



OPENING KEYNOTE

Sunflower crop and climate change in Europe: vulnerability, adaptation and mitigation potential

Climate change is characterised by higher temperatures, elevated atmospheric CO₂ concentrations, extreme climatic hazards and less water available for agriculture. Sunflower, a spring-sown crop often cultivated in southern and eastern regions of Europe, could be more vulnerable to the direct effect of heat stress at anthesis and drought during its growing cycle, both factors resulting in severe yield loss, oil content decrease and fatty acid alterations. Adaptations through breeding (earliness, stress tolerance), crop management (planting dates), and shifting of growing areas could be developed, assessed and combined to partly cope with these negative impacts. New cultivation opportunities could be expected in northern parts of Europe where sunflower is not grown presently and where it could usefully contribute to diversify cereal-based cropping systems. In addition, sunflower crop could participate to the mitigation solution as a low greenhouse gas emitter compared to cereals and oilseed rape.

Sunflower crop models should be revised to account for these emerging environmental factors in order to reduce the uncertainties in yield and oil predictions. The future of sunflower in Europe is probably related to its potential adaptation to climate change but also to its competitiveness and attractiveness for food and energy.

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UNDERSTANDING THE
GENOME FUNCTIONING
TO ADAPT SUNFLOWER
TO CLIMATE CHANGE



UNDERSTANDING THE GENOME
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Sunflower Breeding strategies in relation to climate changes

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Increase of GHG (greenhouse gases) has major impact on global warming. This is caused by carbon dioxide increase but also variations of methane, nitrous oxide, black carbon, and various fluorinated gases. Climate change will act on sunflower in many ways, depending on the territories, the crop will have to face variations of temperature and carbon dioxide content, water availability scarcity and more globally stresses impact shifted along the crop cycle. The fact is that breeding is long cycle. If we consider the expected average temperature will rise up by 60C by the year 2050 then only four to six breeding cycles are left. Under those constraints the breeding community will have to increase its efforts to sustain sunflower yield across territories and respond to oil industrial demand. Development of Doubled-haploid (DH) technology is currently unavailable for sunflower. But the application of Genome Wide Selection (GWS) and/or Marker Assisted Recurrent Selection (MARS) should be used to accelerate the breeding effort and reduce time for one breeding cycle. In 2017, the sunflower reference genome was published. Molecular genetics and associated technologies will continue to be key in the discovery and understanding of novel traits. In the future, big data knowledge and technologies associated with high-throughput sequencing will have to become part of the process driving breeding to high-efficiency. Climate change also implies changes in abiotic as well as biotic interactions. Sunflower crop is affected by many different pathogens. Climate change will directly and indirectly impact development, spreading and distribution of some pathogens which will additionally be a challenge for the breeding community (e.g. downy mildew, charcoal rot, black rust, and broomrape). Current use of new tools such as high through put phenotyping (outdoor or in-controlled conditions) will bring a strong precision improvement necessary for traits development under climatic constraints. This will require the development of new mathematical approaches, new methodologies and new technics to facilitate the integration of complex events into transferable knowledge directly usable by the breeding. In such a challenged environment expected in the coming years sunflower diversity will play a very important role. The Helianthus genus is a rich of many species which gave us in the past lots of good opportunities in different breeding programs (disease resistance, adaptation etc.). The use of crop modelling to assess and characterise environments under abiotic constraint associated with phenomic approaches will give to the breeding community the necessary support of sustainable production, and suitable evaluation. Plant breeding plays a major role in the enhancement of yield and product quality and has been developing sunflower varieties for heat and drought since many years. However the current context will drive more severe and frequent challenges and this will require multidisciplinary approaches.



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Genomics of Stress Adaptation in Wild Sunflowers

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Crop domestication is frequently accompanied by a reduction in resistance to biotic and abiotic stress. This decline in resistance represents a major impediment to global efforts to increase crop productivity, especially in the context of climate change and heightened competition for land and water. To reduce stress-induced yield losses, attention has increasingly turned to crop wild relatives, which often have evolved mechanisms to cope with environmental stress. Here I will discuss a series of projects underway to identify alleles underlying stress resistance in extremophile wild sunflower species and to move these alleles into cultivated germplasm. At the center of this effort is a genome wide association study involving whole genome sequencing data for circa 1800 genotypes representing 175 accessions of three wild species. Searches for associations between genotypic variation, phenotypic traits, and climate and soil characteristics are underway. We also have created pre-bred lines containing introgressions from the same wild species to evaluate the effects of candidate resistance alleles in a cultivated genetic background. Lastly, I will report on the development of modified Multiparent Advanced Generation Inter-Cross (MAGIC) mapping populations that include both cultivated and wild sunflower donors. These populations are expected to facilitate the efficient characterization of resistance traits in sunflower and to produce materials containing exotic alleles that can be readily deployed in breeding programs.



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Broadening the view of cultivated sunflower genomic diversity as a mean to enhance adaptation to rapidly changing environments

The cultivated sunflower is known to be highly variable genetically despite having passed through domestication and improvement bottlenecks in the past 4,000 years. Sunflower is cross-compatible with numerous wild species, which are prized by breeders due to their enhanced tolerance to both biotic and abiotic stress. To determine the extent and nature of genetic diversity in cultivated sunflower, and to quantify potential contributions from introgression with crop wild relatives, we sequenced 476 sunflower genotypes, including 287 cultivars and 189 wild accessions representing 11 wild species. The outcome of these introgressions is variation among lines in the genomic composition and presence/absence of specific genes. Here we present the cultivated sunflower pan-genome which is comprised of 61,205 genes, of which ca. 27% vary across genotypes and represent so-called 'dispensable' genes. At least 10% of the cultivated sunflower pan-genome is derived through introgression from wild sunflower species, and ca. 1.5% of genes originated solely through post domestication introgression. Annotations and GO functional analyses further indicate that genes associated with biotic resistance are over-represented among introgressed regions, an observation consistent with breeding records. We further provide examples in which wild introgressions have contributed to resistance to both biotic and abiotic stress.

