

Présentation des projets financés au titre de l'édition 2009 du
 Programme « Knowledge Based Bio Economy »

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Programme «KBBE »

Edition 2009

ADYSARC	Advancing yield stability and resource efficiency of crop plants
Résumé	<p>This industry-driven project aims to improve yield stability of crop plants under limiting environmental conditions that are of agronomic, ecologic and economic relevance in modern agriculture. Agricultural productivity will become limiting over the next 20 years due to the growing world population and increased meat consumption. At the same time continuous global warming will amplify existing abiotic stress conditions and create new, combined abiotic stresses which will lead to significant crop losses. Of major and increasing agronomic importance is the development of crops with improved tolerance to heat stress and combined heat and drought stress. This project will use Arabidopsis for gene identification and as test model system for transferring results to the crops rice and tomato (using transgenic and non-GM approaches).</p>
Partenaires	INRA UMR 1165- HIRT Heribert
Coordinateur	Heribert HIRT – INRA UMR 1165 heribert.hirt@evry.inra.fr
Aide de l'ANR	450 k€
Début et durée	36 mois
Référence	ANR-09-KBBE-001

Programme « KBBE »

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dnRNAguard

Host-induced gene silencing by RNAi in fungal and oomycete pathogens for healthier and safer food

Résumé

Crop yield and the quality of plant products are affected by a plethora of plant diseases. The agronomically most important losses are caused by fungal and oomycete pathogens. These diseases are mostly controlled by the use of partially resistant cultivars and phytopharmaceutical compounds. However, these crop-protection strategies have important limitations such as breaking of dominant plant resistance genes by virulent pathogen races, or tolerance of fungi and oomycetes towards chemical control agents. Gene technology offers an important alternative for efficient and durable crop protection, as demonstrated by the success of insect-resistant maize and cotton cultivars carrying Bt toxin. Members of this consortium recently discovered that silencing of key fungal transcripts by plant-expressed RNAi constructs provides protection against important pathogens such as powdery mildew, rice blast or *Fusarium* sp. This discovery has an enormous application potential for the production of healthier and safer food without the need of chemical disease control, and has been protected by patent applications from Bayer Crop Science (BCS) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). The aim of the RNAguard consortium is to study the mechanisms of host-induced gene silencing (HIGS) in major fungal and oomycete diseases of mono- and dicots, and to develop and optimize the technology for its future application in agricultural practice. The consortium will follow a broad approach to explore the agronomic potential of HIGS, including major crop plants such as wheat, maize, potato and tomato, and important fungal and oomycete pathogens such as *Fusarium*, *Colletotrichum*, and *Phytophthora*. In three overlapping phases of the project we propose to identify new HIGS targets in these pathogens by genome-wide transcript profiling during in planta growth, by *Agrobacterium*-mediated targeted as well as random mutagenesis, and by a hypothesis-driven approach. Potential targets will be initially validated in transient gene silencing assays. In the second phase of the project, transgenic events will be generated in all four crop plants, based on newly identified top target candidates plus first pre-existing (at partners), validated candidates. The transgenic lines will be challenged by different pathogens in the greenhouse and molecularly characterized. We also propose to test novel approaches to target multiple diseases by double or triple RNAi

constructs, or to enhance the transgene effect by simultaneously targeting multiple genes in a single pathogen. During the third phase of the project, we will conduct field trials in wheat and maize with the four most promising events in Germany and/or Spain, and in the USA. The expected results of the project will be:

- A better understanding of pathogenicity and virulence factors in major crop pathogens.
- A better understanding of the mechanism(s) underlying the HIGS phenomenon.
- A solid validation of the agronomic potential of HIGS as novel crop-protection strategy.
- Transgenic lead events for the direct development of new cultivars by backcrossing into elite material.
- Additional intellectual property (IP) for lead events.

