SafePGR mid-report

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1st call for projects

Intermediate Report T0+18

This document is to be filled out by the coordinator in collaboration with the project partners. It must be sent by the coordinator, within the deadlines specified in the acts of award, to the Joint Call Secretariat.
*It reports on the activity of **all the project partners**. All the partners must have a copy of the version sent to the JCS.*
This model must only be used for the intermediate reports. There is also a specific model for the final report.

SafePGR

A IDENTIFICATION 1
B PUBLIC SUMMARIES 2
C PROGRESS REPORT 6
C.1 Initial objectives of the project 6
C.2 Work performed and results achieved in the period concerned 6
C.3 Work specific to the companies (where applicable) 8
C.4 Significant events and results 8
C.5 Consortium meetings (collaborative projects) 8
C.6 Collaboration with partners 10
C.7 Difficulties encountered and solutions 10
C.8 Work schedule 11
C.9 Deliverables and milestones 12
C.10 Free comments 14
D PROJECT VALORISATION AND IMPACT 14
D.1 Publications and communications 14
D.2 Other valorisation factors 14
D.3 Competitiveness clusters (for ANR funded labelled projects only) 15
D.4 Financial status 16
E APPENDICES (IF NECESSARY) 16

A IDENTIFICATION

Project acronym	SafePGR
Project title	Towards Safer Plant Genetic Resources through improved viral diagnostics
Coordinator (company/organization, country region)	INRA Guadeloupe
Project participants / beneficiaries company/organization, country region) : *1 line/partner*	CIRAD Guadeloupe CIRAD Montpellier CIRAD Réunion INRA Bordeaux University of Azores University of Madeira
Coordinator of the French part of the project (company/organization)	INRA Guadeloupe
Project start date	1st March 21012
Project end date	28 February 2015
Project website (if applicable)	http://www2.antilles.inra.fr/safepgr/

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Date of writing	19 October 2013
Period covered by activity report	1st March 2012 to 4 October 2013

B PUBLIC SUMMARIES

Provide an English summary (300 words max.) of the work done so far, for public dissemination. Include translation in the language(s) of your funding bodies (French, Portuguese and/or Spanish, as appropriate).

Safe-PGR: Towards Safer Plant Genetic Resources through improved viral diagnostics

Biological Resources Centres (BRCs) conserve and distribute plant germplasm for research and development purposes. As such, they play a strategic role by providing breeding programs with genitors that are critical for crop adaptation to ongoing environmental and societal changes. BRCs must guarantee the sanitary status of the resources they distribute, in order to prevent the spread of diseases.
The species conserved in the Guadeloupe, Azores, Madeira, and Réunion BRCs are banana, sugarcane, yam, sweet potato, garlic and vanilla. Being vegetative propagated, these crops do not benefit from the sanitation occurring through a seed cycle, particularly for viral diseases. Sanitation methods exist for recovering virus-free plants but their implementation depends on the availability of sensitive, polyvalent and reliable diagnosis tests for all relevant virus species.

The objective of Safe-PGR is to improve the knowledge of the diversity of viruses infecting the crops addressed by the partner's BRCs. It will be used to develop classical or new diagnostic techniques that will permit the safe movement of plants between the project partners and beyond.

The project will explore the molecular diversity of the viral families affecting the targeted crops, optimize classical diagnostic methods taking into consideration data generated through this analysis of viral diversity and develop new multi-pathogen diagnostic methods based on metagenomics and deep-sequencing technologies.

Results

Initial efforts have already resulted in the discovery and partial genome characterization of several novel viruses in Garlic, Sugarcane, Yam and Vanilla, for which new and efficient detection assays are currently being developed and implemented. Screening of partners germplasm collections using broad range molecular diagnosis techniques is 90% complete, leading to an extensive inventory of virus species belonging to viral genera known to infect targeted plant species.

Test and comparison of 8 extraction methods were carried out in order to develop metagenomics studies. Two complementary methods were selected, based on the extraction of double-strand RNA and viral particles. We decided to use both techniques for the screening of 1500 plants from the CRB germplasm collections.

The transfer of dsRNA extraction methods has been successfully achieved during the interim meeting. All partners are now in capacity to set up the techniques in their own labs (Guadeloupe, Madeira, Azores, Réunion).

The bioinformatics tools have been successfully developed and allowed to analyze the preliminary data obtained from the metagenomic methodological step.

Perspectives

- To carry out the protocols for metagenomics analyses.
- To feed-back Bioinfoinformatics and Metagenomics results to diagnostic and characterization.
- To analyze the virome of the 1 500 targeted plants.
- To prepare the publications.

Safe-PGR: Amélioration du diagnostic viral pour sécuriser les ressources génétiques végétales

Les Centres de Ressources Biologiques (CRB) conservent et diffusent du matériel végétal, à des fins de recherche et développement. Ils jouent donc un rôle stratégique pour fournir des géniteurs aux programmes d'amélioration génétique et ainsi répondre aux enjeux d'adaptation aux changement environnementaux et sociaux actuels et futurs. Les CRB doivent garantir la qualité sanitaire des ressources qu'ils distribuent, afin d'éviter la propagation de maladies des plantes.

Les espèces conservées dans les CRB de Guadeloupe, des Açores, de Madère et de la Réunion sont les bananiers, cannes à sucre, ignames, patates douces, aulx et vanilles. Ces espèces se multiplient par voie végétative, et ne sont ainsi pas assainies de virus car il n'y a pas de passage par les graines. Des méthodees d'assainissement existent mais leur mise en oeuvre passe par la disponibilité de tests de diagnostic sensibles, polyvalents et efficaces pour détecter les principales espèces virales.

L'objectif de SafePGR est d'acquérir des connaissances sur la diversité des virus infectant les espèces végétales d'importance pour les CRB partenaires. Ceci permettra de mettre au point des méthodes classiques ou innovantes pour sécuriser la diffusion de matériel végétal entre les pays partenaires et au-delà.

Le projet explorera la diversité moléculaire des espèces virales cibles, optimisera les méthodes de diagnostic classique en s'appuyant sur les données issues des analyses de diversité et développera des méthodes de diagnostic multi-pathogènes, basées sur les technologies de métagénomique et de séquençage profond.

