complete their genetic work on this species. They have expertise, a very large collection of vegetable beets and an important phenotypic evaluation of them.

The AKER programme thus provides them with a great deal of genomic data allowing, in addition to diversity analyses, to make an obvious link between genetics and the USDA phenotyping results.

Red beets and other colours.





Landscape genetics:  
distribution of  
accessions according  
to geographic areas.

## Adapt programme: use of data in space

The AKER programme has been enriched with a module, landscape genetics, which consists of studying the way each plant has adapted to its environment (soil, climate, light, humidity, altitude...). This research should make it possible to identify alleles for adaptation to a given environment, and therefore to be able to then transfer them by crossing into elite material in order to make it more conducive to growth under specific environmental constraints.

The work on landscape genetics is carried out by the CNRS and the University of Montpellier (Biogeography and Ecology of Vertebrates Laboratory), based on the numerous molecular data generated by the AKER programme.

The idea is to combine the molecular distribution of all the accessions of the genetic resources located on a map, by associating with it all the environmental parameters available (climatic conditions, day length, salt content of the littoral zones, temperatures, rainfall, etc.). The genetic distribution distinguishes an advantageous allele from a neutral allele, alleles changing or not of frequency depending on environmental conditions.

By applying this distribution to different parameters, we find SNP (Single Nucleotide Polymorphism) candidates correlated with different



A researcher at the Center for Functional and Evolutionary Ecology in Montpellier, Marco Andrello has collaborated on the AKER programme in a whole new discipline: landscape genetics.

## MARCO ANDRELLO, GENES IN THE LANDSCAPE

Of Italian origin, Marco Andrello came to do his biology thesis in Grenoble. Since then, he has worked on the genetic analysis of data from the AKER programme. His subject? Landscape genetics.

Marco explains: “We put the genes in the landscape, that is to say we study the distribution of alleles in geographic space, to try to detect the mechanisms behind this distribution such as gene flow or natural selection.”

Landscape genetics is a very recent science, and lies at the crossroads between geography, geostatistics, ecology and genetics.

By relying both on geographic and environmental information systems, and on new molecular biology tools, landscape genetics offers new means and models to identify and, if necessary, treat “genetic discontinuities” within a population, but also to detect atypical genetic variants.

As part of the AKER programme, Marco Andrello has taken up accessions from around the world. “We have found correlations between certain genetic markers and drought, which makes it possible to suppose that these markers reveal the presence of alleles involved in mechanisms of resistance to this trait,” concludes Marco.

levels of adaptation to drought, salinity, and extreme temperatures. By studying the genetic profiles of the accessions of a particular environment (for example a given market, a European country growing beet), we can highlight genomic regions that may be useful to give better adaptation and therefore greater robustness of future varieties.

## Be Domino programme: use of data over time

To study beets and their domestication, a project called Be Domino (*Beta Domesticated In Side Organization*) has been set up, led by the AKER programme and in collaboration with the USDA (United States Department of Agriculture) and the University of Padua (Italy).

Wild beet has a number of alleles. During the various domestication and selection processes, some of these alleles disappeared, others appeared in the cultivated varieties. The evolution of the allelic frequency seems to be, according to the work of the AKER programme, in direct relation to the time scale. Based on this observation, two practical studies have been developed.



### ***Search for resistance alleles***

The first approach consists in searching, in the exotic compartment, for resistance alleles already used by breeders. The objective is twofold: on the one hand, to find the source (location) of these exotic beets, and on the other hand, to characterize more finely the allele(s) of the gene and its close genetic environment. Thanks to this, we hope to find new sources of resistance to a disease and/or the same source with a close but different genetic environment (avoiding linkage drag).

The proof of this concept is found on the *Rz2* allele, used for resistance to rhizomania, a very serious and globally widespread viral disease, the infectious agent of which is a virus transmitted by a soil fungus. It is an allele of another gene than that used historically, *Rz1*.

This *Rz2* has been found in the genome of wild beets located in several very different geographical areas. The more precise molecular approach showed that the resource currently in use came from a *Beta maritima* growing on the Danish coast. Other different alleles (demonstrated with several markers internal to the gene) and also differently “surrounded” (demonstrated using neighbouring markers) have been identified in other geographic areas.

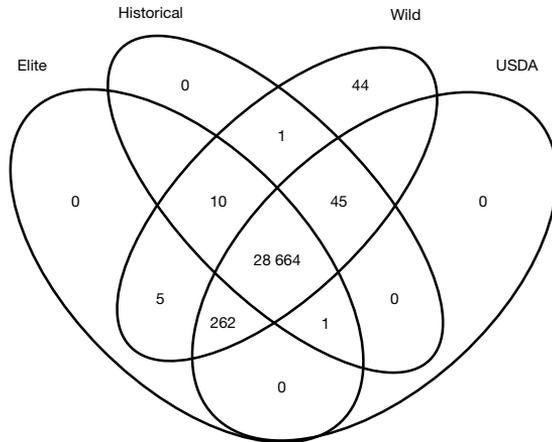
It would indeed be difficult to separate neighbouring genetic defect (linkage drag) without having to use colossal numbers needed to develop recombination between close genes. It should therefore rather be verified that these beets are very resistant and, if this is the case, that they constitute an interesting new source of variability.

The same approach has been applied for the *Rz1* site and for resistance to nematodes, without, however, having the gene itself but only an estimate of its location.

### ***Comparative approach to accessions***

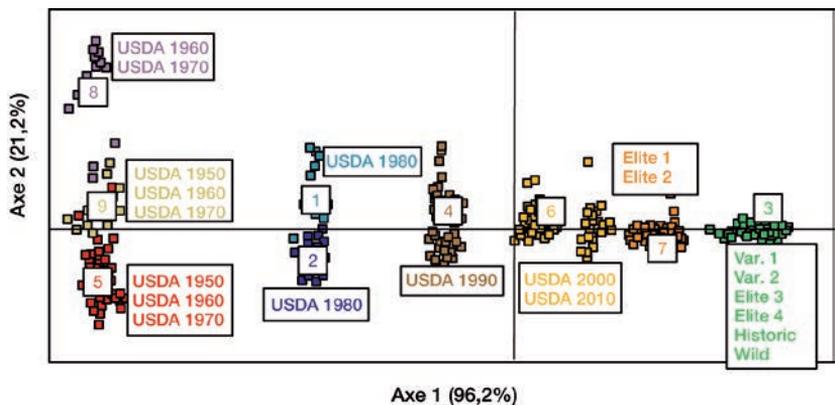
The second approach is to compare accessions belonging to four distinct groups: the wild beets, the USDA material (which can be considered as preselection), many historical varieties and finally recent elite material.

The statistical study shows a clear genetic differentiation of the groups, and a continuum going from the group of the wild beets to the elite material via the USDA material. This confirms the successive process of domestication from the wild beet in USDA material, then their exploitation by breeders.



Comparative study of the genetic composition of the different groups: elite, historical, wild, USDA.

On the other hand, the more precise study of the USDA accessions, by assigning them the date on which they are made available to the breeders (release date), allows a temporal association of the markers. In other words, from the genetic analysis of a release, we can precisely determine or in any case estimate its release date. It is the dynamics of incoming (selected) and outgoing alleles (either counter-selected or simply lost) that allows this dating.