To further explore variation on the chromosome level, the XRQ reference assembly was compared with a new version of the HA412 genome assembly. The extent of rearrangements observed between the two reference genomes imply that sunflower is among the most structurally variable crop species. The extent of diversity on different genomic levels suggests that sunflower has the necessary breeding flexibility to cope with challenges posed by global climate changes.

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UNDERSTANDING THE GENOME
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The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution

The domesticated sunflower, *Helianthus annuus* L., is a global oil crop that has promise for climate change adaptation because it can maintain stable yields across a wide variety of environmental conditions, including drought. Even greater resilience is achievable through the mining of resistance alleles from compatible sunflower wild relatives, including numerous extremophile species. Here, we report a high-quality reference for the sunflower genome (3.6 gigabases), together with extensive transcriptomic data from vegetative and floral organs. The genome is mostly comprised of highly similar, related sequences and required single-molecule-real-time sequencing technologies for successful assembly. Genome analyses enabled reconstruction of the evolutionary history of the Asterids, further establishing the existence of a whole-genome triplication at the base of the Asterids II clade and a sunflower-specific whole genome duplication ~29 million years ago. An integrative approach combining quantitative genetics, expression and diversity data permitted development of comprehensive gene networks for two major breeding traits, flowering time and oil metabolism, and revealed new candidate genes in these networks. We found that the genomic architecture of flowering time has been shaped by the latest whole genome duplication, which suggests that ancient paralogs can remain in the same regulatory networks for dozens of million years. This genome represents a cornerstone of future research programs exploiting genetic diversity to improve biotic and abiotic stress resistance and oil production while considering agricultural constraints and human nutrition needs.

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Sunflower transcription factors in abiotic stress responses as tools to improve performance of different crops

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Sunflower was reported as a crop tolerant to drought conditions; this and other abiotic stress responses are regulated at different levels involving diverse biomolecules. Among these molecules, transcription factors (TFs) play key roles in early steps. These proteins are more abundant in the plant kingdom than in animals and other kingdoms, suggesting that the transcriptional process is the major step in the regulation of gene expression in plants. When compared to model plants, sunflower has a remarkably number of divergent TFs belonging to different families. With the hypothesis that, at least in part, such divergent TFs could be responsible of the sunflower adaptation capability, we decided to perform functional genomics with selected TFs belonging to the homeodomain-leucine zipper (HD-Zip) subfamily I, unique to plants. Members of HD-Zip I subfamily were assigned roles in the plant response to abiotic and biotic stresses.

HaHB4 and HaHB11, both sunflower divergent HD-Zip I TFs, were firstly introduced in *Arabidopsis* as transgenes driven either by the 35S CaMV promoter or their own inducible promoters. HaHB4 and HaHB11 conferred drought and salinity tolerance without yield penalty under such stresses, whereas transgenic HaHB11 *Arabidopsis* also exhibited improved performance under flooding stress, including waterlogging and submergence situations as well as enhanced yield in standard growth conditions.

HaHB4 was used to transform soybean and wheat, and both crops showed improved yield across contrasting environments in field trials. Transgenic HaHB4 soybean was recently approved by regulatory organisms both in Argentina and the US and will probably be a commercial product during 2018, becoming the first transgenic crop in which the gene donor is another plant. HaHB11 was introduced in maize and rice. Transgenic maize plants were assessed both in the greenhouse and in the field. Current results indicate that HaHB11 transgenic maize plants exhibit some of the traits observed in *Arabidopsis* but at different extents.

The morphological and architectural observation of transgenic plants bearing HaHB4 and HaHB11 indicated that stem width and vascular bundles anatomy are major traits determining seed yield. This observation was corroborated by applying weight to sunflower seedlings in early developmental stages. The obtained results were in agreement with observations performed in field trials by other research groups.

We can conclude that sunflower TFs can be considered as biotechnological tools to improve other crops but also to recover seed yield progress in this species.

SESSION
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PESTS
AND
DISEASES

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AND
DISEASES

Understanding the mechanisms of the interaction between *Orobanche cumana* (sunflower broomrape) and sunflower for a more sustainable resistance

Sunflower faces parasitism by sunflower broomrape (*Orobanche Cumana*), mainly in Europe but also in Asia and in North Africa. Sunflower broomrape is an obligate parasitic plant, part of the *Orobanchaceae* family. *Orobanche cumana* doesn't develop roots and is unable to perform photosynthesis. The germination of its seeds depends on specific chemical compounds from sunflower root exudates. After germination and connection to the vascular system of sunflower roots, *Orobanche cumana* begins to use water and nutrients from sunflower to develop an underground tubercle before emerging a flowering stem, as new sinks for sunflower. Up to hundred parasitic plants can fix on a single sunflower when highly susceptible, causing severe yield loss. But breeding for resistant hybrids has been successful. However, new races overcoming the resistances appeared, requiring the identification of new resistance sources.

Both genomes of sunflower and broomrape have been sequenced and resources are available for both species, making *H. annuus/O. cumana* a unique pathosystem to understand parasitism.

Giving an overview of recent results on the interaction between sunflower and *O. cumana*, I will illustrate how the characterization of the interaction at the genetic and molecular level can provide new way to control broomrape.

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Recent advances on the characterization and control of sunflower soilborne pathogens under climate change conditions

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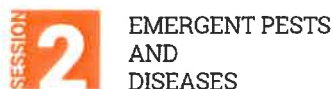
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The control of soilborne pathogens affecting crops is conditioned by the limited management options due to difficult access to active infection courts and by the restrictions in the use of synthetic pesticides in Europe. In the case of some of these pathogens affecting sunflower, an effective management heavily relies on genetic resistance which is, however, hindered by new pathogen populations (new races). In this scenario, special emphasis is put on (i) continuous and updated monitoring and characterization of sunflower pathogens and (ii) enlargement of the set of tools for efficient disease management.