Partenaires INRA UMR 118-Jean-Eric CHAUVIN
INRA UMR 1301- Franck PANABIÈRES
BayerCropScience - Roland BEFFA

Coordinateur Jean-Eric CHAUVIN INRA UMR 118
Jean-Eric.C Chauvin@rennes.inra.fr

Aide de l'ANR 781k€

Début et durée 36 mois

Référence ANR-09-KBBE-002

Programme « KBBE »

Edition 2009

EULAFUEL

Production of energy-rich triterpenoids in *Euphorbia lathyris*, a potential crop for third generation biofuels

Résumé

Aims: In the late 70's *Euphorbia lathyris* was proposed as a novel source of biomass for biofuel production. The latex of this plant accumulates large amounts of energy-rich triterpenoids, hydrocarbon-like compounds that can be extracted and converted to a gasoline-type biofuel. Surprisingly, no significant progress has been made during the last decades to further characterize the biochemical, molecular and cellular processes involved in the biosynthesis and accumulation of triterpenoids in the latex of *E. lathyris*. Moreover very little is known on the differentiation and development of the laticifers, the specialized cells that produce and store latex. The aim of this proposal is to combine the knowledge and expertise of different research groups with the use of different "omic" approaches to get new insights into the biochemical, molecular and cellular aspects related with the biosynthesis and accumulation of latex triterpenoids and laticifer differentiation in *E. lathyris*. The generated knowledge, together with the parallel development of a genetic transformation method for *E. lathyris*, will set the basis for the development of strategies to increase latex triterpenoid content through genetic manipulation.

Partenaires

UDS UMR 7177- Michel ROHMER
CNRS UPR 2357-Thomas J BACH

Coordinateur

Michel ROHMER - UDS UMR 7177
mirohmer@chimie.u-strasbg.fr

Aide de l'ANR

543 k€

Début et durée

36 mois

Référence

ANR-09-KBBE-003

Résumé

Barley is the second most important crop species in Europe, next to wheat both in production and area. Its importance is underscored in Germany, France and Spain, which together account for over 20% of the World production, and over 50% of the EU27. The socio-economic weight of barley in our continent is strengthened by the wide diversity of economic sectors where it plays a significant role. Barley is currently a multi-purpose crop, primarily used for animal feed (grain and forage) and for malt but, increasingly, for direct human consumption due well-documented benefits for health. However, barley – like other crops - is hit by many fungal and viral diseases causing high economic losses. In this respect, resistance is considered as the most cost-effective, consumer-friendly and environment-sound way of plant protection. Therefore, resistance is a prerequisite for the production of safer and healthier food. Breeders must resort continuously to new sources of resistance to overcome the rising virulence of the pathogens. This proposal is built with the aim to exploit new and potentially durable resistances to some of the most important pathogens of barley, i.e. *Blumeria graminis*, *Rhynchosporium secalis*, *Puccinia hordei*, *Pyrenophora teres*, *Ramularia collo-cygni* and the aphid transmitted Barley yellow dwarf virus (BYDV). To achieve this, (i) diagnostic markers for newly discovered resistance genes and QTL against the above mentioned pathogens will be developed, (ii) these markers will be validated on a set of unrelated germplasm and (iii) respective resistance will be marker based incorporated in adapted breeding lines to broaden the genetic basis of disease resistance in European barley breeding. Besides this, (iv) high resolution mapping populations as a base for an enhanced isolation of resistance genes based on the physical map and the whole genome sequence of barley, which will be available in a foreseeable time, will be developed. In addition to this (v) the HaploChiP methodology will be applied in combination with bioinformatics to detect allelic variation involved in cis-regulation of genes expressed in response to pathogen stimuli. At the end (vi) all the markers and genes identified will end up in an integrative map of resistance in barley. In summary, this approach will facilitate a considerable improvement of the resistance level in barley cultivars against important pathogens and will contribute to an enhanced isolation of resistance genes and the identification of allelic variation.

The results of this project will enable the breeders in the consortium to rapidly transfer the new resistances to adapted cultivars based on the closely linked markers developed, whereas for the academic partners this project provides the basis for gene isolation in a mid term view and provides deep insight into the allelic variation of cis-regulation of genes involved in resistance.