Genetic structuring by clustering of different USDA accessions with their release date.

## TIME AND SPACE

By combining the approach of landscape (spatial) genetics and that of domestication (temporal) genetics, we can now start to retrace and locating all the events that participated in the establishment of modern sugar beet. Like paleontological studies, dating and locating fossils, genetics can also be used to date an already localized genetic resource.

## ■ Answers to new questions

The AKER programme provides answers to the questions that arise today, and will respond to new ones that will appear tomorrow. This is so with the response to questions relating to beet diseases.





Growing societal pressure to reduce the use of phytosanitary products has led to the banning of certain active substances. This is particularly the case with the removal of neonicotinoids intended to fight against virus yellow diseases. Agricultural production in general (and sugar beet in particular) thus faces real challenges in controlling certain pathogens and pests with new methods: genetic resistance is being used more than ever.

### **WHAT DO YOU REMEMBER FROM THE AKER PROGRAMME?**

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*“The AKER programme was built by the beet–sugar sector when it anticipated the exit from quotas, with the aim of bridging the competitiveness gap between sugar beet and sugar cane. It is now finalized, when the sector has been out of quotas for three years. But the economic context is particularly gloomy and beet faces new challenges: the emergence of new diseases, the loss of phytosanitary solutions, the necessary agroecological transition. The contribution of selection in this specific sector is essential: AKER will reinforce its interest, by offering new genetic diversity bringing solutions for the future and by further accelerating genetic progress.”*

Christian Spiegeleer, President of SNFS  
(National Union of Sugar Manufacturers)

### **Offer resistant varieties**

The mission of the seed producer is to offer farmers the varieties that, on the whole, are the most resistant to pests. However, the breeder no longer designs individual solutions, but market solutions: a plant in its growing environment, intended to receive certain phytosanitary treatments. In any selection programme for varieties resistant or tolerant to a disease, breeders – all plant species combined – must anticipate the phytosanitary molecules under development, as well as those that will be less used or will disappear from the market.

In addition, the diseases themselves evolve in their frequency, their impact, and their virulence. What will be the major beet disease or pest tomorrow? Climate change, changes to technical itineraries (development of different types of agriculture), regulations, etc., all contribute to an evolution of the agricultural environment. The widest possible genetic diversity is therefore necessary to consider responding to it. A kind of all–risk insurance!

## ONE HEALTH: PLANT, ANIMAL AND HUMAN HEALTH

The concept of One Health encourages researchers to think about the health of plants differently, to get out of the field. Protecting a crop also means protecting the animals and the people who eat it. When acting on a gene, the impact on the entire food chain must be considered. It is necessary to be vigilant on all the families of molecules synthesized naturally by the plant. For example, the genes that allow it to protect itself could also be responsible for a defect in the quality of the product. Reducing this inconvenience to satisfy progress and the health of consumers might amount to disarming the plant. It's all about dosage and balancing the expectations of nutrition, health and genetics experts.

### Reconciling resistance and performance

Diversity alone cannot answer all of the questions. In fact, we find that most exotic material, if used directly in varieties, would have a negative impact on yield. It is therefore complicated to play on two counts: resistance being expressed by a genetic cost, an important work of selection is to be carried out. Since resistant plants represent only an often minimal fraction of diversity, the potential for diversity necessary for genetic gain for other favorable traits such as yield decreases. Thus, in terms of genetic progress, the more numerous the complementary solutions are, the less it will be necessary to mobilize part of the variability, and the more the compromise to protect plants will be easy to identify.

The resistance traits are sometimes of the monogenic type, that is to say that few genes are involved. However, these genes can produce large effects, unlike polygenic characters, such as yield, which are generally governed by many genes but each with small effects. Introducing them into elite material is therefore easier and certainly has less negative impact on performance. This would constitute an area to prioritise with NBT (New Breeding Technologies), and in particular the editing of genes. But this solution is currently prohibited in Europe for release into commercial agriculture – even if it is authorized for basic research.

### Combine all the levers

Another important aspect: in conventional plant breeding, the time steps for varietal improvement are quite long (10–15 years) and do not

allow great reactivity in the face of environmental changes. It's time to take advantage of the tools available, optimize them and speed up the selection cycles. Introgression tools must be provided for the breeder, in order to offer her or him the possibility of accelerating the processes of introgression of resistance genes.

Genetics will not be able to solve all problems, but it is an essential link in the multifactorial response to current challenges: it would be very foolish not to make the most of it. If we want to target the “zero pesticides”, we cannot, however, rely solely on genetics. It will have to be combined with all the other levers of agroecology available (prophylaxis, cultural practices, etc.) which do not exclude the use of biocontrol products. It will also probably be necessary to accept a certain level of disease, which will remain below the harmfulness threshold. The desire to go “disease-free” has probably been pushed to excess. Monitoring of pest populations should be considered to ensure the sustainability of the genetic resistance deployed.

### New sources of yellows resistance

The sixteen reference plants of the AKER programme were not initially phenotyped for their resistance to diseases. Whether they are resistant or not, they are all considered a resource to be assessed in an elite genetic context. During the AKER programme, the 3,000 hybrids were evaluated against powdery mildew and *Cercospora*. The ban on



Close-up on a sick beet with yellows.

Aphid vector of yellows.





neonicotinoids makes it essential to use biotests to detect new sources of resistance to yellows (to viruses or the aphids that are vectors of the disease). The material introduced in these tests consists of elite pollinators introgressed with exotic fragments and not the 3,000 hybrids; sorting is thus earlier and will allow to test multiple hybrid combinations.

Beet field  
infested with  
yellows vectors.

### **AKER IS A PIONEER PROGRAMME**

One may wonder, at the end of the AKER programme, what it would have been if it had not existed, and one can reasonably say that there is a before and after AKER. Beet is certainly a soundly-based crop thanks to the long-term work carried out by research, the remarkable adaptability of the plant, the technicality of the farmers and the quality of professional organizations in the sector. However, the AKER programme makes it possible to create a break with the past in terms of genotyping (genome analysis), phenotyping (evaluation of traits) and data processing (bioinformatics). So sugar beet will be able to leap ahead and end up in the front row of cultivated plants.

## ■ A dynamic contribution to modern agriculture

Sugar beet has benefited from the gems of public and private research and the efforts of all stakeholders since the 19<sup>th</sup> century. The AKER programme is part of the 21<sup>st</sup> century in this strong dynamic.



Beet is an exceptional sector by its organization, its influence, and its constantly improving performance. It is a modern sector that is open to the world, in direct contact with social debates. It is a sector that takes responsibility, which can be proud of the work accomplished, while being aware of the road it still has to travel at the international level.

AKER thus brings its positive and innovative scientific contribution to a productive agriculture, respectful of the health and the environment of our fellow citizens.

The programme provides society with the passion of its researchers who have learned to work together for eight years in a collaborative approach.



Sugar is synonymous with pleasure.

Alcohol, from sugar beet, is used in the composition of perfumes.



It uses the highest technologies (genetics, digital, bioinformatics...) in the service of agriculture in compliance with current regulations (non-GMO) and with concern for the changes needed (genetics vs phytosanitary, loss of neonicotinoids, etc.).