In the last years increases of sunflower wilt diseases, mostly associated to *Verticillium dahliae*, are being observed in some countries of Europe. On the basis of genetic, molecular and pathogenic traits of *V. dahliae* two groups are clearly differentiated in the pathogen, the first group including isolates from Western Europe and the second one those from Eastern Europe. Besides, some wilted plants collected in Russia were processed and the fungus consistently obtained was morphological and molecularly identified as *Cadophora malorum*. The confirmation of Koch's postulates in greenhouse allowed its identification as a new pathogen of sunflower. Third, and since downy mildew attacks on resistant hybrids are frequent in Spain and Portugal in the last years, samples of *Plasmopara halstedii* were collected in both countries and their race determined by artificial inoculation of the nine internationally accorded differentials of races. Plants were maintained under controlled conditions until symptoms assessment. All the isolates were identified as 705, a new race virulent on HA304 (universal susceptible), RHA265 (PI1), and RHA274 (PI2) lines, as well as HAR4 (PI16) and HA335 (PI6) lines.

On the other hand, entomopathogenic fungi (EF) are used in Integrated Pest Management strategies due to their efficacy in controlling insect pests affecting many crops. Besides, new ecological roles of EF have recently been reported. Some species, such as *Beauveria bassiana* (Bb) and *Metarhizium brunneum* (Mb) have antifungal effect against crop pathogens. The effect that strains of Bb and/or Mb can exert on sunflower pathogens is currently being addressed by our research group. Initial dual culture results show that the mycelial growth of *V. dahliae* and/or *C. malorum* is affected depending on the pathogen as well as on the EF species (Bb or Mb), or on particular strains of either Bb or Mb. Antibiosis and/or competition for ecological niche seem to be operating in EF-pathogen interactions. Mechanisms involved in these interactions will be studied in the near future.

In summary, pathogen characterization is essential for genetic resistance for worldwide environments of sunflower production. Moreover, the security of sunflower yield and profitability is dependent on effective genetic resistance together with additional new control options that can be included in successful strategies of sunflower disease management.



Influence of the environmental conditions on virulence and distribution of *Orobanche cumana* Wallr. in the Republic of Moldova

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In the last years, more aggressive races of *Orobanche cumana* Wallr. have evolved and the parasite has rapidly spread to new areas in Southern Europe, as well as in North Africa and China. A similar trend is observed at the level of the Republic of Moldova. At the beginning of 1950s, the broomrape was detected in the southern areas of the country. The latest data reveal a migration and the expansion of *O. cumana* on new areas from center and north. During the expeditions conducted in July 2014 along 24 administrative districts from different parts of the country, the broomrape was present in 14 of 22 localities (63%) from the south, 17 of the 36 localities (47%) from the central part and only in 4 of 22 localities (18%) in the northern regions. Moreover, populations collected from the central part of the republic are less virulent, mostly E or \leq E (about 65%), while populations in the southern and northern regions are characterized by increased virulence, more than 60% of them being classified as aggressive race G and H. The spread of *O. cumana* beyond its present geographical range might cause major economic damage.

Based on these results and the data reported by other authors, related to the impact of climate change on the phytosanitary scenario, we further focused on the interdependence between the climate in the Republic of Moldova and the distribution of parasite *Orobanche cumana* Wallr. on the country's territory, as well as extent of the sunflower infestation by different populations of broomrape.

We mapped the spatial distribution using computerized techniques to allow for visualisation of areas characterized by the presence of high virulent broomrape populations. By the automatic overlapping of outbreaks of *O. cumana* spreading with cartographic modeling of maximum temperature and relative air humidity, the specificity of the manifestation of favorable climatic conditions, which accelerated the development of this parasite, has been highlighted.

We conclude that the prevalence of broomrape infection mainly in the southern and central part of the Republic of Moldova and its sporadic presence in the northern part can be influenced, not only by the intensive processing technologies of the agricultural lands and the type of cultivated sunflower hybrids, but also by the weather conditions represented by higher temperature values and lower humidity in the south and center. We infer that, over the years, these conditions could have contributed to the development of the parasite in these areas. To assess current and future risks of broomrape range expansions we analyzed the multiannual data (1961-2016) and highlighted the trends specific for the last periods, characterized by the increase of the temperature and the decrease of the relative humidity. According to the data, the persistence climate change could potentially, create favorable conditions for infecting the sunflower in all cultivated areas, including the expansion of broomrape to the north of Moldova.

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Pathogenesis-related (PR) proteins expression during IMI herbicide and ABT xenobiotic treatment in young sunflower roots

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Plant response to environmental stresses is mediated by the regulation of gene expression. A major abiotic stress encountered by cultivated sunflower (*Helianthus annuus* L.) is application of several classes of herbicides, including the imidazolinones.

Proteomic studies were carried out in order to characterize the plant response to imazethaphyr herbicide (IMI) in 15 day-old sunflower roots. Seeds of the HA89 sunflower inbred line were grown in pots filled with perlite and watered by capillarity with 1.1 g L⁻¹ of Murashige and Skoog's basal salts while incubated in controlled conditions (27±2 °C, 16/8 hr light/dark). After 8 days, the plants were treated with 0 and 1 µM IMI and a combination of 1 µM IMI + 70 µM 1-aminobenzotriazole (ABT), a cytochrome P450s inhibitor. After 15 days, plants were collected and their roots were washed to remove perlite debris. Total root protein extracts were analyzed via liquid chromatography combined with electrospray tandem mass spectrometry on an LTQ Orbitrap (Thermo Fisher). Data were analyzed against the sunflower complete proteome sequences(1) and a set of commonly observed contaminants. Protein identification was performed using Proteome Discoverer (Thermo Fischer) and Max Quant software(2). Visualization of protein abundance was performed using Perseus software(3). Functional categorization was performed using MapMan software(4).