Résulats

Les travaux ont d'ores et déjà permis de découvrir et de caractériser en partie le génome de plusieurs espèces nouvelles de virus, sur l'ail, la canne à sucre, l'igname et la vanille. De nouvelles méthodes de diagnostic sont en cours de mise au point à partir de ces résultats.

Le screening des collections de ressources génétiques des partenaires a été conduit à 90%, en utilisant des méthodes à large spectre. Ceci conduit à un inventaire élargi des virus infectant nos plantes cibles.

Huit méthodes ont été testées et comparées, afin de mettre au point les études de métagénomique. Deux méthodes complémentaires ont été choisies, basées sur l'extraction d'ARN doubles-brins, et de particules virales. Nous avons décidé d'utiliser les deux techniques sur 1 500 plantes des collections.

Nous avons formé les partenaires à ces méthodes durant le second séminaire du projet, ils sont maintenant en capacité de les utiliser dans leurs propres laboratoires en Guadeloupe, à Madère, aux Açores et à la Réunion.

Les outils bio-informatique ont été mis au point et ont permis l'analyse préliminaire des données obtenues durant l'étape de mise au point méthodologique en métagénomique.

Perspectives

- Conduire les protocoles d'analyse métagénomique.
- Utiliser les résultats de bio-informatique et de métagénomique pour alimenter en retour le diagnostic et la caractérisation virale.
- Analsyer le virome des 1 500 plantes.
- Rédiger les publications.

Safe-PGR: Rumo Safer Recursos Fitogenéticos através de melhores diagnósticos virais

Os Centros de Recursos Biológicos (CRB) conservam e distribuem germoplasma vegetal para fins de pesquisa e desenvolvimento. Como tal, desempenham um papel estratégico, fornecendo programas de melhoramento genético com genitores que são essenciais para a adaptação das culturas às mudanças ambientais e sociais em curso. Os CRB devem garantir o estado sanitário dos recursos que distribuem, a fim de evitar a disseminação de doenças.

Entre as espécies conservadas nos CRB das ilhas de Guadalupe, Madeira, Açores e Reunião estão a banana, inhame (*Discorea* spp.), batata-doce, cana-de-açucar, alho e baunilha. Sendo propagadas vegetativamente, estas culturas não beneficiam do saneamento que ocorre através do ciclo de produção de sementes, particularmente para as doenças virais. Existem contudo métodos de saneamento para a recuperação de plantas isentas de vírus, mas a sua implementação depende da disponibilidade de testes de diagnóstico suficientemente sensíveis, polivalentes e fiáveis para todas as espécies virais relevantes.

Objetivo do projeto SafePGR é melhorar o conhecimento da diversidade dos vírus que infetam as culturas abordadas pelos CRB parceiros. Este conhecimento será aplicado no desenvolvimento de técnicas clássicas de diagnóstico, bem como de novas técnicas, que permitam a circulação segura de plantas entre os parceiros do projeto e para além destes.

O projeto irá analisar a diversidade molecular das famílias de vírus que afetam as culturas alvo, otimizar métodos de diagnóstico clássicos, tendo em consideração os dados gerados pelo estudo da diversidade viral, e desenvolver novos métodos de diagnóstico multi-patógeno com base em metagenómica e novas tecnologias sequenciação.

Resultados

Os esforços iniciais já resultaram na descoberta e caracterização parcial dos genomas de diversos novos vírus no alho, na cana-de-açúcar, no inhame e na baunilha, para os quais novos testes de deteção estão sendo desenvolvidos e implementados. Também, já foi concluída 90% da triagem das coleções de germoplasma dos parceiros utilizando uma vasta gama de técnicas moleculares de diagnóstico, originando um extenso inventário de espécies de vírus pertencentes a géneros conhecidos por infetar as espécies vegetais em estudo.

Foram testados e comparados oito métodos de extração a fim de desenvolver estudos de metagenómica. Foram selecionados dois métodos complementares com base na extração de ARN de cadeia dupla e de partículas virais.
Foi decidido utilizar ambas as técnicas de extração para o rastreamento de 1500 plantas das coleções de germoplasma dos CRB.

A transferência do método de extração de dsRNA foi alcançada com sucesso durante a reunião intermedia. Todos os parceiros têm agora a capacidade de estabelecer a técnica nos seus laboratórios (Guadalupe, Madeira, Açores e Reunião).
As ferramentas bioinformáticas foram desenvolvidas com sucesso e permitiram analisar os resultados preliminares obtidos na etapa de desenvolvimento metodológico da metagenómica.

Perspetivas

- Executar os protocolos destinados à análise metagenómica.
- Interligar os resultados da análise bioinformática e metagenómica ao diagnóstico e caracterização.
- Analisar o viroma das 1500 plantas a estudar.
- Preparar publicações.

C DELIVERABLES AND MILESTONES

When applicable for the project, reproduce the table of milestones and deliverables provided at the start of the project. Indicate all the deliverables, including any deliverables deleted or added with respect to the initial list.