AKER offers an additional opportunity to improve the competitiveness of the French beet sector thanks to an exceptional investment of €18.5 million (including €5 million public grant) in the framework of Investments for the Future Programmes.

Finally, the benefit that the consumer and the taxpayer can expect from the AKER programme lies in the local production of quality sugar. This production maintains a sector and employment on French and European territory, in favourable economic conditions for farmers, industrialists and consumers.

## CAN WE STILL CONSUME SUGAR TODAY?

*"Sucrose is a natural sugar made up of a molecule of glucose and fructose. Glucose is primarily an energy substrate for muscle work, but it is also the brain's preferred fuel. That is to say that it is useful. But can it be harmful? All carbohydrates promote cavities in case of poor oral hygiene. If energy expenditure (physical activity) is lower (on a regular basis) than intake, this can lead to weight gain. As part of a balanced diet and in conjunction with regular physical activity, there is no reason to banish sucrose, especially since it is a vector of eating pleasure. As for the true addiction to sugar, it is observed especially in the event of a food restriction."*

Doctor Jean-Michel Lecerf, Department of Nutrition and Physical Activity, Institut Pasteur of Lille

## WHAT IS THE OTHER ADVANTAGE OF SUGAR BEET?

*“Certainly, beets produce sugar. However, sugar produces alcohol and bioethanol by fermentation. Agricultural alcohol finds its place in spirits, but also in pharmacy, perfumery, cosmetics, industry.. As for bioethanol, incorporated in SP95-E10, or superethanol-E85 for flex fuel cars, or ED95 fuel for heavy vehicles equipped with dedicated diesel engines, it has demonstrated its environmental advantages. Improving the competitiveness of sugar beet is also having an impact on all links in the alcohol-bioethanol sector.”*

Sylvain Demoures, General Secretary of the SNPAA  
(National Union of Agricultural Alcohol Producers)

## ■ A partnership, collaborative, multidisciplinary programme

The expanded partnership, multidisciplinary collaboration, long-term installation (eight years) have made AKER a dynamic and evolving programme, a guarantee of sustainability.



## Partnership

The AKER programme brings together eleven partners, from both the public (five INRAE units, including the former Irstea, Geves) and the private sector (the Florimond Desprez seed company), applied research and development, the academic world (Universities of Angers and Lille, Agrocampus Ouest), serving the beet sector as a whole represented by the ITB (Technical Institute for Sugar Beet).

In addition, farmers and industrialists were associated in the implementation of the programme guidelines, in a strategic committee. They hijacked the project by exchanging on its priorities and anticipating the dissemination of its results.

## Collaborative

The strength of the AKER programme lies in its collective nature. It brought together a hundred collaborators from all disciplines, who learned to work together. It has enabled the various partners to collaborate, to create new tools and new methods, to publish in scientific journals, in a word to accelerate research by combining forces.

The coordinating committee of the AKER programme.





AKER is not a sum of individual projects, but a joint laboratory. It is characterized by a great coherence focused on a thematic in the service of an objective: to improve the competitiveness of sugar beet. The geographic dispersion of collaborators was compensated by the discussions initiated during the annual coordinating committees, and by regular interpersonal communication.

## Multidisciplinary

AKER is at the crossroads of many disciplines studied by its different partners, and finds its richness in this diversity. These include genetics, physics, optics, mathematics, physiology, biochemistry, bioinformatics, robotics, agronomy, etc. It involves partners who are not necessarily beet specialists, but who bring their expertise in their chosen field, promoting “open innovation”.

This multidisciplinary facilitated the permanent adaptation of the collaborators to the evolution of technologies and knowledge throughout the programme.

### WHAT IS THE LEVERAGE OF THE AKER PROGRAMME ON THE FRENCH MARKET?

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*“Sugar beets have experienced one of the biggest yield increases in decades, thanks to genetics. The AKER programme derives its efficiency both from the richness and diversity of the genetic heritage of wild plants, and from the power of available technologies in classical selection. Building on these strengths, AKER will enable Cristal Union’s cooperators to respond to the dual challenge of increasing sugar yields per hectare and combating pests, while contributing to environmental challenges.”*

Olivier de Bohan, Chairman of the Board  
of directors of Cristal Union

## ■ A transversal programme

The AKER programme is transversal in nature thanks to its new and original approach to the exploitation of exotic genetic resources, extrapolable to other cultivated species.

AKER is already producing well-known selection tools and methods in other fields (medical, plant, veterinary, etc.) or which can be extrapolated



to other species. Here we are thinking not only of marking and sequencing, but also of genome analysis methods, imaging techniques for phenotyping, genomic selection, etc.

The dynamics of obtaining genetic material more efficiently may benefit other plant species. Indeed, sugar beet is a species with a fairly small genome. The accumulated knowledge is, for the most part, generalizable or even adaptable to species with larger and often more complex genomes, such as wheat.

Diversified agricultural landscape where sugar beet find its place in a farming system.





The peculiarity of sugar beet also lies in the fact that it is an industrial plant with roots, and which is biennial. All of the knowledge accumulated on its vegetative phase, and in particular on the root, its architecture, its physiology, etc., will be transposable to species with taproots or even more complicated. Its reproductive phase, its seed carriers, its flowering physiology, and its seeds... are also all models for other plants in which we seek to improve these characters.

The AKER programme therefore not only repositions beets in the leading group of cultivated species, but also allows other orphan research species, with reference collections, to join the peloton by providing them with the methods and tools developed for beet.

### **WHAT IS THE LEVERAGE OF THE AKER PROGRAMME ON INTERNATIONAL MARKETS?**

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*“The liberalization of the market has made our farms and our sugar factories more vulnerable to fluctuations in the price of sugar and, in recent years, climatic vagaries have been linked. This is why genetics still has a lot to offer us. Genetic progress should enable us to secure a high level of yield despite environmental hazards (drought, climatic shocks, parasitism, frost, etc.) and societal changes. The AKER programme has been a gamble, a mark of confidence in the industry and in the state towards research, and it is a gamble that seems to be on the verge of being successful. AKER will be one of the tools that will open up the field of possibilities and help secure sugar beet production.”*

Jean-Charles Lefebvre, Chairman of the Supervisory board of Tereos

## **■ A research–education–business continuum**

**Agrocampus Ouest and the University of Lille have offered a week-long training course each year from the start for master’s degree students in sugar beet and the AKER programme.**

An original collaboration between a Grande École and a university has brought together more than 200 students in total over a period of eight years, face-to-face in Rennes at Agrocampus Ouest and by videoconference at the University of Lille. The proposed master level module (1 and 2) is entitled: “Selection assisted by markers and genomic selection: application to an allogamous plant, sugar beet”.



The originality of this collaboration lies both in the fact of integrating training into a research programme, and in the modalities and operation of this training. It alternates theoretical courses, practical applications, tutorials, and round table sessions to broaden exchanges thanks to the transversality enabled by the AKER programme.

This round-trip theory-practice allows the bringing together not only of teachers, but also research partners and other actors of the AKER programme. The roundtable discussions, at the request of the students, made it possible to widen the range of speakers to Inra researchers, breeders (Florimond Desprez, Limagrain), agricultural technical institutes (ITB, Arvalis – Institut du végétal, Terres Inovia), as well as to other researchers.