Changes in protein expression levels (min. 2-fold) were detected in several metabolic pathways including aminoacid turnover, xenobiotic detoxification and cell wall synthesis. In addition, biotic stress-related proteins were highly expressed in response to IMI treatment. These protein families involved cell wall-located chitinases, PR-6 endopeptidases and endochitinase precursors, among others. All of them are reported to be involved in defense response to fungus and systemic acquired resistance. EP3-chitinases present in root epidermis and related to hypersensitive response, were also detected. In contrast, no expression changes were found in these protein families in response to ABT.

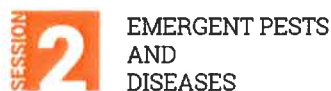
Recent changes in climatic conditions all over the world and specifically in Argentina included a raise in annual precipitations, favouring the incidence of fungal pathogens. A rapid adaptation or innate tolerance mechanisms in sunflower would protect further development of the plant and would be important in fighting against emergent pathogens. PR-related proteins detected in this preliminary study included those involved in fungal defense suggesting that herbicide treatment triggers some stress response pathways shared with those ones corresponding to biotic stresses.

Common plant defense mechanisms would play important roles in fighting against new pests, weeds, as well as abiotic stress in sunflower.

(1) - Badouin, Helene et al. The sunflower genome provides insights into oil metabolism, flowering and Asteroid evolution. *Nature*, 2017 Jun 1;546(7656):148-152. doi:10.1038/nature22380

(2) - Tyanova, S. et al. *Proteomics*, 2015, 15, pp 1453-1456. doi:10.1002/pmic.201400449

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Genetics and Genomics applied to disease resistance breeding in sunflower

The Instituto Nacional de Tecnología Agropecuaria (INTA) has a long tradition in sunflower breeding with emphasis on quantitative disease resistance. By exploiting the diversity of a broad range of foreign genetic resources in combination with introgressions of wild *Helianthus* species, INTA has pioneered sunflower breeding in Argentina and has become one of the most prolific sunflower breeders in the country.

Fungal diseases are a major constraint for sunflower grain and oil yield worldwide, but the relative importance of pathogen incidence depends on the characteristics of the growing region. In the Argentinian Southern Pampas, an area that produces 70% of national sunflower production, the most relevant diseases are Verticillium Wilt (VW) and Sclerotinia Head Rot (SHR). VW is an endemic disease caused by the soil-borne fungus *Verticillium dahliae* (Kleb.), spread over an extensive area covering up to 1.2 million hectares in this particular region. Meanwhile, the SHR is a common constraint of the temperate regions caused by *Sclerotinia sclerotiorum* (Lib.).

The Sunflower Breeding group at INTA seeks to apply and integrate the concepts and technologies of genomics to assist crop breeding. We are working on the identification and characterization of genetic resistance sources to VW and SHR through a wide range of complementary genomic approaches, including classical QTL-Linkage Mapping, eQTL mapping, Genome-Wide Association Studies (GWAS), and functional genomics tools like Tilling, and differential expression studies on selected genotypes.

In this report, we present a comprehensive analysis of the resistance response of the INTA Association Mapping Population (AMP) against SVW and SHR. This panel consists of 137 inbred lines, comprising both local and international accessions. The main goals of this work were (a) to evaluate the response of the AMP to biotic stresses by multivariate analysis, and (b) to assess the relationship between resistance response and genetic structure.

To evaluate the AMP disease responses the phenotypic inbred means were adjusted for each one of the phytopathological variables scored (Incidence, Severity, Intensity, AUDPC and Incubation Period) using Generalized Linear Mixed Models (GLMM). In parallel, the population sub-structure, and genetic diversity were estimated using a complete matrix of 18161 imputed GBS-derived-SNPs. Principal component analysis was conducted using the adjusted phenotypic means of each variable scored, partitioning the data by different criteria (i.e., country of origin, genetic cluster, maintainer-restorer status) to establish their relationship with the distribution of phenotypic variation. Results for both VW and SHR will be presented.

This integrative analysis of phenotypic and molecular diversity constitutes a cornerstone for the guidance of our future breeding efforts. These results allowed us to choose the parental inbreds for a multi-parent advanced generation inter-cross (MAGIC) population that will serve as a new tool for a more in-depth approach to disease resistance GWAS.

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The races type identification and genetic diversity analysis of sunflower broomrape (*Orobanche cumana* Wallr.) in China

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Currently, sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic seed plant and caused dramatic yield loss annually. It has become one of the most important constraints in sunflower production in China. The most effective way to control this disease is to apply resistant varieties. So, it is rather urgent to make clear the race type composition and distribution of sunflower broomrape in China so as to facilitate the breeders to arrange the resistant varieties against different race type reasonable.

To identify the race types of *Orobanche cumana* in China, 29 samples (broomrape seeds) collected from different locations in the main sunflower production region in China. With the standard differential lines provided by Dr. Skoric, the identification tests were conducted using plastic cup under greenhouse condition. Our results showed that there are five race types of *Orobanche cumana* in China: Race A, D, E, F and G. Among them, the race G is the highest level of physiological race and all from Inner Mongolia region (Linhe, Wulateqianqi and Xixiaozhao). Race F is only found at Siwangziqi of Wulanchabu region. Race E mainly distributes in Xinjiang and Inner Mongolia. Race D is the most dominant race type, which is mainly distributed at Xinjiang, Inner Mongolia and Jinlin. Race A is mainly distributed at Shanxi, Hebei and Shaanxi and the Tuzuoqi of Inner Mongolia, where the local cultivars planted.