Partenaires SAS Florimond Desprez Veuve & Fils - Pierre DEVAUX

Coordinateur Pierre DEVAUX - SAS Florimond Desprez Veuve & Fils
pierre.devaux@florimond-desprez.fr

Aide de l'ANR 61 k€

Début et durée 36 mois

Référence ANR-09-KBBE-004

Programme « KBBE »

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ROOT

Root enhancement for crop improvement

Résumé

The plant root system is an important yet underscored agronomic trait. Plant roots perform many essential functions including water and nutrient uptake, storage of reserves, anchorage to the soil and establishment of biotic interactions in the rhizosphere. The size and architecture of the root system determine the plant's ability to fulfil these functions. In particular, water and nutrient shortages limit growth in many agricultural ecosystems and pose increasing problems. For example, the phosphate concentration is too low in 80% of agricultural soils. Similarly, access to nitrogen is vital during vegetative growth but often limiting. These substances are added through fertilization. Fertilization is expensive, energy consuming (50% of the energy used in agriculture) and often causes environmental problems such as eutrophication. Root length and/or root density are positively correlated with mineral uptake and access to water in many instances. Thus the negative impact of the aforementioned factors may be reduced by optimizing root system architecture (RSA). Root enhancement is difficult to assess with classical breeding strategies and RSA cannot yet be determined in the field. We present an alternative approach to overcome these limitations. Recent work has revealed that the plant hormone cytokinin and certain evolutionary conserved miRNAs are single factors controlling RSA. Importantly, these factors can be manipulated in a targeted fashion and proof has been obtained in model plants that genetic engineering generates an enhanced root system, improved drought resistance and nutrient accumulation in the aerial organs. A central aim of this project is, because of their high agricultural relevance, to transfer these novel approaches to a cereal plant. We selected barley (*Hordeum vulgare* L.) as an important European cereal crop plant. Spain, Germany and France are the leading barley growing nations in Europe with total surface of >6 Mill ha and >20% of the total world yield. Global climate changes pose increasing drought problems, in particular during early summer (seed filling) and barley shares the common agricultural problem associated with fertilization. Barley is a diploid plant and used as a genetic model, it can be transformed and increasing genomic resources are available. Importantly, barley can serve as a reference for wheat, the most relevant European crop but currently less amenable for experimental work. In addition to the generation of lines with enhanced root systems we will study the interplay of different factors in determining root architecture, focusing on nitrogen

and phosphate, and we will develop novel methods to visualize and study RSA. These studies will involve work in Arabidopsis as it is an advantageous system to study crosstalk between factors that modulate root architecture and to identify novel genes. Specific activities in the project will be: · Generation of transgenic barley plants with an enhanced root system based on knowledge recently gained in model plants. · Understand the regulation of RSA by different intrinsic regulatory pathways (hormones, miRNA) and identify their interactive mode of action with exogenous cues (nitrogen, phosphate). · Refine and apply optical imaging and tomographic methods to study root architecture. In order to achieve the research objectives, this proposal brings together a consortium with a complementary set of expertises in genomics and molecular biology, nutrient physiology and innovative imaging physical methods. The project exploits the most recent technologies and will provide valuable training to participating young scientists. The results have a high commercial potential as breeding lines with improved performance under limiting conditions are an important breeding aim. More generally, the project will provide important insights into the function of several parameters regulating RSA and their interaction. Features of the project which correspond to the PLANT-KBBE programme include genomic approaches to identify genes that are relevant to control root growth, an important parameter to determine yield under abiotic stress and in low input systems. An improved root system will increase yield stability compared to near-isogenic lines lacking this trait and reduce the use of fertilizers thus contributing to better and healthier food production. We will not only work in a model plant (Arabidopsis) but also transfer recent knowledge in which partners of this project are specialists into a cereal, barley. Potential application beyond barley is an expected outcome. Thus this proposal supports the overall objectives of the PLANT-KBBE programme