The strong involvement of stakeholders in the beet sector in this module brings real added value to the training. It is an example of concrete feedback from the new selection methodologies developed in companies. This training is also the time for an active participation of the students, in particular via the oral synthesis/summarising of the highlights of the week that they make and discuss with the different partners of the AKER programme.



Director of the Institute of Genetics, Environment and Plant Protection (Igepp) and professor of genetics and plant breeding at Agrocampus Ouest, Maria Manzaneres-Dauleux makes the link between research, training and companies. This is verified within the framework of the AKER programme.

### MARIA MANZANERES-DAULEUX: *CONTINUUM*, CO-CONSTRUCTION, PARTNERSHIP

Originally from Spain, Maria Manzaneres-Dauleux studied at the Complutense University of Sciences in Madrid, then completed a DEA in plant improvement genetics at Ensa in Rennes, and finally a doctoral thesis on artichoke resistance to viruses. From the 2000s, she worked at Inra Rennes before taking over as a professor of genetics and plant breeding at Agrocampus Ouest.

Through Igepp, the largest joint plant research unit located in Brittany, Maria believes that a *continuum* must be established between basic and applied research. “This *continuum* makes it possible to respond to rapid developments in science, to translate innovations in teaching and to transmit them as quickly as possible to students.”

According to her, training must be the result of a joint construction between universities, schools and companies, if only through internships, theses, etc. “This involves partnerships, like what has been done with Agrocampus Ouest, the University of Lille and Florimond Desprez for the construction of a training module within the framework of the AKER programme”, she explains.

This triptych “*continuum*, co-construction, partnership” is also experienced by Maria Manzaneres-Dauleux in other sectors where the interactions between industries and companies work well. And, to close the circle, she concludes: “Innovations must be accepted by society.”

Students in training on the AKER programme.



## JEAN-LOUIS HILBERT: PREPARING THE NEXT GENERATION OF PLANT RESEARCHERS

“We must attract young people to research, make plants attractive, train future researchers beyond the AKER programme and prepare the next generation,” says Jean-Louis Hilbert, trained at the University of Nancy and deputy director of the Terra-Viollette cross-border joint research unit at the University of Lille. First of all, it is a matter of promoting existing training modules and instilling in future researchers the “AKER spirit”, for example on new tools in genomics, sequencing, the study of the expression of genome, diversity analysis, etc.

“This approach was implemented for master’s degree students, but also for technical staff who wanted to update their knowledge of the plant,” said Jean-Louis. It has been extended to active researchers at the engineering level, supplied by external contributors, whether or not they are linked to the AKER programme.

The particularity of these training courses at all levels is that they concern both the University of Lille and Agrocampus Ouest, with the collaboration of Florimond Desprez. “We have pooled our training,” adds Jean-Louis Hilbert, even if each of the clusters has its particularities: genomics for Lille, genetics for Agrocampus Ouest. The link between research and teaching is through pedagogy, an intrinsic dimension of the AKER programme.



Jean-Louis Hilbert, university professor at the University of Lille, making the link between teaching and research, a feature and an asset of the AKER programme to guarantee the next generation.

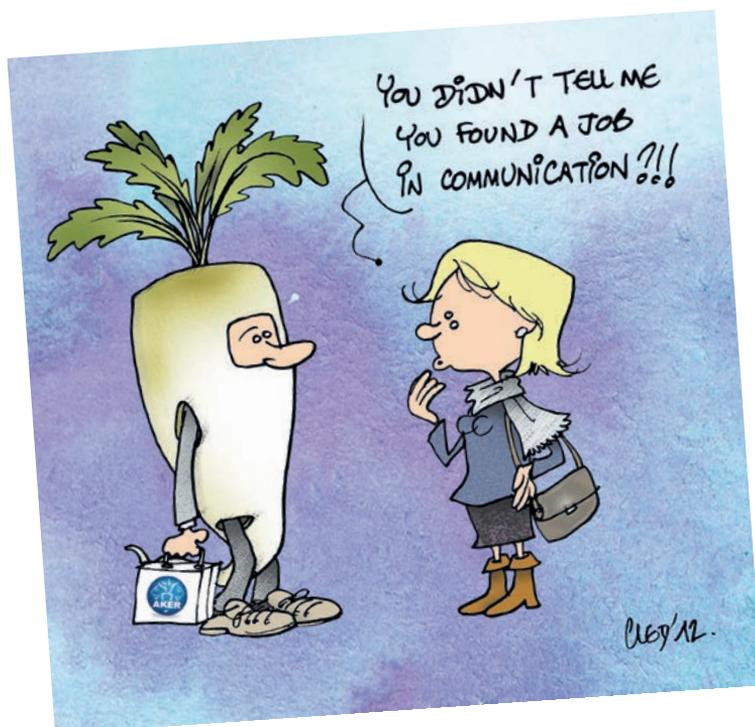
The opening up to other Investments for the Future Programmes with the same concerns made it possible to compare strategies, means and interpretations. In 2016, the actors working on rapeseed as part of the Rapsodyn programme were invited. This sharing of experiences was renewed in 2017 with researchers working on peas as part of the PeaMust programme.

In addition, numerous interventions took place during the AKER programme in other agricultural or agronomic higher education establishments, whether at the technical or engineering level (UniLaSalle, ISA Lille, Institut de Genech, etc.).

This research–training approach will undoubtedly be carried forward to any new research programme, evolving towards the concept of co–construction (codesign). The innovative pedagogy thus implemented starts from the description of the problems, continues with work carried out by the students and ends with a critical exchange with the experts.

## ■ The power of communication

The AKER programme has a real communication plan which involves creating an identity, defining key messages, implementing multi-year programming and supporting employees over the long term.



Initially, the AKER programme joined the services of a communication manager and an editorial committee, comprising researchers and communicators, responsible for piloting/validating communication. A graphic identity was defined and helped shape the image and reputation of the programme.

The priority was to build, through internal communication, a team spirit with collaborators who did not know each other initially and who built the project together. They themselves have acquired the reflex of participative communication.

The researchers were associated with external communication and supported in their contribution by co-producing numerous articles,

portraits, files, etc. The communication approach implemented starts from the principle that researchers are best placed to talk about objectives, methods, work and results... without waiting for the end of the programme. AKER has therefore adopted a multi-year plan, highlighting a communication theme each year, and giving rise to a substantial dossier, with the collaboration of each of the partners concerned.

Press trip organized on a phenotyping platform.



Presence of AKER at professional events like Betteravenir, an exhibition organized by the ITB.





Vidéos



Récolte des sèves de phénotypage



Utilisation du drone pour le phénotypage de champ



Mise en place des essais de phénotypage



Mode en place des essais de phénotypage

www.aker-betterave.fr



AKER, cap sur 2020

AKER contribue à améliorer la compétitivité de la betterave



www.aker-betterave.fr



Some visuals of the communication supports.

A website was created from the outset to carry all of the messages and content. It was later reinforced by publishing regular newsletters and setting up a Twitter account. Posters and brochures were published at key stages of the programme, and numerous videos were produced. A launch meeting for the AKER programme was organized in 2012 and the 2020 international scientific symposium will have closed it. The synthesis of the work is found in the publication of this work.