The genetic diversity study was performed with 96 samples from different sunflower growing areas together with 12 ISSR primers, which were screened out from 100 primers based on their high polymorphism and good reproducibility. A total of 147 bands were detected, which 90 were polymorphic bands. The percentage of polymorphic bands (PPB) was 61.22%. The genetic diversity analysis showed that the

the diversity of *Orobanche cumana* from Inner Mongolia and Xinjiang was relatively higher. The Shannon diversity index was 0.5560 and 0.5067 respectively. The cluster analysis showed that the *Orobanche cumana* from 6 different provinces can be divided into two subgroups, of which Shanxi, Shaanxi and Hebei provinces were clustered into one subgroup. Jilin, Inner Mongolia and Xinjiang were clustered into another subgroup. The genetic distance between Shanxi and Hebei was the closest, while distance between Hebei and Xinjiang was the longest.



Phomopsis stalk canker disease in North America: correlation with climate and solutions through breeding

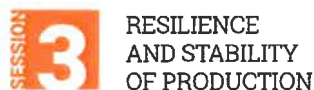
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Climate change has the potential to affect agriculture directly through agroecological shifts driving pest elimination or proliferation. Land use changes due to farmers perceiving changes in growing season length and available soil moisture also has an effect on agroecology, through the crops that are grown and their sequence. In the Plains of North America, we have seen this result in pest reduction, e.g. the reduced range and numbers of sunflower beetle (*Zygogramma exclamationis*). However, Phomopsis stalk canker disease (caused by *Diaporthe* spp.) has proliferated under recent climate conditions and in the context of our current crop sequences. We will discuss how this has changed the relative importance of Phomopsis stalk canker from being a relatively minor pest to being a major pest in sunflower production in this region. Assuming that this will continue, at least in some years and environments, underlines the importance of producing Phomopsis resistant plant materials and improving marker-assisted approaches. The goal is to use resistance sources in a way that can also maintain or enhance yield and quality, irrespective of disease prevalence, in future sunflower hybrids. We will discuss progress on this front.

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SESSION
3

RESILIENCE
AND
STABILITY
OF PRODUCTION



Biogemma research to tackle sunflower adaptation to changing environment

Climate change is a big issue whose effects in agriculture are becoming a reality. Crops are subject to different biotic and abiotic stresses and breeding must adapt to meet these new challenges. These stresses act in a combined way and this dimension must be integrated into the approaches implemented by the breeder. Because of its growing area, sunflower is particularly affected by climate change and the resulting water and heat stress. The crop is also susceptible to many pests and notably broomrape whose area of presence increases with its recent arrival in France in particular. Biogemma has developed a research program integrating these different components, targeting in particular sunflower tolerance to water stress and resistance to broomrape. Ecophysiological modeling for environmental characterization and molecular tools for broomrape population's characterization, are approaches developed by Biogemma to tackle these challenges.

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RESILIENCE
AND STABILITY
OF PRODUCTION

Combined approach of quantitative genetics and crop modelling to understand sunflower tolerance to drought

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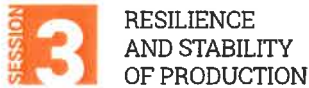
As the result of climate changes, more variability is expected from location to location in the timing and quantity of water availability for crop production. On the long term period, the competitiveness of sunflower chain - seeds, farmers, coops, oil seed industry - is highly depending on the stability of oil yield across years and locations.

Since 10 years, we develop in the frame of public-private partnership, interdisciplinary projects (SUNYFUEL, OLEOSOL, SUNRISE) at the interface between genetics and agronomy to identify the genetic basis of yield plasticity in response to abiotic stresses such as drought. Our goals are to understand the molecular and physiological processes involved in drought, identify novel genetic material to improve the elite germplasm tolerance and being able to predict performance in different climatic scenarios and therefore tolerance based on genomic information.

I will present up-to-date results combining crop modelling, quantitative genetics and genomics that allowed us to identify a genomic region involved in drought tolerance for grain yield, describe its physiological effect and how it relates to modern hybrid development.

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Map and sequence-based candidate gene analysis of the male fertility restoration gene *Rf5* in sunflower

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A single nuclear gene, *Rf5* originating from sunflower wild species of *Helianthus annuus* accession PI 596476, restores PET1 cytoplasmic male sterility in sunflower. The *Rf5* gene was previously mapped to linkage group (LG) 13 of the sunflower genome. The flanking SSR markers defined the *Rf5* gene to an interval of 5.8 cM of genetic distance. In the present study we screened 192 F₂ individuals of the original mapping population using 21 publicly available SNP markers mapped in this region and narrowed down the gene interval from 5.8 to 0.8 cM in length. Fine mapping was performed using lines derived from 2,447 F₂ plants and screened with the SNP marker SFW03371 and the SSR marker ORS728 which cover a 1.8 cM interval. A total of 87 recombinants were detected from 4,894 gametes in this region, and 30 progeny plants from each recombinant line were tested in the field for male fertility restoration in order to determine the position of the *Rf5* gene relative to the recombination points in each line. Based on the position of the flanking SNP markers we extracted eight contig sequences covering the *Rf5* gene interval, chromosome13_sequence248 to 252 and chromosome13_sequence254 to 256, from the earlier version of the HA412-HO reference genome sequence. A total of 45 SSR primer pairs were designed from those sequences. Fine mapping resulted in the mapping of *Rf5* gene to a 58- and 60-kb interval on the HA412-HO and XRQ genome assemblies, respectively. Whole genome re-sequencing was performed on the sunflower line HA-R9 carrying the *Rf5* gene using Illumina HiSeq PE150 generating sequence data with 40x coverage of the sunflower genome. SNP/InDel calling was conducted in the *Rf5* gene interval using both HA412-HO and XRQ sequences as reference genome. An additional 48 SNP markers were developed from this region and tested in the recombinant lines. Eight SNP markers were close to the *Rf5* gene and located on a 15-kb region based on both HA412-HO and XRQ genome assemblies. Two candidate genes, Ha412v1r1_13g048240 and Ha412v1r1_13g048260, were found in this interval of HA412-HO genome, which encodes pentatricopeptide repeat proteins, a gene family related to *Rf* genes in higher plants. Genome assembly of the *Rf5* interval in the donor line HA-R9 is underway.