Green : done, **Black** : to be done, **Orange** : delayed

No.	Designation	Nature*	Date of supply			Partners (underline the responsible partner)
			Initially planned	Re-scheduled	Delivered	
PROJECT MANAGEMENT						
1	M0-1: Website developed	Milestone	01/03/12		01/03/12	P1
2	M0-2: Kick off meeting (Madeira)	Milestone	28/03/12		28/03/12	All P7
3	M0-3: Action plan 1 released	Videoconference report	01/05/12		20/06/12	All P1
4	M0-4: 6 month report released and consortium agreement signed	Milestone	31/07/12		21/08/13	All P1
5	M0-5: Interim report available	Milestone	31/10/13			All P1
6	M0-6: Final report available	Milestone	31/05/15			All P1
7	M0-7: Meeting 2 (Montpellier)		12/09/13	29/09/13	29/09/13	All P3
8	M0-8: Action plan 2 released	Videoconference report	01/07/13		3/04/13	P1
9	M0-9: Meeting 3 (Guadeloupe)		26/01/15			All P1
10	D0-1: A follow-up project website	Website	1/03/12		1/03/12	P1
11	D0-2: Communication supports (talks, web pages, press release)	Pages and files in the website	Throughout the project			All P1
12	D0-3: The consortium agreement	Document	31/08/12		21/06/12	All P1
13	D0-5: The kick-off meeting report	Report	31/08/12		20/07/13	All P1
14	D0-6: The mid-report	Report	31/10/13			All P1
15	D0-7: The final report	Report	30/04/15			All P1
16	D0-8: Joint scientific and technical publications	Publications	Throughout the project			All
VIRAL DIVERSITY						
17	Staff for coordination of analyses in Guadeloupe hired	Milestone	1/09/12		3/09/12	P1
18	M1-1: Plant samples analyzed and sequences generated	Milestone	15/09/13	31/12/13		P1, P2, P4, P5, P6, P7
19	D1-1: A catalogue of viruses infecting accessions of BRCs	Files in the website	31/03/14	31/12/13		P1, P2, P4, P5, P6, P7
20	M1-2:Polyvalent tools available, specificity of diagnostic tools verified by sequencing	Milestone	31/01/15			P1, P2, P4, P5, P6, P7
21	D1-2: Sequences comparisons and phylogenetic analyses of identified viruses	Data	31/01/15			P1, P2, P3, P4, P5
22	D1-3: Optimized protocols for viral diagnostic	Files in the website	Throughout the project			P1, P2, P4, P5
METAGENOMICS						
23	Staff for metagenomics development hired	Milestone	01/05/12		01/05/12	P3
24	M2-1: metagenomic approach selected	Milestone	15/09/13		30/09/13	P3
25	M2-2: metagenomic approach transferred	Milestone	30/09/13		04/10/13	All P3
26	D2-1: Protocols for obtaining plant viromes	Files on the website	15/09/13		04/10/13	P3
27	D2-2: Training session in the use of metagenomics	Training session	20/09/13		04/10/13	P3
28	M2-3: plants from the 6 target crops processed	Milestone	31/01/15			P3
29	D2-3: Inventory of viruses present in the BRCs	Milestone	End of the project			All P3
BIO-INFORMATICS						
30	M3-1: screening of ESTs for viral sequences completed	Milestone	1/10/12	31/07/13	Yes	P3, P5
31	D3-1: Results of the screening of	Data	1/10/12	31/07/13	Yes	P3, P5

No.	Designation	Nature*	Date of supply			Partners (underline the responsible partner)
			Initially planned	Re-scheduled	Delivered	
	crops ESTs for viral sequences					
32	D3-2: Sequence information on novel viral agents to WP 1	Data	1/10/12	31/07/13	Yes	P3, P5
33	Staff for informatic development hired	Milestone	1/10/12	1/04/13	1/04/13	P5
34	M3-2: pipelines established	Software	31/3/13	20/09/13	20/09/13	P5
35	D3-3: Processing and annotation pipelines for 454 pyrosequencing and Illumina siRNA reads	Data	31/3/13	20/09/13	20/09/13	P5
36	M3-3: analysis of the NGS data from WP 2 / task 3 completed	Data	30/6/13	20/9/13	20/9/13	P5
37	D3-4: Training of partners to the use of pipelines	Training session	20/9/13		20/09/13	P5
38	D3-5: Results of analysis of all NGS data from the validation step of WP2	Data	31/01/15	31/01/15		P5
39	M3-4: analysis of the sequences data from WP2/task 5 completed	Data	31/1/15			P5

** milestone, report, software, prototype, data, etc.*

D PROGRESS REPORT

D.1 INITIAL OBJECTIVES OF THE PROJECT

Maximum 10 to 20 lines.

The project aims at developing improved plant virus diagnostic approaches, in particular using next generation sequencing (NGS)-based on plant viral metagenomics, that can be used for routine indexing of BRCs resources. The project will:

· **Compare classical diagnostics and various high-throughput next generation sequencing (NGS) approaches** on a limited number of accessions from each target crop (sugarcane, yam, vanilla, garlic, sweet potato and banana) in order (i) to identify the best NGS strategy (taking into account benefits, costs and constraints) for the detection of known and unknown viral agents and (ii) to compare its efficiency with that of classical diagnostics for the detection of known viral agents.

· **Use the plant viral metagenomic approach over several quarantine and BRC plant collections** located in Guadeloupe, Réunion, Madeira, Azores and Montpellier in order to get a first detailed assessment of the sanitary status of the plants maintained in these collections (sugarcane, yam, vanilla, garlic, sweet potato, and banana).

· **Describe the new virus species and the diversity of known or poorly know viral species and use this information** to further develop or improve efficient diagnostic approaches, in particular so that the contribution of novel agents to important diseases can be evaluated.

D.2 WORK PERFORMED AND RESULTS ACHIEVED IN THE PERIOD CONCERNED

Maximum 1 page. Describe the work performed and the results obtained during the period concerned, conformity of work progress with initial schedule. Clearly indicate who performed the work, and do not omit to mention the work performed by the other partners.

WP1: Knowledge development on viral diversity

TASK1: MOLECULAR DIVERSITY WITHIN VIRUS FAMILIES KNOWN TO INFECT BANANA, GARLIC, SUGARCANE, SWEET POTATO, VANILLA, YAM

BRC collections were screened using classical molecular techniques based on PCR and the use of degenerate primers. Primers designed by partners P3, P4 and P5 based on datamining of ESTs and Genbank were used upon availability (see below Task 6). A total of 3805 indexings were performed. Amplification products are being sequenced, either directly or following cloning. Preliminary results are available for a limited number of viral species and crops but do not allow yet drawing conclusions on viral diversity.

TASK2: OPTIMIZATION OF EXISTING POLYVALENT DIAGNOSTIC TOOLS OR DEVELOPMENT OF NEW ONES ABLE TO COVER THE MOLECULAR DIVERSITY WITHIN VIRUS FAMILIES KNOWN TO INFECT BANANA, GARLIC, SUGARCANE, SWEET POTATO, VANILLA, YAM

Diagnostic methods were optimized for the detection of badnaviruses in banana, sugarcane and yam, that of criniviruses in sweet potato and potexviruses in yam. Diagnostic methods were designed and implemented for the detection of tobamoviruses in banana, foveaviruses in garlic, nucleorhabdoviruses in sweet potato, mastreviruses and flexiviruses in sugarcane,

begomoviruses in sweet potato, potexviruses in vanilla, closteroviruses, macluraviruses and sadwaviruses in yam.