AKER participated in many professional events, including the Betteravenir professional event organized by the ITB in 2016. Contacts were maintained regularly with journalists, and two press trips were organized during the programme: on the theme of seed phenotyping in Angers (2014) and on a field phenotyping platform (2018). This communication plan has contributed to the pride of belonging for employees of the AKER programme, and to its notoriety during its duration and beyond.

## HÉLÈNE DORCHIES: SHOW EMPATHY

When she discovered AKER, Hélène Dorchies was enthusiastic: "It is a beautiful programme, very ambitious in terms of results, methods, partnerships. I arrived during the results phase; it is the richest in terms of communication."

Hélène holds a master's degree in marketing and communication, she likes the job, but she realizes that she lacked writing skills. She then completed a master's degree in communication and journalism in work-study. After going through a public relations agency, she returned "to the advertiser" at the Livestock Institute, then at ITB.

She believes that she is at the service of an agricultural technical institute to disseminate the results of the applied research work of her colleagues and, thereby, participate in improving practices. She insists on the term of service and continues: "Establish the relationship between the planters, the industry and my colleagues, listen and show empathy, send a message so that the receiver hears it and above all listens to it." But not just anyhow. "The problem is not so much what to say, but how to say it," she said.

Coming from the world of livestock, Hélène Dorchies observes acutely the evolutions of agriculture and society. She notes that the criticisms leveled against the animal sector which appeared several years ago are reaching more and more the plant. This comes, she says, from the fact that society has an image out of touch with reality. Hélène concludes: "The agricultural world must show what it does, explain how our food is produced, show how farmers work: the technical nature of their job and their constant concern to provide quality products."



One of the keys to the job of Hélène Dorchies communication manager at ITB is empathy. In addition, the agricultural world must show society its know-how and the quality of its work.



Communication manager at Geves (Variety and Seed Study and Control Group), Nathalie Augé attaches great importance to teaching and promoting the work of plant experts.

## NATHALIE AUGÉ: COMMUNICATING AN EXPERT OPINION

An agricultural engineer, Nathalie Augé began her career as a teacher of scientific subjects in an agricultural educational establishment, then as establishment manager. “Transforming scientific data into simple elements of understanding has always fascinated me, pedagogy is very useful to me today,” says Nathalie.

She defines the mission of Geves with this communicating signature: “Expertise, from the gene to the plant via the seed, on all of the cultivated species”. She likes to give her visitors a concrete vision of this organization, by restoring her action from a sector perspective, thanks to a frieze where she gathered all the stages and all the partners of the seed activity.

“In terms of communication, we have many requests from our colleagues, but we always start by focussing them: who do you want to address? Why? How?” Nathalie led the construction of the new website with a different approach: “We wanted to start from the needs of the users, and not from the description of our missions.”

Through the National Seed Testing Station, within Geves, Nathalie has followed the AKER programme on the technical level (phenotyping of seeds and seedlings) and she participates in the communication dynamic initiated among the partners.

## ■ An international dimension

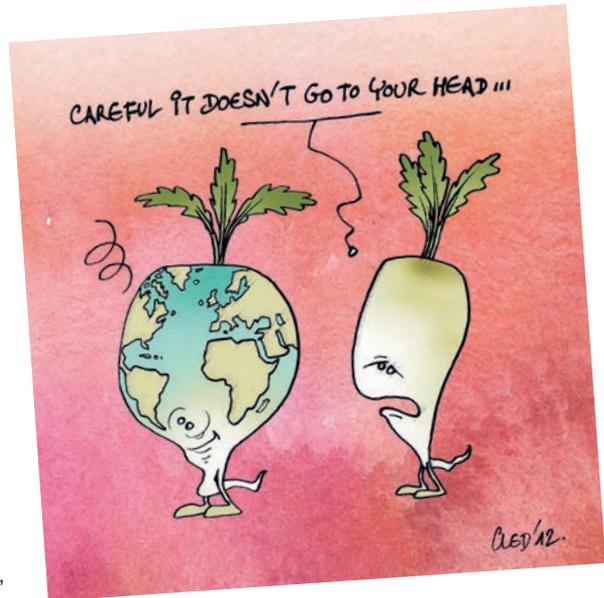
If the vocation of the AKER programme is national, its dimension is international, because its results are of interest for the whole scientific community and will allow the current limits of beet to be pushed back.

The AKER programme aims to strengthen the competitiveness of beet compared to cane, which is dominant worldwide, even though sugar beet is grown in around 50 countries. The AKER programme managers set up a scientific committee from the start, made up of ten members, half of whom are foreign scientists recognized for their skills and international approach to beet research or genomics.

Researchers have travelled widely to present the objectives and results of the AKER programme at scientific conferences, for example at the PAG (Plant & Animal Genome) in San Diego (California), at the general assembly

of ASSBT (American Society of Sugar Beet Technologists). Closer to home, in Europe, many AKER researchers participated in the regular congresses of the IIBR (International Institute of Sugar Beet Research) and others during the congresses of the CIBE (International Confederation of European Beet Growers) and CEFS (European Committee of Sugar Manufacturers). Florimond Desprez, for his part, hosted the congress of the WABCG (World Association of Beet and Cane Growers).

Beet research is not isolated, researchers work in an interdependent world, and the results of the AKER programme can be used in varied contexts. By analysing the adaptation capacities of beets and pushing their limits, we can imagine conquering new geographic areas, new countries, new markets.



The congress of the World Association of Beet and Cane Growers (WABCG) received at Florimond Desprez.





## AND NOW, WHAT IS THE 360° PERSPECTIVES FOR THE AKER PROGRAMME?

Christian Huyghe, scientific director of Agriculture at INRAE, AKER project manager, concludes this work by affirming that the prospects of the programme are of unprecedented richness for the beet world and, beyond, fuel the debates of the scientific world with exemplarity.



“The AKER programme was a wonderful scientific adventure, launched in 2012, in an agricultural and economic context widely described in this book and whose conditions anticipated in 2012 were largely confirmed, namely the global competition on the sugar market and the environmental performance requirements for productions.

The scientific gamble won by AKER was based on the idea of widening the genetic diversity available for the selection of sugar beet, made possible by molecular biology techniques, by high throughput phenotyping technologies, starting from the seed and seedling to the root through the development of plants in the field. It was also based on the know-how of all those involved in the work, making this scientific adventure a formidable human adventure. The 360° perspectives for the follow-up to the AKER programme are of unprecedented richness.

In terms of genetic progress, the very significant increase in diversity makes it possible to envisage significant progress for major agronomic traits, under polygenic control, for several decades. It should be the same for characters under oligogenic control whose alleles are infrequent, in particular resistance to pests.

In terms of genetic improvement methodology and variety characterization, the AKER programme has created a unique resource of genotyping and phenotyping technologies. They will be usable by breeders and seed companies. They will also have to be used when registering varieties in the National and European Catalogue. The fact of having worked closely with the CTPS and with the Geves, partner of the project, is therefore a considerable asset which will have to be played quickly.

Finally, at the moment when lively discussions stirred the scientific world on the place to be given to New Breeding Technologies (NBT), the AKER programme drew, with exemplarity, a new line of reconciliation between, on the one hand, a methodical exploration of genetic resources using the most recent technologies, and, on the other hand, an implementation of these genome editing techniques where no genetic variability exists.