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High-throughput and high-effort phenotyping reveal the genetic basis of key traits associated with drought, nutrient and salinity resistance in cultivated sunflower

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The need for high yielding stress resistant crop varieties is readily apparent with climate models predicting increasingly frequent and severe droughts, an increasing demand for high yield on marginal lands, and problems with increased soil salinity due to poor irrigation practices. Research at UGA on cultivated sunflower (*Helianthus annuus*), the world's 4th largest oilseed crop, has shown substantial resistance to drought, low nutrients and salinity stress being present in this species.

In order to identify the traits and their genetic basis that confer resistance to those stresses, several large scale experiments have been conducted with a mapping population of 288 genotypes, >90% of the allelic diversity in sunflower. Across several field and greenhouse experiments on drought, salinity and low nutrients stress, a combination of high-throughput (field) and high effort (field and greenhouse) phenotyping revealed a considerable level of variation in resistance and associated traits being present in the mapping population.

Results from the field showed that when subjected to drought a strong drought avoidant, deep rooting, strategy allowed most genotypes to tap deeper available water leading to only minor effects on yield. Results from the greenhouse showed that with low nutrient and high salinity stress, a genotypes suite of morphological and physiological traits were a good predictor of performance and response to stress. Genome wide association of >600k single nucleotide polymorphisms (SNP's) showed multiple genomic regions underlying the expression of multiple physiological and morphological traits but more interestingly also identified different regions associated with the plasticity of trait expression under stress.

Taken together results highlight the potential for breeding for desirable traits conferring stress resistance. How trait expression and its plasticity/rigidity is linked to performance under ideal and stressful conditions and whether there are trade-offs and synergisms in resistance to drought, salt and low nutrient stress is a focus of ongoing analyses.



Changes in sunflower seed germination phenology in the context of global warming

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Seeds are at the core of agriculture and biodiversity, and most human food and animal feed is derived directly or indirectly from seeds. The efficiency of seed germination and seedling emergence is vital to crop yield and efficiency. Shifts in germination phenology resulting from global climatic changes, mostly including higher temperatures, erratic precipitations and prolonged periods of drought, will directly influence population dynamics and productivity of all agrosystems.

The environmental conditions prevailing during seed production can have a marked influence on subsequent seed vigour and dormancy status and thus interact with post-dispersal environmental factors that will determine germination phenology. Our work shows that a water stress applied on the mother plant during sunflower seed development can confer a better tolerance to the same stress during seed germination. The use of a comparative RNAseq and methylome analysis allowed us to identify some of the molecular bases of this process. Global warming is also expected to negatively affect seed germination and thus to penalize stand establishment. A screening of seed germination under stress of 80 sunflower hybrids showed that resistance to water constraint at this developmental stage had a genetic component. As expected tolerance to stress at the germination stage was also related to metabolism of reactive oxygen species. Proteomic, metabolomic and transcriptomic experiments have been performed on two contrasting genotypes and the results indicate that seed tolerance to stress is associated with specific changes in gene expression and in metabolite profiles but not with marked changes in protein patterns. In conclusion we will discuss the additive effects of climate change on both seed development and seed germination and their possible consequences on seed production.

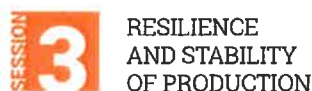


RESILIENCE
AND STABILITY
OF PRODUCTION

Non-invasive methods for biotic stress phenotyping in sunflowers

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In order to more quickly and effectively respond to threats from disease and climate change, high-throughput and highly accurate phenotyping and screening methods are needed. This is particularly true for biotic traits, where rapid, precise, and reproducible quantification of symptoms is required for estimating the severity of infection and evaluation of host resistance. Up until now, qualitative visual assessments have often been used to quantify disease severity. They require rating scales to be finely tuned, and raters to be trained, which decreases reliability and increases the cost and the time devoted to phenotyping projects. In contrast, automated sensor-based diagnostics provides a powerful alternative to conventional visual screening. In particular, recent advances in thermal imaging have made it feasible to assess plant-pathogen interactions by monitoring patterns of surface tissue temperature. Here, we report preliminary results of a study aimed at testing of the practical utility of thermal imaging for resistance screening of some of the most important diseases in sunflower: rust, *Phomopsis* stem canker (PSC) and *Sclerotinia* head rot (SHR). Additionally, we used a high-resolution high-throughput sensory system developed by Ecoation that combines pulse-modulated fluoroscopy, Raman spectroscopy and non-invasive chemical analysis. Ecoation uses various Machine Learning techniques to analyze the big data harvested by their platform. The efficiency of these technologies was compared to some of the available alternatives, as well as to most widely used method - visual screening. The results of this study will bring us a step closer to the development of more accurate and efficient strategies both for large scale phenotyping and for precision agriculture in sunflower.



Sunflower outdoor phenotyping facilities in Toulouse

INRA Toulouse research center has developed two outdoor phenotyping platforms that are well adapted to sunflower.

Heliaphen can host 1300 pots under a 600 m² bird protected area. Plants can be grown and characterized from sowing to seed maturity in 15L pots equipped with individual pot shelters. Unlike most glasshouse platform, the Heliaphen light environment is comparable to field conditions. A unique robot system enables precise management of the water content of each pot. Throughout an experiment, environmental conditions are automatically recorded (temperature, wind, precipitation, evaporative demand) and the robot irrigates plants individually according to a pre-defined drought scenario. Pot weight is measured automatically and plant water status (i.e., the fraction of transpirable soil water) is calculated on a daily basis. The Heliaphen platform also phenotypes plants daily using a laser scanner (stem diameter), and a light barrier (plant height, leaf area, senescence). Finally, transpiration rate can be determined by combining this information.