WP2: Development of new tools for a global diagnostic of viruses

TASK3: METHODOLOGICAL STEP: ASSESSING THE HIGH-THROUGHPUT SEQUENCING METHODS

Eight NGS-based protocols were assessed for the detection of plant viruses on all six crops targeted by the project. Information about virus species known to infect each of these 6 crops in the BRCs and quarantine facilities managed by project partners were used to confirm the efficiency of the metagenomics methods described hereafter, together with data collected in the frame of WP 1. Sixteen plants for each of the six target crops were used in this task. Briefly, the tested methods are:

1. Purification of dsRNA with CF11 cellulose, amplification assays based on tagged random RT-PCR primers and pyrosequencing
2. Purification of dsRNA using CF11 cellulose (method developed by P5) followed by amplification assays based on tagged random RT-PCR primers and pyrosequencing
3. Purification of dsRNA using CF11 cellulose, amplification assays based on tagged random RT-PCR primers and pyrosequencing
4. Purification of viral particle (method developed by P3) followed by amplification assays based on tagged random RT-PCR primers and pyrosequencing
5. Purification of viral particle (method developed by P3) followed by amplification assays based on tagged random RT-PCR primers and pyrosequencing
6. Purification of RNAs (Trizol method) followed by Illumina sequencing of small RNAs
7. Purification of RNAs (Phenol: Chloroform method) followed by Illumina sequencing of small RNAs
8. Purification of RNAs (Commercial Kit MirVANA) followed by Illumina sequencing of small RNAs

Bioinformatics tools developed in WP3 (see below) allowed to begin the comparison of the efficiency between the eight different deep sequencing approaches tested in this task. Collectively, all partners decided to select two methods for Task5, namely method #1 developed by P5 and method #4 developed by P3 because results obtained from these two methods were complementary and enabled an efficient inventory of plant viruses present in each tested plant.

TASK4: TECHNOLOGY TRANSFER

The methodological step corresponding to Task 3 was completed by September 2013. A training session was organized in Montpellier in order to transfer method #2 (see above) to all project partners. This transfer will allow running an interlaboratory validation step of this method in Guadeloupe (sugarcane, yam, and banana), Reunion Island (vanilla, garlic and sweet potato), Montpellier (quarantine yam and sugarcane), Azores (sweet potato and garlic) and Madeira (sweet potato) in the frame of Task 5, using a total of 1500 plant samples. Fresh samples of the same plants will be shipped by project partners to P3 who will use them to assess method #4. Combining both methods #2 and #4 will ensure a comprehensive screening of plant viruses present in the analyzed 1500 plant samples.

WP 3: Data analysis and bioinformatics

TASK 6: DATA MINING: SYSTEMATIC SEARCH FOR VIRAL SEQUENCES AND UNKNOWN VIRUSES IN EST DATABASES OF THE CROPS TARGETED IN THE PROJECT

A systematic search of public available EST resources for **sugarcane, vanilla, yam, sweet potato, garlic** and **banana** was performed by partners P3 and P5 to identify yet uncharacterized viral agents. For all newly identified viruses, the EST sequences were downloaded, assembled and the taxonomic affinities of the detected viruses ascertained as best as possible. The assembled sequences were also used to design primer pairs that were transferred to the partners involved in WP1 to screen germplasm collections of the relevant crops for the presence of the agent. Overall a total of 17 new viruses have been tentatively identified: 2 ***Foveaviruses*** in **Garlic**, 1 ***Macluravirus,*** 1 ***Closteroviridae*****,** 1 ***Secoviridae****, 2* ***Betaflexiviridae, 1 Luteoviridae,*** *2* ***Potexiruses, 1 Rhabdoviridae*** *and 1* ***Potyvirus*** in **Yam,** 1 potential new ***Tobamovirus*** in **Banana**, 1 tentative ***Betaflexiviridae***, 1 ***Closteroviridae*** and one tentative ***Ilarvirus*** in **Sugarcane** and 1 tentative ***Rhabdoviridae*** or ***Varicosavirus*** in **Sweet potato.**

TASK 7: DEVELOPMENT OF NGS DATA ANALYSIS PIPELINES FOR A DIAGNOSTICS APPLICATION

As decided during a coordination meeting, for siRNA data, a commercial package (CLC Genomics workbench) was used while for 454 pyrosequencing data two tools have been developed: one integrates demultiplexing of reads into individual samples, trimming of tags and low quality sequences; another one, a pipeline, performs de novo assembly of reads and automated annotation of contigs and singletons. The pipeline was presented to partners during the training session organized in the frame of the coordination meeting, which took place in Montpellier in October 2013.

TASK 8: ANALYSIS OF NGS DATA COMING FROM WP2

The bioinformatic analysis of the sequencing data generated by the pilot phase of WP2 (siRNAs, first batch of 454 pyrosequeuncing) has been performed, allowing the identification of novel viruses in **Garlic** (two strains of a ***Foveavirus*** previously identified in Garlic ESTs, 1 ***Luteovirus*** and 2 tentative **Umbraviruses**), in **Sugarcane** (a ***Mastrevirus***, previously identified by partner P3 and 1 ***Closterovirus*** previously identified in ESTs), in Sweet Potato (2 new **Mitoviruses** and novel parts of previously known ***Sweet potato symptomeless mastrevirus***), in Yam (one ***Potexvirus*** previoulsly identified in ESTs, at least one new ***Macluravirus***, 2 or 3 new ***Closteroviridae*** and 1 new ***Flexiviridae***) and in **Vanilla** (a ***Potexvirus*** and a ***Flexiviridae***, probably belonging to the ***Allexivirus***). It has also allowed beginning the comparison of the efficiency of different deep sequencing approaches tested in WP2. The sequences of the novel viruses identified and detection primers based on these sequences have been transferred to project partners who have developed PCR-based detection assays for these novel agents.

D.3 WORK SPECIFIC TO THE COMPANIES (WHERE APPLICABLE)

Company xxx

10 to 20 lines maximum per company. Describe the project activities of each company in the consortium, focusing on the contributions, collaborations and outlooks associated with the project. Specify in particular the industrial or technological application prospects, the economic and commercial potential, prospects for integration in the industrial activity, etc.