This progress also underlines the extreme attention that should be paid to the characterization and preservation of genetic resources, thus consolidating the dynamic implemented today in the field of plant genetic resources and drawing attention to the implications and potential negative consequences of the difficulties of Tirpaa (International Treaty on Plant Genetic Resources for Food and Agriculture).”



# GLOSSARY

**Accession.** Identified batch of seeds from a seed collection (bank).

**Allele.** Variant of the same gene at the same locus. Within a species, the genomes of individuals are all different. This genetic polymorphism is due to the appearance of mutations which are variations of the nucleotide sequence. Several natural sequences may therefore exist in natural populations for the same locus. We are talking about different alleles.

**Association genetics.** Recent methodology that allows access to complex characters, without the need to sequence all the genes involved. It also makes it possible to test a large number of genotypes and to explore a broad spectrum of genetic diversity. It consists in statistically “associating” the variations of the characters (phenotype) with the variations of the genome.

**Backcrossing.** Crossing of a hybrid with one of its parents or with an individual genetically similar to one of its parents, so as to obtain a descendant having a genetic identity closer to that of the parent.

**Big data.** Combination of technological progress, linked to the massification of data, and innovations in the use of this data. Over the past few years, a set of technological innovations has transformed the way in which data is generated (with exponential volumes and speeds in particular), transmitted, stored and used, and this is becoming increasingly distributed (via the cloud).

**Bioinformatics.** Science at the interface of digital disciplines (computer science and mathematics) and life sciences (biochemistry, biology, microbiology, ecology, epidemiology). Life scientists are generating an increasing number of new data on genomes, biomolecules, organisms, their interactions and their evolution.

**Cell hybridization.** Cell fusion or cell hybridization is the *in vitro* formation of a single hybrid cell by the union of two cells from different species. In the hybrid cell, the donor nuclei may remain separate or may merge but, during subsequent cell divisions, a single mitotic spindle is formed, so that each of the daughter cells will have a single nucleus containing complete or partial chromosome sets of each parental line.

**Chromosome.** Microscopic structure formed by the association of proteins with a DNA molecule representing all or part of a genome.

**Collection (of genetic resources).** Gathering of domesticated individuals (traditional varieties, ancient and modern cultivars, and improved lines) and related wild or weed species.

**Crossover.** Also called crossing-over or spanning, is a genetic phenomenon that takes place during meiosis and that contributes to genetic mixing during reproduction (genetic recombination).

**Cross-pollination.** Allogamous crossbreeding system in which crosses are made between less related individuals than the average pairs chosen at random from the population.

**DNA (deoxyribonucleic acid).** Macromolecule carrying genetic information, composed of two strands associated in a right helix and made up of a chain of deoxyribonucleotides.

**DNA sequencing.** DNA sequencing involves determining the sequence of nucleotides for a given DNA fragment.



**Gene.** Deoxyribonucleic acid (DNA) sequence that specifies the synthesis of a polypeptide chain or a functional ribonucleic acid (RNA). You can also define a gene as a unit of genetic information. This is transmitted from cell to cell during the process of mitosis, after duplication of genetic material (chromosome(s)). The entire genetic material of a species constitutes its genome.

**Gene bank.** *Ex situ* conservation device for genetic material. In the case of plants, this can be done by freezing cuttings taken from the plant, or seeds.

**Genetic distance.** Degree of kinship between different genomes.

**Genetic diversity.** Variation in the genetic makeup of individuals of one species or of individuals of different species; heritable genetic variation within or between populations.

**Genetic engineering.** Set of techniques for modifying the gene pool of a cell by manipulating genes *in vitro*. All the tools and techniques of molecular biology allowing, in a controlled manner, the study of the modification of genes: their isolation, their cloning, their sequencing, their cutting... for the purpose of fundamental or applied research.

**Genetic erosion.** Loss of genetic diversity over time between or within populations, or reduction in the genetic base of a species.

**Genetic imprint.** Fine structural characteristic of a specific region of DNA allowing the identification of a cell and its parentage.

**Genetic labeling.** A genetic marker in or close to a gene.

**Genetic map.** Construction of a map, either localized around a gene, or based on a large genome. More generally, it is a question of determining the position of a locus (gene or genetic marker) on a chromosome as a function of the rate of genetic recombination. Its unit of distance is the centimorgan (cM).

**Genetic marker.** Site on a physically identifiable chromosome, the transmission of which can be followed. Character which serves as experimental evidence to identify an individual or one of its characteristics.

**Genetic polymorphism.** Variation between individuals in the gene sequence.

**Genome.** A set of hereditary material made up of nucleic acids (DNA or RNA) from a cellular organelle, an organism or a species. The nuclear genome of a plant is carried by its chromosomes.

**Genomic selection.** Genomic selection aims to predict the genetic value of candidates for selection. The predictive model is calibrated on a set of individuals using their assessments, usually phenotypes, and their genotypes. It is then applied to candidates for selection whose genotype is known for the same markers and who have generally not been evaluated.

**Genotype.** All of an individual's alleles. In the narrow sense, all of an individual's alleles at particular loci.

**Genotyping.** Molecular determination of the genotype or fraction representative of the genotype of an individual.

**Homologous chromosomes.** Homologous chromosomes (a pair of homologous chromosomes forms a bivalent), also called autosomes (only for non-sex chromosomes), are chromosomes belonging to the same pair, of the same size, having the same genes but not necessarily the same alleles. One of the homologous chromosomes in diploid organisms is of maternal origin, the other of paternal origin.



**Hyperspectral imagery.** Spectro-imagery, also called hyperspectral imagery as opposed to multispectral or super spectral imagery, is a technology allowing the representation of a scene according to a large number of spectral bands (generally more than a hundred), narrow ( $\leq 10$  nm) and contiguous.

**Intergenic DNA.** Non-coding DNA, increasingly studied and which seems to be involved in the structure of chromatin. More specifically, the latest research has shown a crucial role for these regions in regulating gene expression by modifying the state of chromatin on large chromosomal regions.

**In silico.** Latin-inspired neologism designating research or a test carried out using complex computerized calculations or computer models. This expression is used in the fields of genomics and bioinformatics.

**In situ.** From Latin “on the spot”. Expression used to designate an operation or a phenomenon observed in the place where it takes place (without removing or moving it), as opposed to *ex situ*.

**In vitro.** Experiments made in test tubes – literally “in glass”. Not in a living organism.

**In vivo.** From Latin “within the living”. Expression qualifying research or examinations carried out on a living organism, as opposed to *in vitro* or *ex vivo*.

**Karyotype.** The karyotype (or karyogram) is the standard arrangement of all the chromosomes in a cell, from a microscopic shot. The chromosomes are photographed and arranged in a standard format: in pairs and classified by size, and by position of the centromere.

**Linkage disequilibrium.** The linkage disequilibrium is related to the physical proximity of the markers to the genome. If two markers are physically close, the disequilibrium between them is significant; if they are far apart, the disequilibrium is small.

**Linkage group.** All the loci that appear linked by analysis of their hereditary transmission. Typically, a chromosome.