Agrophen is the field phenotyping platform of the French Phenotyping Platform Network located near Toulouse. It is equipped with a UAV system and with a unique field robot system named Phenomobile. The UAV is a hexacopter with 15-20 min flight autonomy and automatic flight capabilities. The sensor configuration is either a high-resolution (24 Mpix) RGB camera or a 6-channel multispectral camera (1280*960) which can be associated with a Flir Tau2 thermal camera (640*480). The field robot is equipped with a 12 m long telescopic arm with a ground clearance of 4 m. The movements of the robot and its arm are fully programmable and controlled by cm-level precision GPS. The sensor equipment consists of 3 RGB cameras (nadir and lateral), 2 6-band multispectral cameras and 3 LiDARs (nadir and lateral). The system has 5 high-power flashes to achieve high repeatability of the images acquisition conditions. The throughput is around 100 plots/h. The target traits of these two systems are plant density, cover fraction, canopy height, Green Area Index, fraction of intercepted PAR, Chlorophyll Content and leaf surface temperature (UAV only). The two systems are complementary as the field robot brings high quality and high spatial resolution which gives access to canopy structure information whereas UAV system brings high throughput and the possibility to assess rapidly within day changes such as midday wilting for example.

The research effort is now on developing automated data analysis pipeline for the various data sources to compute biologically sound traits from raw sensor data.

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SESSION
4 PREDICTING
PRODUCTION
FROM GENES
TO FIELD



Genomic prediction is a useful tool to predict sunflower hybrid performances and their stability

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Climate variation is one of the actors that has driven the process of adaptation in the past, more rapid climate shifts, as predicted in the future, will certainly induce significant evolutionary changes. To cope with this issue, the creation of new varieties, more tolerant to water or heat stress and that present a yield stability over good or bad years, is one possibility that can be already worked. However, to correctly estimate the stability of a variety or to find more tolerant varieties, it is necessary to observe many varieties in many environments or at least to be able to predict their performances.

Prediction of hybrid performance using the general combining ability (GCA) of the parents has been widely used in breeding program but is not very accurate when the GCA is imprecise. Genomic prediction refers to the prediction of unobserved variety performance using a model based on markers covering the whole genome. A training population that is both genotyped and phenotyped is used to train the model that links genotype to phenotype. So, genomic prediction can be accurate even when parents of a hybrid are not well characterized.

We will present results concerning 452 sunflower hybrids from an incomplete factorial design of 36 female and 36 male lines observed in a multi-environment trial (MET) over three years, leading to nine different environments. Genotyping was obtained by re-sequencing of parental lines. We identified 468,194 non-redundant SNPs that were heterozygous in both males and females. Hybrid genotypes were inferred from parent genotypes.

We compared genomic predictions to GCA-based predictions then we included, into genomic model, functional information such as genes involved in known metabolic network or SNPs found by GWAS. We focused on a highly heritable trait, the oil content and a more complex one, the senescence speed.

Genomic prediction is accurate in the SUNRISE design and it outperforms GCA-based prediction when parents of hybrids are not well characterized. Adding functional information can be as accurate as whole genome prediction and it dramatically lessens the required number of markers, thus enabling rapid and inexpensive genotyping. In addition, genomic prediction can accurately predict hybrid performance across all MET environments, providing the opportunity to examine hybrid stability across environments.

SESSION 4
PREDICTING PRODUCTION
FROM GENES
TO FIELD

Predicting sunflower production from traits to fields: application to assessment and recommendation of cultivars.

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Plant breeding programs design new crop varieties which, while adapted to distinct population of environments, are nevertheless grown over large areas. At the farm level, the crop is exposed to highly diverse stress patterns caused by climatic uncertainty and various management options. Therefore, such production situations can often lead to decreased crop performance regarding to the expected level (yield gap). Phenotypic plasticity is defined as the range of phenotypes a single genotype can express as a function of its environment. At the population level, this process underlies the relative variation in performance of varieties across environments. It is commonly referred as genotype (G) x environment (E) interactions in plant breeding and agronomy and can explain up to 20% of total yield variance observed in multi-environment trials.

In this study, our aim is to assess how a finer spatial management of genetic resources could reduce the genotype-phenotype mismatch in farming environments and ultimately improve the efficiency and stability of crop production. We used crop modeling and simulation to predict the crop performance resulting from the interaction between cultivar growth and development (G), climate and soil conditions (E), and management actions (M). We designed a computational experiment that evaluated the performance of a collection of commercial sunflower cultivars in a realistic target population of cropping environments in France. Optimization methods were then used to search for cultivars \times environments combinations that lead to increased yield expectations.

Results showed that when cropping conditions are not properly characterized, choosing a cultivar with an important plasticity is a robust strategy for a large number of production situations. However, when the knowledge of cropping conditions improves, tuning the choice of cultivar significantly impacted the crop performance at national scale. We argue that this approach, while being operational on current genetic material could act synergistically with plant breeding as more diverse material could allow access to cultivars with distinctive traits that are more adapted to specific conditions.

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**PREDICTING PRODUCTION
FROM GENES
TO FIELD**

Modeling damage caused by multiple pests on sunflower under climate change: methodological reflections

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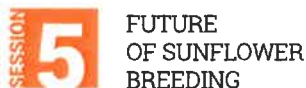
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Climate change will affect not only crop development, but also pest dynamics, along with cropping practices. It is therefore crucial to have tools and methods to predict changes in sunflower-based agroecosystems. This presentation will provide insights on how to tackle this challenge. In particular, a focus will be made on the modelling of yield loss caused by multiple pests on sunflower, along with the modelling of injury profiles under various climate change scenarios and changes in cropping practices. The discussion will highlight the need for data collection on various biophysical processes in order to be able to fully address the question.