Company	Xxx
Author (name + e-mail address)	
…	

D.4 SIGNIFICANT EVENTS AND RESULTS

Write a few lines for each significant event or result. This could be used for public outreach about the project, with the agreement of the project coordinator.

Virus discovery

The bioinformatic analyses of plant EST databases and of the first deep sequencing results generated by WP2 allowed the tentative identification of a total of 24-26 new viruses:
1 potential ***Tobamovirus*** in **Banana**, 2 ***Foveaviruses,*** 1 ***Luteovirus*** and 2 tentative **Umbraviruses** in **Garlic**, 1 ***Mastrevirus***, 1 ***Closteroviridae,*** 1 tentative ***Betaflexiviridae*** and one tentative ***Ilarvirus*** in **Sugarcane**, 2 new **Mitoviruses,** 1 tentative ***Rhabdoviridae*** or ***Varicosavirus*** in **Sweet potato**, 1 ***Potexvirus*** and 1 ***Flexiviridae***, probably belonging to the ***Allexivirus*** in **Vanilla** and 1 or 2 ***Macluravirus,*** 2 or 3 ***Closteroviridae,*** 1 ***Secoviridae****, 2* ***Betaflexiviridae, 1 Luteoviridae,*** *2* ***Potexiruses, 1 Rhabdoviridae*** *and 1* ***Potyvirus*** in **Yam.**

Development and optimization of 10 new PCR-based diagnostic tools.

These tools are now available to all partners.

Training session: metagenomics method transfer

The training session was organized in Montpellier in order to transfer to all partners the so-called "Bordeaux dsRNA purification method". As mentioned above, this transfer will allow performing an interlaboratory validation step (Task 5), which will result in the screening of plant viral biodiversity present within the targeted 1500 plants.

Training session: bioinformatics pipeline transfer

The viral sequences analysis and annotation pipeline developed within WP3 for the analysis of deep sequencing data for the identification of viruses has been presented to partners during the training session organized during the coordination meeting in October 2013 in Montpellier.

D.5 CONSORTIUM MEETINGS (COLLABORATIVE PROJECTS)

List the consortium meetings, physical or by audio or video conference.

Date	Place	Partners present	Subject of the meeting
26-28 March 2012	Madeira	**P1 – INRA Guadeloupe** Claudie PAVIS **P2 – CIRAD Guadeloupe** Pierre-Yves TEYCHENEY **P3 – CIRAD BGPI Montpellier & Guadeloupe** Philippe ROUMAGNAC Denis FILLOUX Jean-Heinrich DAUGROIS **P4 – CIRAD La Réunion** Michel GRISONI **P5 – INRA Bordeaux** Thierry CANDRESSE Armelle MARAIS **P6 – University of Azores CbA** Duarte MENDONCA	Kick-off meeting: - Talks on the general objectives of the project and of the meeting, presentations of the partners, inorder to know each other. - General planning of the project - Detail of the allocation of tasks and contributors in each work package. - Visits of the ISOPlexis facilities, and field crops.

Date	Place	Partners present	Subject of the meeting
		P7 – University of Madeira ISOPlexis Miguel CARVALHO Manhaz KHADEM Teresa SANTOS Emmanuel SILVA	
20 June 2012	Video conference	**P1 – INRA Guadeloupe** Claudie PAVIS **P2 – CIRAD Guadeloupe** Pierre-Yves TEYCHENEY **P3 – CIRAD BGPI Montpellier & Guadeloupe** Philippe ROUMAGNAC Denis FILLOUX Jean-Heinrich DAUGROIS **P4 – CIRAD La Réunion** Michel GRISONI **P5 – INRA Bordeaux** Thierry CANDRESSE **P6 – University of Azores CbA** Artur MACHADO Duarte MENDONCA **P7 – University of Madeira** ISOPlexis Miguel CARVALHO Teresa SANTOS Emmanuel SILVA	Follow-up of the planed tasks.
26 September 2012	Video conference	**P1 – INRA Guadeloupe** Claudie PAVIS Lydiane BONHEUR **P2 – CIRAD Guadeloupe** Pierre-Yves TEYCHENEY **P3 – CIRAD BGPI Montpellier & Guadeloupe** Charlotte JULIAN Denis FILLOUX **P4 – CIRAD La Réunion** Michel GRISONI **P5 – INRA Bordeaux** Thierry CANDRESSE **P6 – University of Azores CbA** Duarte MENDONCA **P7 – University of Madeira** ISOPlexis Miguel CARVALHO Teresa SANTOS Emmanuel SILVA	Follow-up of the planed tasks.
3 April 2013	Video conference	**P1 – INRA Guadeloupe** Claudie PAVIS Lydiane BONHEUR **P2 – CIRAD Guadeloupe** Pierre-Yves TEYCHENEY **P3 – CIRAD BGPI Montpellier & Guadeloupe** JEAN-EHNRI DAUGROIS Denis FILLOUX Charlotte JULIAN Philippe ROUMAGNAC **P4 – CIRAD La Réunion** Michel GRISONI **P5 – INRA Bordeaux** Thierry CANDRESSE S. CONTRERAS ARMELLE MARAIS S. THEIL **P6 – University of Azores CbA** ARTUR MACHADO	Follow-up of the planed tasks.

Date	Place	Partners present	Subject of the meeting
		Duarte MENDONCA **P7 – University of Madeira** ISOPlexis Miguel CARVALHO Emmanuel SILVA	
30 September - 4 October 2013	Montpellier	**P1 – INRA Guadeloupe** Claudie PAVIS Lydiane BONHEUR **P2 – CIRAD Guadeloupe** Pierre-Yves TEYCHENEY **P3 – CIRAD BGPI Montpellier & Guadeloupe** Jean-Henri DAUGROIS Denis FILLOUX Charlotte JULIAN Philippe ROUMAGNAC **P4 – CIRAD La Réunion** Michel GRISONI **P5 – INRA Bordeaux** Thierry CANDRESSE Sandy CONTRERAS Armelle MARAIS Sébastien THEIL **P6 – University of Azores CbA** Duarte MENDONCA **P7 – University of Madeira** ISOPlexis Miguel CARVALHO Emanuel SILVA	Training on Metagenomics and Bioinformatics. Follow-up of the planed tasks.