**Locus.** Specific position on a chromosome where a particular gene, group of genes, or segment of DNA is located.

**Microsatellite DNA.** Repeated type of DNA made up of repeats of very short motifs such as di-, tri- or tetranucleotides. Also called simple sequence repeats (SSR).

**Molecular hybridization.** Association of single strand nucleic acid chains to form double strands.

**MRNA.** Messenger ribonucleic acid, messenger RNA or mRNA is a transient copy of a portion of DNA corresponding to one or more genes. It is used as an intermediary by cells for protein synthesis.

**Multispectral imagery.** Multispectral imagery records multiple wavelengths in a single shot that are isolated for specific analyses and recombination techniques. This allows for much finer detail analysis and visualization of details not visible to the naked eye.

**Ontology.** Universal vocabulary and structured information that allows better sharing of data acquired by different researchers and above all for automated searches. It is at the same time a dictionary, a lexicon, a tree structure, a map of relationships between terms which works with cascading logic.

**PCR (polymerase chain reaction).** A polymerase chain reaction is a technique used to obtain large quantities of a specific DNA sequence from a DNA sample. This amplification is based on the replication of a double stranded DNA template. It breaks down into three



phases: denaturation, hybridization with primers and elongation. The products of each synthesis step serve as a matrix for the following steps: an exponential amplification is thus carried out.

**Phenotype.** Set of observable characteristics of an individual. For a given trait, the phenotype is the result of the interaction between the genotype and the environment.

**Phenotyping.** Determination of the phenotype of one or more individuals.

**Physical map.** Sequencing of overlapping cloned fragments reconstructing the starting DNA molecule. It is from this map that the minimum set of fragments will be chosen ensuring complete coverage of the genome to be sequenced. The distances between the different markers, measured in base pairs (bp), are said to be absolute.

**Plant improvement.** Multiplication and genetic manipulation by hybridization or deliberate crossing of plants in order to select improved progenies.

**Polymerase.** Enzymes which have the role of synthesizing a strand of polynucleotide (DNA or RNA), most often using a complementary strand as a template and nucleotide triphosphates (NTP or dNTP) as monomers.

**Population genetics.** Quantitative study and measurement of populations in statistical terms. For example, studying genetic phenomena in terms of standard genetic parameters such as frequency tables and distributions, means, variances and standard deviations.

**QTL (Quantitative Trait Locus).** A more or less large region of DNA closely associated with a quantitative characteristic, that is to say a chromosomal region where one or more genes are located which are at the origin of the characteristic in question.

**Recessive (vs dominant).** In genetics, we speak of recessivity when an allele cannot give a phenotype if it is the only representative in the chromosomes of the cell considered (except in the case of haploidy).

**RNA (ribonucleic acid).** Polymer consisting of a linear chain of ribonucleotides.

**Selfing.** Reproductive system by which a plant reproduces with itself.

**SNP (Single Nucleotide Polymorphism).** The nucleotide polymorphism or polymorphism of a single nucleotide is, in genetics, the variation of a single base pair of the genome, between individuals of the same species.

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# AKER PARTNERS

## INRAE, Scientific Direction of Agriculture

The National Research Institute for Agriculture, Food and the Environment (INRAE) is a French agricultural research organization founded in 1946, having the status of a public scientific and technological establishment (EPST), and placed under the double supervision of the ministry in charge of Research and the ministry in charge of Agriculture. First agricultural research institute in Europe and second in the world in number of publications in agricultural sciences and in plant and animal sciences, INRAE conducts finalized research for healthy and quality food, for sustainable agriculture, and for a preserved and valued environment. It is the result of the merger between Inra and Irstea. [www.inra.fr](http://www.inra.fr)

## INRAE, LIPM, Toulouse

The Plant–Microorganism Interactions Laboratory (LIPM) is a joint CNRS–INRAE research unit. The LIPM primarily explores the interactions between plants and symbiotic or pathogenic microorganisms, through concerted studies of microbial and plant partners. This research is carried out on a small number of model species (*Arabidopsis thaliana*, *Medicago truncatula*), but also more recently on models of agronomic interest such as sunflowers, tomatoes and beets. <https://www6.toulouse.inra.fr/lipm>.

## INRAE, Urgi, Versailles

The Genomics–info research unit (Urgi) is dedicated to the genomics of plants and their pathogens. Its research activity focuses on the structure and dynamics of the genome. Urgi currently comprises two teams: one dedicated to the development of tools for the analysis of the genomes of plants and fungi, the other to the development of the information system and data integration. The unit hosts a bioinformatics platform belonging to the French Institute of Bioinformatics (IFB). <https://urgi.versailles.inra.fr>

## INRAE, IRHS, Angers

The Horticultural and Seed Research Institute (IRHS) brings together the main players in Angevin research in plant biology, under the supervision of INRAE, Agrocampus Ouest and the University of Angers. This laboratory brings together the expertise of geneticists/genomicians, phytopathologists, physiologists/ecophysiologicalists, biochemists, modelers, bioinformaticians and biophysicists at the service of the quality and health of horticultural species, and seed production. <https://www6.angers-nantes.inra.fr/irhs>

## INRAE, ITAP, Montpellier

The ITAP UMR (Information and technology for agro–processes) develops science and technology with the aim of developing equipment for sustainable agriculture that respects the environment. Within ITAP, the COMIC laboratory (Optical sensors for complex environments) is interested in the design of optical systems and data processing methods in complex environments: multi– and hyperspectral imaging; instrumental optics and spectrometry; chemometrics. <https://itap.irstea.fr>





### University of Angers, Laris



The Angers Laboratory for Systems Engineering Research (Laris) is part of the University of Angers and works in the field of information science and technology. On the Angers region, the Laris is participating in the development of the instrumentation and imaging platform for the plant “Phenotic”. Different imaging techniques are being explored for their contribution to phenotyping, some are intended to contribute to automated high-speed phenotyping, others are more exploratory. [www.univ-angers.fr](http://www.univ-angers.fr)

### Geves, Angers



The Variety and Seed Study and Control Group (Geves) is responsible for carrying out the studies necessary for the approval of new plant varieties for their inclusion in the French Official Catalogue, legal protection of plant breeders’ rights, and certification of seeds before marketing in the case of species submitted to regulatory certification. It acts for the description of varieties, the evaluation of genetic progress and the assessment of seed quality. [www.geves.fr](http://www.geves.fr)

### Agrocampus Ouest, Rennes



Agrocampus Ouest has a triple vocation of training–research–innovation. The establishment puts the skills of its teacher–researchers at the service of students enrolled in four engineering courses and other training courses ranging from bachelor’s to doctorate degrees, to train living professionals, managers and committed citizens. These missions are declined in a double approach: integrative and multidisciplinary, from the gene to the agro–system; territorial, from local to global. [www.agrocampus-ouest.fr](http://www.agrocampus-ouest.fr)

### University of Lille



The University of Lille, a multidisciplinary university of excellence in the heart of Northern Europe, is rich in an exceptional cultural and scientific heritage inscribed in the history of the Hauts–de–France Region. With its 72,000 students, 6,700 employees, 66 research units and a training offer that covers all disciplinary fields, it has established itself as a major player in the region for training, research and innovation, and through its commitment to social issues. [www.univ-lille.fr](http://www.univ-lille.fr)

