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
Funding Scheme:	Large collaborative project
Date of latest version of Annex I against which the assessment will be made:	14th July, 2012
Periodic report:	1st ☐ 2nd ☐ 3rd X 4th ☐
Period covered:	from 1st January, 2012 to 31th December 2012
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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 of SmartCell is to gain a comprehensive understanding of the extraordinarily complex biochemical capacity of plants, leading to the development of enabling technologies that will facilitate rational pathway engineering in plants and plant cells so that valuable secondary metabolites can be produced systematically in a predictable and reproducible manner. These enabling technologies include platforms for multigene transformation and metabolomics, as well as bioinformatics tools that can handle the large data sets generated during the project. We also aim to establish and optimize large-scale production platforms for plants and plant cell cultures prior to demonstration activities that will be carried out in the last months of the project. The specific aims for the third reporting period were:

- to continue validating the best subset(s) of genes and subcellular localization strategies for the optimised production of 10-hydroxygeraniol (fast track)
- to analyse the sequence data (deep sequencing) of *Catharanthus roseus* plants and cells to identify new candidate genes for advanced track transformation experiments
- to validate new genes encoding enzymes, transporters and transcription factors in the isoprenoid and TIA pathways
- to optimise the production platforms based on tobacco and *C. roseus*
- to establish scaled-up production and downstream processing systems for plants and plant cells to be utilised in our demonstration activities (WP7)
- to establish a platform for experimental data repository using real experimental data
- to ensure the proper handling of IPR using our IP database and FTO analysis
- to disseminate the results in terms of scientific publications and also general articles

1.2 A description of the work during the third reporting period

The SmartCell project has progressed according to the work plan during the third reporting period, with some minor deviations explained and justified in Section 2 of the current periodic report. The collaboration between partners has been active and fruitful. The reporting period included two project meetings in Lleida (Spain) and Saariselkä (Finland).

The terpenoid pathway engineering component of the project is divided in two themes: a fast track leading to 10-hydroxygeraniol and an advanced track leading to secologanin. Whereas we focused on establishing the sequencing platform and generating data during the previous reporting period, in this third reporting period we focused on validating the sequencing results greatly accelerating the amount of useful data. RNA sequences from elicited/transgenic cell cultures and plant tissues were assembled in a database, which was searched to identify genes co-expressed with those already known to be active in the early iridoid pathway. This information was integrated with leaf epidermis and mesophyll proteomic data, revealing a set of promising candidates for the different predicted steps in the secologanin pathway. Some prime candidates have been selected for cloning and expression in *Escherichia coli* (soluble proteins) and yeast (membrane-anchored cytochrome P450 enzymes). A set of 15 enzymes has thus been expressed and screened for activity against predicted substrates. With the exception of one step characterized by a competing group (Geu-Flores et al., (2012) Nature 492: 138-142), all pathway enzymes were successfully characterized in the SmartCell screen and the complete pathway is now validated. Initial attempts

to reconstruct the pathway in *Nicotiana benthamiana* have been encouraging. The expression context of the early iridoid pathway genes has been optimised in *N. benthamiana*, and we are now making progress towards the expression of the same metabolic cassette in stably transformed tobacco and *C. roseus* plants. RNA sequencing and co-expression analysis also led to a set of 20 candidate transcription factors and 28 transporters that are currently under investigation.

The SmartCell partners have created a large number of transformants carrying diverse combinations of genes so that their role in the TIA pathway can be characterized using proteomics and metabolomics approaches (LC-MS, GC-MS and NMR). New bioinformatics tools have been developed using several linear and non-linear methods so that spectral differences between groups of tobacco and *C. roseus* samples with different genetic modifications and growth conditions can be detected, characterised and visualised. The results show that the best insight was gained using an interactive procedure, whereby the different detection methods and smooth threshold values could be visually explored by researchers before committing and reporting their conclusions. A cloud-based application for inspecting the project-related NMR data was implemented with using a tool stack developed by VTT to combine the bioinformatics methods with easy-to-use web-based interfaces.

Different tobacco production platforms including whole plants grown in greenhouses, *in vitro* hairy roots and suspension cell cultures have been evaluated to optimise geraniol production. We found that hairy root and suspension cell cultures provide the highest product yields in the shortest time. Furthermore, both systems can be cultivated under controlled conditions in bioreactors enabling the reproducible and homogenous production of geraniol. We have optimised biomass accumulation and product yields in whole plants, hairy roots and suspension cells even further by improving the nutritional and physical culture conditions in factorial design experiments. Fermentation processes using plant tissue and cell cultures has also been carried out using disposable bioreactors to allow larger-scale production. Our initial purification strategies produce geraniol with purity in excess of 99%.

Our final aim is to demonstrate that our optimized plant systems and procedures can be used to manufacture approximately 1 g of geraniol. Tobacco hairy roots and suspension cells already producing up to 200 µg geraniol per gram dry weight of biomass were chosen as potential manufacturing platforms. We found that stirred and orbitally-shaken bioreactors were the most suitable bioreactors for suspension cells, whereas wave-mixed bioreactors were preferable for hairy roots. After testing and validating different parameters, we achieved an average yield of 8 g geraniol per litre of hairy root culture.

Thus far, no exploitable results have been identified by the Exploitation Committee. However, it is likely that exploitable results will be achieved now the relevant genes have been validated. The most likely exploitable results would be novel compounds and/or newly-discovered genes validated based on sequencing data and functional characterization.

SmartCell has actively been involved in dissemination of the results of the project. A large number of original research articles, reviews, popular articles, interviews and other dissemination activities have been published, and SmartCell researchers have been presented many posters and talks at scientific congresses and educational events.

1.3 The expected final results and their potential impact and use

The SmartCell project addresses the use of plants as green factories for sustainable non-food products. It focuses on the development of enabling technologies allowing the manufacture of plant-based products in rationally-engineered plants and plant cells, especially pharmaceuticals and speciality chemicals. This will have a positive impact on the environment and will promote sustainable economic improvement and international competitiveness. The inclusion of plant genomics, proteomics and metabolomics in SmartCell will significantly improve the productivity and composition of plant raw materials in terms of known and new high-value added products. The expected impact in Europe is the significant gain of knowledge concerning the molecular, biochemical, genetic and physiological aspects of plant metabolic pathways at the systems level allowing the rational, efficient and sustainable production of important industrial compounds. A major component of this exercise is the generation, protection and exploitation of intellectual property and the integration of regulatory and biosafety issues.

The cutting edge of basic plant research is rapidly evolving from understanding the function of individual genes to studying networks of genes that control complex systems-level biological processes such as the production of metabolites. The SmartCell demonstration component (WP7) started in month 42, and involves the manufacture of a terpenoid end product (10-hydroxygeraniol) in an optimised large-scale system. This provides SmartCell with a unique opportunity to embark on the transition from fundamental science to applications, thus validating (within the lifetime of the project) concepts, tools, tangible materials, resources, intellectual property and regulatory/biosafety issues that affect the commercial manufacture of value-added products from plants. When the project is complete, the gene bank, metabolomics/pathway database, compound library and cell culture collection developed during the project will be made available to the wider academic and industrial communities. The direct involvement of companies in SmartCell will support the competitiveness of European industries, specifically those dealing with the industrial application of new technologies, processes and products.

1.4 The address of the project public website

http://www.smart-cell.org