D.6 COLLABORATION WITH PARTNERS

Maximum 1 page. Describe the work performed in collaboration with the partners of the project, highlighting the added value of the collaboration. Mention the difficulties encountered, if any.

The SafePGR project relies on the idea of a collaborative network, at all levels. It effectively worked like that, and materials and methods have been efficiently shared between all partners.

On the other hand, innovative methods such as bioinformatics and molecular analyses have been developed by P3 and P5, and then transferred to each partner during the interim meeting held in October 2013 in Montpellier.

The valorization of the results in the form of scientific publications is already planned. All partners will be co-authors of most of the publications resulting from project activities.

D.7 DIFFICULTIES ENCOUNTERED AND SOLUTIONS

Maximum 1 page. Any difficulties encountered and the solutions envisaged, e.g.: technical deadlock, service provider default, failure to meet deadlines, budget control. Does the project content need reviewing? Does the project schedule need reviewing?

Technical and financial adjustments

Eight methods were tested in WP2 / Task3 instead of 5 methods as planned in the full proposal of the project. We have chosen to strengthen the scope of Task3 in order to perform a reliable overview of the metagenomics approaches currently available (some of them were not yet known when the proposal was submitted). The end of this task was therefore slightly delayed. Based on thorough comparative analyses of results, 2 methods have been selected and will be used in Task 5: one will be processed overseas and the other one will be processed at UMR BGPI. This choice will lead to a substantial increase of P1 and P3 involvement in Task5. This slight delay and increase in workload led to a 6 month extension of both assistant engineers hired by P1 and P3, in order to fulfill the goals of Task3 and Task5. Extra costs resulting from these extensions, which amount to 22k€, will be covered by P1 and P3 from other funding sources than SafePGR budget

Financial problems encountered by P7

Since the beginning of the project, the Regional government of Madeira has encountered serius financial problems, which prevent it from making any payment of project funds to P7. Therefore, the University of Madeira had to advance funds to P7. Due to this recurrent problem, the University decided to stop all activity of P7 related to the project. Consequently, the partner was not allowed to attend the interim meeting. A solution was found in two days by the Managing Board. P1 got the authorization from the Dean and P3 advanced the funds for covering traveling expenses of P7 (3 500 €).
The team leader from Madeira informed us that the local authorities are expected to release the funds to the University very soon, but we are still waiting for their decision.

Timing of recruitment of non-permanent staff

The planned hiring of a bioinformatician within WP3 was delayed because of difficulties to identify a suitable candidate. The person has now been recruited and will stay until the end of the project. Despite this small problem, no major delays that cannot be dealt with in the frame of the project are identified and we will be able to deliver the final results on time as planned.

D.8 WORK SCHEDULE

Maximum 1 page. Describe the work plan for the rest of the project, stemming from the work already performed.

WP1: Knowledge development on viral diversity

- Remaining indexings will be completed by December 2013 and nucleotide sequences of amplified products will be generated according to the project's schedule, in order to fuel phylogenetic analyses and comparisons between classical molecular and NGS-based diagnosis.
- New primers developed by P3 and P5 for the detection of new virus species will be used for developing detection methods upon availability.
- The catalogue of virus species infecting accessions of partners BRCs will be completed by December 2013.
- Polyvalent diagnostic tools will be made available by January 2015 after verifying their specificity by sequencing.
- Manuscripts of publications describing the characterization of novel viruses will be prepared in collaboration with other projects partners.

WP2: Development of new tools for a global diagnostic of viruses

TASK5: VALIDATION STEP: USE OF METAGENOMICS TO SCREEN PLANT COLLECTIONS

This task was modified during the intermediate meeting that was held in Montpellier in October 2013. All partners reached the following decisions:

- 1536 plants will processed at this stage, including 64 plants from Azores (20 banana, 2 garlic and 42 sweet potato), 740 plants from Guadeloupe (190 banana, 300 sugarcane and 250 yam), 100 from Madeira (36 banana and 64 sweet potato), 322 from Montpellier (200 sugarcane, 50 yam and 72 control sugarcane samples), 273 from Réunion (46 garlic, 19 sweet potato, 200 vanilla and 8 yam).
- A schedule regarding shipments of plants from overseas territories to Montpellier was adopted during the intermediate meeting. Plants from Madeira, Azores, Réunion (yam, sweet potato, and part of vanilla collection), and Guadeloupe (yams) will be sent during October/November 2013, the remaining plants will be sent by mid-December 2013 (Réunion) and putatively by end of January 2014 (Guadeloupe). All 1536 plant samples will be stored at UMR BGPI at -80°C.
- Five positive controls will be sent to all partners, including 3 plants from Montpellier (2 sugarcane and 1 yam), 1 vanilla from Réunion, and 1 tobacco from Bordeaux

- The selected 1536 plants will be processed by all partners using the dsRNA CF11 purification developed by INRA Bordeaux
- All 1536 plants will be processed by BGPI using the viral particle purification developed UMR BGPI
- cDNA obtained using dsRNA purification and first step RT-PCR will be sent from Réunion, Guadeloupe, Madeira and Azores to Montpellier (no later than May-June 2014)
- The final PCR step using tagged primers and cDNA sent from partners will be carried out at Montpellier
- 4 454 Roche plates will be processed and sequencing results are expected along the first semester of 2014.

WP3: Data analysis and bioinformatics

- If new EST data become available for the 6 plant species, these will be screened for evidence of the presence of novel viruses, using the strategies and tools already developed for this task in the project.
- For the novel viruses already identified (from EST screening or the first rounds of deep sequencing data), detection and characterization primers will be designed and transferred to partners involved in WP1 to develop PCR detection assays targeting these novel agents.
- We will further improve the annotation and virus identification pipeline until March-April 2014, when the new 454 sequencing data from the validation phase of WP2 become available
- Then the pipeline will be used, until the end of the project, to identify novel or known viruses present in the partner genetic resources analysed (ca. 1500 plants, two different sequencing strategies). This will allow (1) to further compare and validate NGS indexing techniques, (2) to compare them to PCR-based assays, (3) to identify the range of viruses (virome) present in the partner collections of each of the 6 species targeted by SafePGR.
- Prepare manuscripts of publications describing the methods developed and the results obtained, including the characterization of the novel viruses identified

D.9 DELIVERABLES AND MILESTONES

Reproduce the table of milestones and deliverables provided at the start of the project. Indicate all the deliverables, including any deliverables deleted or added with respect to the initial list.