SESSION
5

FUTURE
OF
SUNFLOWER
BREEDING



Value of sunflower crop wild relatives' habitat diversity for sunflower in a changing world

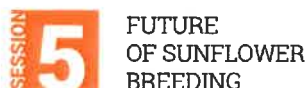
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Genetic resources are the biological basis of global food security. Preservation and utilization of cultivars, landraces, and wild relatives of important plant species provides the basic foundation to promote and sustain agriculture. The domesticated sunflower, *Helianthus annuus* L., is a global oil crop that has promise for adapting to changing environments because it can maintain stable yields across a wide variety of environmental conditions, including drought. The sunflower crop has been proposed as a potential model crop for adaptation to a changing environment. Even greater resilience is achievable through the mining of resistance alleles from compatible sunflower crop wild relatives, including numerous extremophile species. A changing climate is expected to cause significant alterations to weather patterns in traditional sunflower production areas resulting in increased average temperatures, drought, and storm severity. Abiotic challenges generally evoke complex responses involving many genes. This is where the genetic diversity of the crop wild relatives becomes very valuable. Sunflower crop wild relatives grow in extreme environments, tolerating abiotic stresses such as high salt and high temperature in areas with limited precipitation. Some desert dwelling species survive in extremely dry conditions with less than 250 mm of precipitation with their deep rooting ability of primary roots reaching depths of 2 m and more when needed to search out water and nutrients, allowing plants to reach resources not available to other crops. Access to deep soil moisture also provides a heat tolerance factor. Increased storm severity may require a renewed emphasis on incorporating traits which prevent plant lodging. The species of *Helianthus* represent a broad range of plant structures such as sturdy stalks, leaf architecture, sessile leaves, leaf angle, growth habits, flowering times, and colonized endemic habitats. Probably the most useful biotic traits from the crop wild relatives are disease and parasitic weed resistance. The sunflower disease complex will change and evolve with their changing environment. Crop wild relatives have traditionally provided resistance genes for many of the economically important diseases and will continue to provide new sources for current and emerging diseases that may appear with the change in environmental conditions. The sunflower crop stands a very good chance of surviving a changing environment with its broad genetic base and the emerging technologies that will allow the mining of traits and genes from the vast and relatively unexploited wild crop relatives gene pool.

Impact of new targeted genome modification techniques (CRISPR-Cas9) on plant breeding

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Plant evolution relies on spontaneous genome mutations that can potentially give rise to new traits fixed by selection. The resulting genetic diversity has always been the raw material for breeders and they have always taken advantage of the best available science to use, and when possible, increase, this genetic diversity. Beyond their predominant role in human and animal therapy, genome engineering tools have also become essential tools for plant breeding, providing means to introduce almost any type of mutation in a very precise way. This not only empowers the breeders to accelerate and direct crop selection in an unprecedented way; it also opens up the door to an almost unlimited range of possibilities in terms of the combination of new alleles by erasing sexual barriers. However, if these new tools could be integrated in breeding schemes very rapidly in the upcoming years some limiting factors can still be identified. First, reliable and efficient identification of the genes underlying traits of interest is needed, as well as the evaluation of their combination on the value of these traits. For that matter, developing efficient gene function analysis tools and precise high-throughput phenotyping methods are essential. Secondly, agronomic applications rely on the mastery of gene inactivation and gene modification. However, if the knock-out of genes by non-homologous end-joining (NHEJ)-mediated repair of the targeted double-strand breaks (DSBs) induced by the TALEN or CRISPR-Cas9 systems is rather well mastered, the knock-in of genes by homology-driven repair is still challenging in higher plants. In this context, we will present here recent data obtained in the frame of the French ANR GENIUS (Genome ENgineering Improvement for Useful plants of a Sustainable agriculture) project aiming at mastering the techniques of genome editing in a wide range of crop species.



Determinants of Farmers' Choice in Varietal Innovation: a comparative study between French, Hungarian and Bulgarian farmers

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As expressed by the latest IPCC reports, the climate is changing and the human activity is responsible for its acceleration. Whatever the political orientations implemented, it is now noted that its consequences will increase in the coming years. In order to contain global warming below 2 ° C two strategies must be tackled head-on: adaptation to the effects of climate change and the mitigation of its causes. Confronted with these changes, which put at risk the world's food security, the agricultural sector is questioned. Indeed, the agricultural sector, although responsible for 13.5% of global GHG emissions, is directly impacted by climate change: displacement of possible crop areas, modification of the biological cycles of plants, more water and thermal hazards, etc. If different technical, agronomic and genetic possibilities are available, because as said Houiller (2015): "the first, to explore in priority and easy to accept, is the genetic way." In this context, sunflower appears as a plant of interest because it is a low demand crop of inputs, a plant of diversification and a good head of rotation. Furthermore, its tolerance to water and heat stress and its pivotal root also make it a crop that responds quite well to climate change.

The aim of this study is to understand how to develop this crop around Europe. For that, we focus on the determinants of farmers' choices of new seeds in three different countries: France, Bulgaria and Hungary. The method used is interviews with open questions. In order to have a broad view of the determinants, we integrate in the study farmers, but also seed companies, distributors, processors and parallel actors as public stakeholders. As a result, we interviewed 37 persons in France, 15 in Hungary and 11 in Bulgaria.

The main determinants of choice of varietal innovation can be found in the four following categories: 1) the technical needs of the farmer determine his varietal choices, 2) the organization of the sector, 3) the public policies and 4) the climate change, by imposing new needs. However, these four determinants do not have the same importance in each country because of their organization and their history, but also due to their representations. For example, in France, the climate change does not count as a determinant of choice compared to Hungary and Bulgaria. In Bulgaria, the communism time play yet a role in their representations of a market and influence the organization of the sector.

Under these results, we are able to discuss the levers and the constraint of the development of sunflowers in these countries. We will also attempt to generalize our result to the European level