Partenaires

CNRS UPR 2355- Florian FRUGIER
 CNRS UMR 5004- Philippe NACRY

Coordinateur

Florian FRUGIER- CNRS UPR 2355
frugier@isv.cnrs-gif.fr

Aide de l'ANR

520 k€

Début et durée

36 mois

Référence

ANR-09-KBBE-005

SUIT

Engineer SUBerin biopolyester production in potato to Improve stress Tolerance

Résumé

Suberin is a tissue sealing polyester deposited at the plant environment interphase. In peri-derms of aerial plant organs and in the root exodermis and endodermis, suberin functions as a barrier preventing uncontrolled water and nutrient loss and pathogen attack. In addition, controlled suberization occurs in response to wounding, salt or drought stress. In potato these suberin functions are not only crucial for growth dependent yield, but also for post harvest storage properties of tubers. Despite these important functions our understanding of the enzymes and genes involved in suberization is surprisingly limited. Recently, reverse genetics approaches in Arabidopsis and potato identified the first suberin involved genes. These proof of concept studies demonstrated that reductions in predominant suberin monomers result in increased water permeability of potato periderms. Arabidopsis enhanced suberin mutants exhibited an increased tolerance to salt and drought stress conditions. From these studies it can be postulated that targeted engineering of suberization can modify barrier properties of suberized tissues leading to improved yield stability and stress tolerance in crop plants. This project aims are an increased knowledge of the metabolic pathways and the underlying molecular attributes involved in suberin formation, and the production of plants with engineered suberin to improve performance of crops under unfavourable environmental conditions. To reach this goal a complementary approach combining bioinformatics, molecular genetics, recombinant protein bio-chemistry, physiology, and analytical chemistry is proposed with the following main objectives (MO):. MO 1. Discover and validate key suberin genes by reverse genetics in plant models (Arabidopsis and potato) A bioinformatics approach considering tissue specificity, environmental conditions, coexpression with known suberin involved genes and potential functions in processes expected in suberization (e.g. fatty acid modification) will generate a comprehensive list of suberin candidate genes (SG) from Arabidopsis. Expected 80-120 good SG will be short listed to 20-30 by detailed expression profiling. GC-MS based compositional suberin profiling in corresponding knock out and overexpression lines will verify involvement in suberization. For expected 15-25 verified Arabidopsis putative orthologues will be obtained by homology based cloning. Tissue-specific expression profiling of isolated potato SG will be used to verify the specificity for suberizing tissue. Sequencing genomic

5'-regions of SG will identify suberin regulatory elements and enables tissuespecific expression strategies. MO 2. Use heterologous expression systems to characterize the catalytic properties of key enzymes in suberin biosynthesis SG, especially Cytochrome P450 oxygenases involved in ω -oxidation of the predominant suberin monomers, ω -hydroxyacids and α,ω -diacids, will be introduced in engineered yeast and baculovirus expression systems. Catalytic properties will be determined testing collections of potential substrates e.g. fatty acids, acyl-CoAs and acyl-glycerols. This will indicate in vivo substrates and help selecting additional SG to optimize substrate availability in co-overexpression strategies. MO 3. Elucidate the role of SG in suberin macromolecular structure SG characterized in MO 1 will be cloned into optimized expression vectors, enabling ectopic overexpression, RNAi silencing and expression in suberizing peridermal tissues. Large scale transformation generate approximately 600 transgenic potato lines for the selection of 3-5 stably expressing lines from 5-10 SG over expression construct, 5-10 SG silencing constructs and 5-10 Promoter:Reporter constructs. The latter will be investigated for expression in suberizing tissue. Isolated tuber periderms from SG-transgenic potato will be analytically characterized regarding monomer composition, molecular connectivity and macromolecular structure, using mass spectrometry techniques (GC-MS, ESI-MS/MS), FTIR and ^{13}C NMR. The tubers of expected 5-15 SG-transgenic potatoes with quantitatively or qualitatively modified suberin will be investigated for alterations in suberin ultra structure using electron microscopy. MO 4. Determine the impact of compositional components and macromolecular structure on barrier properties Tuber periderms from transgenic potatoes - characterized in MO 3 as being affected in suberization - will be investigated for modified features such as function as an efficient permeation barrier. The permeability will be determined for water and isotopic model tracers. Intact tubers will be used to investigate the effect of suberin modification on post harvest food loss during storage and upon pathogen attack. MO 5. Targeted modification of SG to engineer suberin for improved stress tolerance. The performance of whole potato plants and Arabidopsis with modified root suberin (MO 1 and MO 3) will be tested under unfavourable environmental conditions. These studies will uncover coherences between the composition, macromolecular- and ultra structure of suberin and its barrier characteristics as well as suberin characteristics and tolerance to biotic and abiotic stresses.