Green : done, **Black** : to be done, **Orange** : delayed

No.	Designation	Nature*	Date of supply			Partners (underline the responsible partner)
			Initially planned	Re-scheduled	Delivered	
PROJECT MANAGEMENT						
1	M0-1: Website developed	Milestone	01/03/12		01/03/12	P1
2	M0-2: Kick off meeting (Madeira)	Milestone	28/03/12		28/03/12	All P7
3	M0-3: Action plan 1 released	Videoconference report	01/05/12		20/06/12	All P1
4	M0-4: 6 month report released and consortium agreement signed	Milestone	31/07/12		21/08/13	All P1
5	M0-5: Interim report available	Milestone	31/10/13		31/10/13	All P1
6	M0-6: Final report available	Milestone	31/05/15			All P1
7	M0-7: Meeting 2 (Montpellier)		12/09/13	29/09/13	29/09/13	All P3
8	M0-8: Action plan 2 released	Videoconference report	01/07/13		3/04/13	P1
9	M0-9: Meeting 3 (Guadeloupe)		26/01/15			All P1
10	D0-1: A follow-up project website	Website	1/03/12		1/03/12	P1

No.	Designation	Nature*	Date of supply			Partners (underline the responsible partner)
			Initially planned	Re-scheduled	Delivered	
11	D0-2: Communication supports (talks, web pages, press release)	Pages and files in the website	Throughout the project			All P1
12	D0-3: The consortium agreement	Document	31/08/12		21/06/12	All P1
13	D0-5: The kick-off meeting report	Report	31/08/12		20/07/13	All P1
14	D0-6: The mid-report	Report	31/10/13		31/10/13	All P1
15	D0-7: The final report	Report	30/04/15			All P1
16	D0-8: Joint scientific and technical publications	Publications	Throughout the project			All
VIRAL DIVERSITY						
17	Staff for coordination of analyses in Guadeloupe hired	Milestone	1/09/12		3/09/12	P1
18	M1-1: Plant samples analyzed and sequences generated	Milestone	15/09/13	31/12/13		P1, P2, P4, P5, P6, P7
19	D1-1: A catalogue of viruses infecting accessions of BRCs	Files in the website	31/03/14			P1, P2, P4, P5, P6, P7
20	M1-2:Polyvalent tools available, specificity of diagnostic tools verified by sequencing	Milestone	31/01/15			P1, P2, P4, P5, P6, P7
21	D1-2: Sequences comparisons and phylogenetic analyses of identified viruses	Data	31/01/15			P1, P2, P3, P4
22	D1-3: Optimized protocols for viral diagnostic	Files in the website	Throughout the project			P1, P2, P4, P5
METAGENOMICS						
23	Staff for metagenomics development hired	Milestone	01/05/12		01/05/12	P3
24	M2-1: metagenomic approach selected	Milestone	15/09/13		30/09/13	P3
25	M2-2: metagenomic approach transferred	Milestone	30/09/13		30/09/13	All P3
26	D2-1: Protocols for obtaining plant viromes	Files on the website	15/09/14		3/10/13	P3
27	D2-2: Training session in the use of metagenomics	Training session	20/09/14		3/10/13	P3
28	M2-3: plants from the 6 target crops processed	Milestone	31/01/15			P3
29	D2-3: Inventory of viruses present in the BRCs	Milestone	End of the project			All P3
BIO-INFORMATICS						
30	M3-1: screening of ESTs for viral sequences completed	Milestone	1/10/12		1/10/12	P3, P5
31	D3-1: Results of the screening of crops ESTs for viral sequences	Data	1/10/12		1/10/12	P3, P5
32	D3-2: Sequence information on novel viral agents to WP 1	Data	1/10/12		1/10/12	P3, P5
33	Staff for informatic development hired	Milestone	?	2/04/13	2/04/13	P5
34	M3-2: pipelines established	Software	31/3/13	20/9/13	20/9/13	P5
35	D3-3: Processing and annotation pipelines for 454 pyrosequencing and Illumina siRNA reads	Data	31/3/13	20/9/13	20/9/13	P5
36	M3-3: analysis of the NGS data from WP 2 / task 3 completed	Data	30/6/13	20/9/13	20/9/13	P5
37	D3-4: Training of partners to the use of pipelines	Training session	20/9/13		3/10/13	P5
38	D3-5: Results of analysis of all NGS data from the validation step of WP2	Data	31/01/15			P5
39	M3-4: analysis of the sequences data from WP2/task 5 completed	Data	31/1/15			P5

** milestone, report, software, prototype, data, etc.*

D.10 FREE COMMENTS

Comments from the coordinator

General comments at the coordinator's discretion, e.g. on the state of project progress, interaction between the partners, or the relationship with the funding bodies.

The coordinator is happy: the project already generated significant data and led to the development of new techniques and improved diagnostic tools, which have been successfully transferred to all partners. This will allow continuing work in good conditions.

The collaborative work is carried out efficiently and in a friendly atmosphere. Communication flows between partners is very satisfactory during videoconferences, the two coordination meetings and through the project's website and the shared repertories.

Work package leaders play their role efficiently; they have great qualities for organizing the tasks and sharing knowledge and tools.

Some administrative and technical problems occurred. All were solved except crucial funding problems encountered by P7, for which we hope for a happy ending.

Question(s) posed to JCS and/or to the funding agencies

Questions posed, if any

Funding problems of P7 requires a quick answer from local authorities in Madeira.

E PROJECT VALORISATION AND IMPACT

This section groups elements accrued since the beginning of the project, which will be monitored as its proceeds and taken up in the final project review.