### Technical Institute for Sugar Beet



An applied research organization, the Technical Institute for Sugar Beet (ITB) is the technical reference for the sugar beet sector: beet growers and manufacturers of sugar, alcohol and ethanol. In line with societal and environmental expectations, it conducts studies in four main areas: genetics and varieties, weed control, diseases and pests, agronomy and agro–equipment. The ITB has an experimentation center at Le Griffon (Aisne) and eight regional delegations. [www.itbfr.org](http://www.itbfr.org)

### Florimond Desprez



Florimond Desprez is an independent, family–owned business, that operates as a variety breeder and seed producer. The group is one of the world leaders in beet seeds and is one of the leading European breeders of straw cereals. It is also one of the major players in the potato plant market in Europe. Its Beets and Chicory Laboratory (LBE) is based on the work of the Genetics and Biometrics Laboratory (LGB), whose mission is to bring new genetic material and tools to assist breeders, on that of the Biotechnology Laboratory (LBI), which provides selection assistance tools in cytology, cell biology and molecular biology, and finally those of the SmartPath Laboratory (LSP) which provides its expertise in phytopathology. [www.florimond-desprez.com](http://www.florimond-desprez.com)

# AKER COLLABORATORS

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Anne-Françoise Adam-Blondon, INRAE-Urgi  
Juliette Adrian, ITB  
Évelyne Alavo, ANR  
Françoise Alfama, INRAE-Urgi  
Marco Andreollo, University of Montpellier  
Nathalie Augé, Geves  
Frédéric Baret, INRAE, UMR Emmah  
Stephen Barnes, SESVanderHave  
Michèle Beguiné, Agrocampus Ouest  
Étienne Belin, University of Angers – Laris  
Ryad Bendoula, INRAE – UMR ITAP  
Landry Benoit, University of Angers – Laris  
Suzanne Blocaille, ITB  
Karima Boudheri-Gresse, Geves  
Carole Caranta, INRAE, BAP department  
Frédéric Carlier, FD  
André Chabeniuk, FD  
Danièle Chalain, Geves  
François Chapeau-Blondeau, University of Angers – Laris  
Alain Charcosset, INRAE – Le Moulon  
Baptiste Charlet, FD  
Aurélien Charrier, Geves  
Dorothee Charruau, INRAE-Urgi  
Arnaud Chauveau, FD  
François-Xavier Chombart, FD  
Angélique Clément, FD  
Benoît Dauchy, FD  
Jean-François Dauchy, FD  
Cécile de Scheerder, FD  
Angélique Delanoue, Geves  
Hubert Delesalle, FD  
Alexandre Delivryne, FD  
Didier Demilly, Geves  
Christophe Descamps, FD  
Marie-Claire Descamps, FD  
Anne Despouy, SNFS  
Bruno Desprez, FD  
François Desprez, FD  
Brigitte Devaux, FD  
Pierre Devaux, FD  
Paul Dhallewyn, FD  
Hélène Dorchies, ITB  
André Droulers, FD  
Michel Dubus, FD  
Philippe Dubus, FD

Arnaud Ducanhez, INRAE – UMR ITAP  
Sylvie Ducournau, Geves  
Amandine Dupont, FD  
Audrey Dupont, Geves  
Mélanie Durand, Geves  
Sophie Durand, INRAE-Urgi  
Mylène Durand-Tardif, GIS BV  
Carolyne Dürr, INRAE-IRHS  
Faustine Duyck, FD  
Justine Flament, FD  
Valérie Fortin-Dhellemmes, FD  
Lothar Frese, Julius Kühn Institute  
André Gallais, AgroParistech  
Philippe Gate, Arvalis – Institut du végétal  
Sandrine Germain, FD  
Nathalie Gorretta, INRAE – UMR ITAP  
Ellen Goudemand-Dugué, FD  
Aurélia Gouleau, Geves  
Mélanie Gravelines, FD  
Claire Guerche, INRAE-Urgi  
Sherif Hamdy, Geves  
Karine Henry, FD  
Nicolas Henry, FD  
Céline Herbert, Geves  
Ana Herrero, INRAE – UMR ITAP  
Jean-Louis Hilbert, University of Lille  
Christian Huyghe, INRAE – Scientific director of Agriculture  
Sylvain Jay, INRAE – UMR ITAP  
Mélanie Jubault, Agrocampus Ouest  
José Karramkan, INRAE  
Aminah Olivia Keliet, INRAE-Urgi  
Jetta Keranen, Ayming  
Erik Kimmel, INRAE-Urgi  
Laurence Landais, Geves  
Tania Langon, Ayming  
Anne Laperche, Agrocampus Ouest  
Gaël Laporte, Ayming  
Vincent Laudinat, ITB  
Valérie Laurent, FD  
Laurence Le Corre, Geves  
Joël Lechappé, Geves  
Lydie Ledroit, INRAE-IRHS  
David Lefebvre, FD  
Damien Leroux, INRAE-LIPM  
Sophie Lesaffre, FD

Ghislain Malatesta, ITB  
Brigitte Mangin, INRAE-LIPM  
Maria Manzanares-Dauleux, Agrocampus Ouest  
Annie Masson, Agrocampus Ouest  
Timothé Masson, WABCG  
Fabienne Maupas, ITB  
Mitchell Mc Grath, USDA-ARS  
Dimitri Millon, FD  
Nacer Mohelliabi, INRAE-Urgi  
Gwenaëlle Mortelette, FD  
Martine Mortreux, FD  
Daniel Moura, INRAE – UMR ITAP  
Corinne Murez, FD  
Claire Noël, FD  
Lee Panella, USDA-ARS  
Prune Pegot-Espagnet, INRAE-LIPM  
Philippe Pelzer, communication entrepreneur  
Florence Phocas, INRAE, UMR Gabi  
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Romain Pivon, GIS BV  
Émilie Plus, FD  
Gilles Rabatel, INRAE – UMR ITAP  
Pejman Rasti, University of Angers – Laris  
Pierre Rayé, CGB  
Guy Richard, INRAE, Depe  
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Olivier Robert (+), FD  
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Daphné Verdelet, FD  
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Robbie Waugh, The James Hutton Institute  
Magdalena Wieraszka, ANR

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Sugar beet has entered the age of liberalism with the abolition of production quotas in Europe. It finds itself on the world market and on an equal footing with sugar cane.

France has benefited from the “AKER – Sugar beet 2020, a competitive innovation” Investments for the Future Programme, which aims to double the annual growth rate of the sugar yield per hectare of beet.

It has made a scientific breakthrough by researching all of the genetic diversity available worldwide, and by carrying out genotyping before phenotyping. It is developing new genetic material, available for introduction into future sugar beet varieties. It also offers innovative tools and methods in the fields of genotyping and phenotyping, supporting players in the sector – beet growers and sugar manufacturers – in their imperative improvement in competitiveness.

This book is mainly intended for scientists and professionals, and all those interested in research, development and training in the plant sector. It has just completed eight years of multidisciplinary work bringing together a hundred scientists.

The AKER programme puts for a long time sugar beet in the top tier of cultivated species and helps to provide the consumer with quality sugar produced locally and under environmentally friendly conditions.

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