PROJECT PERIODIC REPORT

Grant Agreement number:	222716
Project acronym:	SMARTCELL
Project title:	Rational Design of Plant Systems for Sustainable Generation of Value-Added Industrial Products
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Periodic report:	1st ☐ 2nd X 3rd ☐ 4th ☐
Period covered:	from 1st July, 2010 to 31th December 2011
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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

1 PUBLISHABLE SUMMARY

1.1 A summary description of project context and objectives

The overall scientific objective is to gain a thorough understanding and exploitation of the extraordinary complexity of the biochemical capacity of plants. This can be done by means of the developed enabling technologies which facilitate rational pathway engineering of plants and plant cells towards desired secondary metabolites systematically in a predictable and reproducible way. These enabling technologies include multigene transformation technologies and developing metabolomics and bioinformatics tools to handle huge data sets which already have been and will be generated in the course of the project. Important element is also to establish and optimize large-scale production platforms for plants and plant cell cultures. The specific aims for the second reporting period were:

- to continue collecting candidate genes for the fast track transformation experiments
- to sequence *Catharanthus* plants and cell to obtain new candidate genes for the advanced track transformation experiments (deep sequencing)
- to validate new enzymatic and transporter genes as well as potential transcription factors in the isoprenoid and TIA pathway
- to develop multigene transformation techniques for tobacco plant and hairy roots with four, validated fast track candidate genes
- to establish relevant analytical platforms for targeted and non-targeted chemical analyses
- to evaluate and optimise the production platforms for tobacco and *Catharanthus*
- to establish scale-up and down-stream systems for plants and plant cells
- to standardise data formats and data processing and to establish experimental data repository with real experimental data
- to continue a widespread dissemination of results
- to ensure the proper handling of IPR and finish an IP database which will help to inform FTO strategies for SmartCell
- to organize training schools for post-docs and PhD students

1.2 A description of the work during the second reporting period

In general, the SmartCell project has advanced according to the work plan with some minor deviations which are explained and justified in part 2 of this periodic report. The collaboration between partners has been active and fruitful. Three project meetings, in Aachen (Germany), Wädenswil (Switzerland) and Strasbourg (France) and three training schools have been organized. The mini-symposium which was organized in connection to the Strasboug meeting was an excellent opportunity to connect SmartCell partners with other distinguished scientists in the field e.g. to Canada.

The terpenoid pathway engineering is divided in two themes: a fast track leading to 10-hydroxygeraniol and an advanced track leading to secologanin. The largest effort in gene discovery during this second period of reporting has been the sequencing experiments. Illumina Deep sequencing of the transcriptome of *Catharanthus roseus* material was carried out. The deep sequencing strategy was designed such that both *de novo* sequence assembly and transcript counting will be possible. The former facilitates subsequent cloning and support proteomics analysis and the latter enables comparative mining of gene expression which in turn allows selecting candidate genes for the advanced track. Deep sequencing data for the cell samples have been now delivered from the subcontractor

FASTERIS and in silico analysis has been started. A total of 416'942'482 reads were sequenced corresponding to 41'694'248'200 bases. This work is now on-going.

A large set of suitable fast track genes leading to 10-hydroxygeraniol has been identified and validated. The most promising genes have been transformed into tobacco plant in different combinations by novel multigene particle bombardment transformation techniques. Additionally these genes have been inserted into tobacco and *Catharanthus via Agrobacterium rhizogenes* to lead to hairy root cultures. A large number of transformants have been analysed at molecular, proteomic and metabolic level. Besides of discovering functional genes the Consortium has studied the potential function of regulatory and transporter genes. To modify the terpenoid structures also some genes coding for terpenoid decorating enzymes have been discovered and functionally tested.

The SmartCell partners have also developed several analytical platforms to profile terpenoid formation and content in different control and transgenic plants and plant cell and hairy root cultures. Chromatographic (UPLC-MS, GC-MS) and spectrometric (NMR) methods as well as basic methods for flux analysis are now available and fully functional for targeted and non-targeted use of analyzing terpenoid end products and their intermediates. The Consortium has made also a lot of efforts synthesizing some of the hypothetical intermediate compounds of the TIA pathway to be used in functional validation tests as well as in analytical experiments. To be able to handle huge data sets which are and will still be generated during the project the data formats and data processing have been standardized and the experimental data repository has been established. The database for repository of secondary metabolite data has also been set and is fully functional now.

Substantial efforts have been made by the academic and industrial partners torwards large scale production of plants and cultivated plant cells and hairy roots in various types of bioreactors. Novel disposable *e.g.* wave-type bioreactors have been tested as well as conventional bioreactors. Technical transfer of the novel bioreactor technology has been done between two partners. Cryopreservation techniques for different cell lines are still on-going and have turned out to be more demanding than anticipated in the beginning of the project. The case study component *i.e.* manufacturing valuable terpenoids, 10-hydroxygeraniol in an optimized large-scale system gives SmartCell a unique opportunity to directly make the transition from fundamental science to application. This part of the project will only start on month 37.

The Consortium has performed an assessment of the commercial relevance for the target compounds to be manufactured in a large scale. So far, no exploitable results have been identified by the Exploitation Committee. However, chances are that exploitable results might be obtained relatively soon. The most likely exploitable results would be novel compounds and/or new genes identified in the deep sequencing experiments.

SmartCell has activiely been involved in dissemination of the results of the project. A large number of scientific papers, public articles, interviews and other disseminations have been published. The scientists have also been active in giving oral presentations both in scientific congresses and different educational events (see in part 2 more in detail).

1.3 The expected final results and their potential impact and use

The SmartCell project addresses specific issues for using plants as green factories for sustainable non-food products. It focuses on developing enabling technologies for plant-based products in rationally engineered plants and plant cells, especially for pharmaceuticals and speciality chemicals. This in turn will impact environmental improvement and

sustainability, economic advancement and international competitiveness. Especially plant genomics, proteomics and metabolomics are included in SmartCell to improve the productivity and composition of plant raw materials to known and new high-value added products. The expected impact for Europe is to exponentially increase our knowledge on molecular, biochemical, genetic and physiological aspects of plant metabolic pathways at the systems level for the rational, efficient and sustainable production of important compounds for industrial use. A major component of this exercise is the generation, protection and exploitation of Intellectual Property as well as taking into account the regulatory and biosafety issues.

The cutting edge of basic plant research is rapidly evolving from understanding the function of single genes to studying networks of genes that control complex biological processes such as production of metabolites in plant cells at the systems level. The demonstration component (WP7), which will start on month 37, i.e. manufacturing terpenoid end product, 10-hydroxygeraniol, in an optimised large-scale system, gives SmartCell a unique opportunity to embark on the transition from the fundamental science to applications, thus validating, within the lifetime of the project concepts, tools, tangible material and resources, and also IP and regulatory/biosafety aspects. After the project a “real life” genebank, metabolomics and pathway map databases as well as compound libarary and cell culture collection, all created in the project, will be made available to the wider academic and industrial communities. Direct involvement of SMEs and large end-user companies in SmartCell will support the competitiveness of European industries, specifically those dealing with industrial applications of new technologies, processes and products.

1.4 The address of the project website

www.smart-cell.org