Partenaires CNRS UPR 2357- Franck PINOT

Coordinateur Franck PINOT - CNRS UPR 2357
franck.pinot@ibmp-ulp.u-strasbg.fr

Aide de l'ANR 196 k€

Début et durée 36 mois

Référence ANR-09-KBBE-006

SUSTAINPINE

Genomic tools in maritime pine for enhanced biomass production and sustainable forest

Résumé

The forest sector has to cope with a progressive impact of the global climate change in a context of increasing global economic competition among industrial forest areas. Local competition for wood is also developing fast as it constitutes an attractive biomass for energy. In this harsh reality biotechnology is likely to provide effective solutions to accelerate forest management for sustainable and competitive production. Recent developments in genomic research are providing increasingly efficient tools for molecular analysis of biological processes. The general aim of this project is the application of the latest technologies towards identification of key genes determining adaptive traits in conifers, which are crucial for forest productivity, conservation and management. The expected major outcome is to propose practical, marker-based strategies to maintain the competitiveness of forest industries by improving biomass and wood productivity of conifer forests even under stress conditions. We also aim at specifically providing new knowledge for the European forest-based-sector that promotes the use of renewable resources in the context of global climate change. We will focus on maritime pine (*Pinus pinaster* Ait), the most advanced conifer model species for genomic research in Europe and the most widely planted species in France, Spain and Portugal. Results on this species would be easily transferred to closely related *Pinus* species and other economical and/or environmentally important gymnosperm species. The proposed research activities are addressed to increase genomic resources and to study candidate genes involved in the regulation of maritime pine development, growth and the response to environmental stress, capitalizing on data from previous projects. The work plan include: i) Isolation of novel maritime pine expressed sequence tags (ESTs) and full-length cDNAs (FLcDNAs); ii) Large-scale expression analysis and a System Biology approach to identify candidate genes; iii) Functional studies of selected candidate genes : Functional analysis of regulatory regions and interaction with transcriptions factors and Gene functional analyses in transgenic trees.; iv) Genetic mapping of selected candidate genes in reference genetic maps; v) Exploration of natural diversity of selected candidate genes in maritime pine populations; vi) Association mapping of selected candidate gene for growth and wood quality. Additionally, we will apply bioinformatic methods to perform the storage, processing and mining of all data delivered

in the project. This will especially facilitate the co-ordinated analysis of structural, expressional and functional data. Additionally, we will apply bioinformatic methods to perform the storage, processing and mining of all data delivered in the project. This will especially facilitate the co-ordinated analysis of structural, expressional and functional data. The expected results from this project are thus multiple integrated data from already or newly discovered candidate genes. Pine breeders will primarily benefit from new, molecular selection criteria to ensure more efficient design of adapted varieties for improved productivity (growth, vigour, wood properties) and sustainability (adaptation to drought or nutritional stresses). Such a better control of tree productivity will ultimately benefit the forest industries and other wood and biomass users. This project is strongly linked to the programme theme "bioenergy" since it aims at contributing to the development of a forest crop with the potential of resource cascading for different energetic and industrial applications (biorefinery concept) in a sustainable bio-based economy. Conifers are important as carbon sinks and as a source of renewable material for biofuel production and biorenewable chemicals. The project will also constitute the first step towards comprehensive Systems Biology in gymnosperms, with a special focuss on nitrogen metabolism.