E.1 PUBLICATIONS AND COMMUNICATIONS

Indicate the publications resulting from the project, using the usual standards for the field. If the publication is accessible online, indicate the website address[1]

List of collaborative publications with partner number		
Scientific	**Peer-reviewed journals**	
	Books or chapters in books	
	Communications (conferences)	
Outreach initiatives	**Popularization articles**	
	Popularization conferences	1. The Guadeloupe germplasm repository. Its role in viral diagnostic & sanitation. C. Pavis1, F. Gamiette1, M. Umber1, D. Roques1, M. Boisseau1, F. Nuissier1, D. Pétro1, T. Candresse5, P. Roumagnac3, P.-Y. Teycheney2 (2013). Poster presented at the 'Yams 2013' Meeting – 3-6 October 2013 – Accra, Ghana.
	Others	

List of single-partner publications		
Scientific	**Peer-reviewed journals**	
	Books or chapters in books	
	Communications (conferences)	
Outreach initiatives	**Popularization articles**	
	Popularization conferences	
	Others	

[1] . *For ANR funded partners*, The ANR encourages the publishing of articles stemming from the projects it funds, in compliance with the rights of the co-authors and editors, in the open multidisciplinary archive HAL: http://hal.archives-ouvertes.fr/

E.2 OTHER VALORISATION FACTORS

The valorisation factors are spin-offs other than publications. The following shall be detailed in particular:

- *national and international patents, licences, and other elements of intellectual property resulting from the project.*
- *software and any other prototype*
- *standardization actions*
- *launching of product or service, new project, contract, etc.*
- *development of a new partnership,*
- *creation of a platform available to a community*
- *company creation, spin-off companies, fund-raising*
- *others (international opening, etc.).*

In this table, give details of the national and international patents, licences, and other valorisation factors resulting from the project, the know-how, any other spin-offs from the project, any partnerships, etc.. See those announced in the technical appendix in particular.

List of factors. Indicate the titles, dates and comments	
International patents obtained	
International patents pending	
National patents obtained	
National patents pending	
Operating licences (obtained / transferred)	
Company creations or spin-offs	
New collaborative projects	
Scientific symposiums	
Others (software**)**	1. VirAnnote : Pipeline for assembly, annotation of metagenomics sequences. Software adaptated from TriAnnote (2013, S. Contreras5 and S. Theil5).

E.3 COMPETITIVENESS CLUSTERS (FOR ANR FUNDED LABELLED PROJECTS ONLY)

For projects labelled by one or more competitiveness clusters.

Project collaboration with the cluster(s) that labelled it

What collaborations have there been between your project and the competitiveness cluster(s) that labelled it?
Detail the activities carried out by the public laboratories using the complementary funding granted on account of the labelling. Indicate in particular the partners involved and the collaborative work conducted with the cluster(s).

…

E.4 PERSONNEL RECRUITED ON FIXED-TERM CONTRACTS (EXCLUDING INTERNS)

This table summarizes project recruitment of non-permanent personnel on fixed-term contracts or equivalent. Fill out one line per person hired for the project when the hiring has been partially or entirely financed and the contribution to the project has lasted at least 3 months, all employment contracts combined, subject to the condition that the Grant may only represent a portion of the person's remuneration over the duration of his or her participation in the project. Interns who have an internship agreement with an educational establishment must not be mentioned.

Additional information on the professional future of the persons concerned will be requested at the end of the project. Their career path may be tracked for up to 5 years after the end of the project.

Identification				**Before recruitment for the project**			**Recruitment for the project**			
Surname and first name	Sex M/F	E-mail address (1)	Date of last contact (if contract is finished)	Last diploma obtained at time of recruitment	Place of studies (France, EU, outside EU)	Prior professional experience (years)	Partner who hired the person	Position in the project (2)	Date of recruitment	Duration of missions (months) (3)
Lydiane BONHEUR	F	lydiane.bonheur@yahoo.fr		MSc Microbial diagnostic	France	2	P1	Engineer	3/09/12	18
Charlotte JULIAN	F	charlotte.julian@supagro.inra.fr		MSc Molecular Biology technics	France	0	P3	Assistant-Engineer	01/05/2012	24
Sandy CONTRERAS	F	sandy.contreras@bordeaux.inra.fr	10/2011 to 3/2013	MsC Bioinformatics	France	1.5	P5	Engineer	2/4/2013	22
Sara ROCHA	F	sararocha@hotmail.com	01/2010 to 12/2012	MsC Agricultural Sciences	Portugal	2	P6	Research Grant	1/11/2012	12
Emanuel SILVA	M	emasil2008@gmail.com	15/09/2013	Pre-Bolonha Graduation Biology	Portugal	5	P7	Technician	15/09/2013	18

Table filling-out aid

(1) E-mail address: indicate an e-mail address that will be as long-lasting as possible
2) Position in the project: post-doctoral, doctoral student, engineer or equivalent, technician, individual contractor, other (specify)
(3) Duration of missions: indicate the total duration in months of the project missions carried out or planned (including missions not financed).

The personal information collected shall undergo computerized data processing for the sole needs of the depersonalized study on the professional future of the people recruited for ANR projects. The information will not be transferred in any way and will be kept by the ANR for a maximum period of 5 years after the end of the project concerned. Pursuant to the Act No. 78-17 of 6 January 1978, amended, relative to Data Processing, Files and Personal Privacy Protection, the persons concerned have a right of access to, correction and deletion of their personal data. The persons concerned shall be informed directly of this right when their contact details are filled out. They can exercise this right by contacting the ANR (http://www.agence-nationale-recherche.fr/Contact).

E.5 FINANCIAL STATUS

Give an indicative account of the grant budgets spent by the partners. Indicate how this complies with the forecasts and explain any significant divergences.

Name of partner	Grant budget spent (in %)	Comments (if necessary)
1 - INRA ASTRO Guadeloupe	49%	Compliant with the forecasts.
2 - CIRAD AGAP Guadeloupe	43%	Compliant with the forecasts.
3 - CIRAD BGPI Montpellier	35%	Compliant with the forecasts.
4 - CIRAD PVBMT La Réunion	59%	Compliant with the forecasts.
5 - INRA BFP Bordeaux	17,7%	A very large part of the budget concerns salary of a bioinformatician whose hiring was delayed (difficulty in finding suitable candidate) but who will work to the end of the project so that budget will ultimately been used as planned.
6 - University of Açores CBA-UAc	51,8%	Compliant with the forecasts.
7 - University of Madeira Isoplexis	73,3%	The local authorities have not released the funds to the University of Madeira. Nevertheless, the University made financial advances to the team, but the context is very difficult for the team.

F APPENDICES (IF NECESSARY)