Partenaires

FCBA- Jean-Luc HARVENGT
INRA UMR 1202- Christophe PLOMION
INRA UPR 588- Marie-Anne LELU-WALTER

Coordinateur

Jean-Luc HARVENGT – FCBA
luc.harvengt@fcba.fr

Aide de l'ANR

457 k€

Début et durée

36 mois

Référence

ANR-09-KBBE-007

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TOAST

Molecular breeding for tolerance to abiotic stress in tomato: a genomic approach for a sustainable agriculture

Résumé

Drought, salinity and low temperatures are the most important environmental constraints limiting plant growth and agricultural productivity. In fact, it has been estimated that the reduction of crop productivity because of these constraints is more than 60%. Therefore, the development of new high-yielding crop varieties showing increased tolerance to abiotic stresses constitutes an essential goal within the frame of a sustainable and environmentally-respectful agriculture. Plants are sessile organisms and have developed sophisticated responses to adapt to adverse environmental conditions. Understanding the molecular basis controlling these responses has not only a basic interest to learn how plants grow and develop, but also the biotechnological potential to generate tools to improve the drought and salt tolerance of important crops. During the last years, genetic and molecular analyses in *Arabidopsis* have allowed to define signal transduction pathways that mediate abiotic stress responses, including low temperature, water deficiency and high salt, and to identify several genes involved in tolerance to these adverse environmental conditions. In the case of crop plants, however, despite the efforts realized through conventional breeding programmes and modern biotechnology to increase their tolerance to abiotic stresses, the results obtained to date have generally been disappointing. Furthermore, the major international Solanaceae consortium, SOL, has only a very limited component dealing with abiotic stress in tomato. Tomato is considered the most economically important vegetable crop in the world, and is of particular importance in European countries. Nevertheless, abiotic stresses promoted by low temperature, salt accumulation and water deficiency, result in significant losses of productivity, mainly affecting countries around the Mediterranean basin. Therefore, the availability of tomato lines bearing cold, salt and/or drought tolerance would be a useful tool for the development of improved varieties better adapted to adverse environmental conditions. These varieties could definitively result in reduced inputs (such as water, heating, etc) and promote a more sustainable agriculture

Partenaires

CNRS UMR 8186 BMOP - Chris BOWLER

Coordinateur

Chris BOWLER CNRS UMR 8186 BMOP
cbowler@biologie.ens.fr

Aide de l'ANR 290 k€

Début et durée 36 mois

Référence ANR-09-KBBE-008

Programme « KBBE »
Edition 2009

ViReCrop

Identification of new plant susceptibility factors whose modification would confer recessive virus resistance and application in barley, tomato and melon

Résumé

Plant viruses are responsible for a significant proportion of crop diseases and are very difficult to combat due to the scarcity of effective countermeasures, placing them among the most important agricultural pathogens. Most emerging infectious diseases of plants are caused by viruses, being emergence provably favored by climate change, increased seasonal weather instability, and conditioned by intensive global trade. Viral diseases can affect food quality as well as reducing yields, yet quality is also affected by measures such as spraying with pesticides to kill off insect vectors. With no doubt, the best strategy for the control of plant viruses consist of the use of crop cultivars or varieties that are genetically resistant. Thus, introgressions of durable resistance to plant viruses into elite cultivars as well as the identification of new sources of resistance are major goals in plant research. About half of the ~200 known virus resistance genes in plants are recessively inherited, suggesting that recessive resistance is more common for viruses than for other plant pathogens. The use of such genes is a very important tool in breeding programs. Based on previous knowledge on recessive resistance to plant viruses generated by the participating teams, we propose the identification of new plant susceptibility factors whose modification would confer recessive virus resistance. We aim at transferring this knowledge to at least three important crop species: barley, tomato and melon.

Partenaires

INRA UPR 1052 – Carole CARANTA
Syngenta Seeds SAS - Julien BONNET

Coordinateur

Carole CARANTA - INRA Unité de Recherche de Génétique et Amélioration des Fruits et Légumes
carole.caranta@avignon.inra.fr

Aide de l'ANR

329 k€

Début et durée

36 mois

Référence

ANR-09-KBBE-009