European Cooperation in Science and Technology
- COST -

Secretariat

Brussels, 16 December 2010

COST 4197/10

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1006: Plant Metabolic Engineering for High Value Products

Delegations will find attached the Memorandum of Understanding for COST Action FA1006 as approved by the COST Committee of Senior Officials (CSO) at its 180th meeting on 1 December 2010.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action FA1006
PLANT METABOLIC ENGINEERING FOR HIGH VALUE PRODUCTS

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4159/10 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is establish a multi-disciplinary network to define and develop rational design strategies to produce known and novel plant natural products of pharmaceutical and industrial interest in a sustainable, economical, and ecological way.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 72 million in 2010 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

A significant amount of knowledge has been gained during the last decades about the biosynthetic capacity of plants and the pathways leading to the formation of plant natural products (PNPs), many of which are of high relevance as pharmaceuticals or fine chemicals for industries. To fully exploit the capacity of engineering plants for the production of high value PNPs this COST Action will support and enhance a Pan-European network which will amalgamate resources, define target pathways and prioritize compounds, disseminate novel technologies and applications, set standards for computational support, and develop synthetic approaches in plant metabolic engineering. Due to its multidisciplinary approach this Action will initiate a European network of experienced as well as early stage researchers which will serve as a base for future research collaborations. Outcomes will help guiding researchers in the design of plants as production host and provide building blocks for pathway engineering. The dialog established within the research community will involve key players in industry, stakeholders, and policy makers guaranteeing the highest momentum for the European research sector as well as the public.

Keywords: Plant secondary metabolism, plant natural products, plant metabolic engineering, synthetic plant biology, medicinal plants

B. BACKGROUND

B.1 General background

Since the beginning of our historical records humans have used plants, not only as food, commodity for clothes, buildings and vehicles, but also as resources for small molecules with highly diverse functions. These so-called secondary metabolites or “plant natural products” (PNPs) serve as nutrients, colorants, flavors, fragrances, cosmetics, crop protectants and most prominently as medicines and therefore they have an unprecedented importance in our lives. However, their availability is often limited which makes them valuable and generates a need for alternative sources.

Although chemical synthesis has advanced tremendously, for complex PNPs it is often uneconomically. Many important PNPs can be obtained from their plant sources in sufficient quantities only with great difficult; in addition a large infrastructure has to be built (e.g. artemisinine, paclitaxel, vincristine, podophyllotoxin). In other cases, especially for substances for non-pharmaceutical use and thus lower prices, the large expensive infrastructure limits their use (e.g. insecticides like natural pyrethroids are only suitable for high priced private consumer applications).

The biochemical and molecular mechanisms underlying the formation of PNPs is highly complex and as diverse as the chemical structures of PNPs found in nature in a vast arry of plant species are. The task of isolation and identification of novel PNPs in the plant kingdom and the development of analytical methods for their determination was initiated more than 200 years ago with the purification and description of morphine. During the last decades, the elucidation of the genetic background and biochemical requirements for PNP production has evolved tremendously. Although our present understanding of PNP formation and its regulation is still far from being complete, the current *status quo* already enables us to envisage the next logical step, the targeted modification of plant pathways to modify and enhance PNP production in their natural or heterologous hosts, or to produce modified PNPs adapted to specific requirements.

It would be an enormous advantage if the biosynthesis of complex PNPs could be enhanced in - or even decoupled from - their natural sources, to provide unlimited resources for valuable drugs and chemicals. Pasting the genetic blueprint for a biosynthetic pathway into a fermentable host like bacteria or yeast or -even more striking- into a high yielding and fast growing plant will provide an unprecedented advantage for the production of valuable PNPs. In the same instance, modifications to the original pathway might lead to increased yields or modified PNP with improved properties. To pave the way for this objective it is of tremendous importance to bring together researchers from the highly diverse branches of PNP research, to centralize and unify scattered resources, to identify compounds of high interest, to evaluate the current possibilities for the modification of their biosynthetic pathways, and finally to define solutions for reaching the goal of developing designer fine chemical producing or medicinal plants. Beside the existing national research activities, as well as the European research frameworks (which usually cover only small fractions of this area, e.g. defined product classes like bacterial polyketides, terpenoids or model plants), COST offers the unique opportunity to generate a higher-order network under the topic “plant natural products”.

This integrative approach has the potential to define new targets and to develop means for overcoming bottlenecks in the provision of unlimited resources for valuable PNPs. This will be a starting point for participants in this COST Action which can then apply for future funding in traditional ways to implement the developed strategies.

B.2 Current state of knowledge

Today, there is still a significant number of high value products, especially drugs and pharmaceuticals, which are obtained from natural sources due to lack of alternative resources (e.g. no chemical synthesis feasible). Scopolamine, an alkaloid from solanaceous plants, serves as a precursor for the production of N-butylscopolaminium bromide, the active ingredient of an antispasmodic medicine with a large market share. Other than for scopolamine, which can be obtained from fast growing, high-yielding plants, expanded and extended use of PNPs commonly is limited by their availability. Numerous PNPs of much higher impact can only be extracted in minute amounts from their natural sources so far, or are constituents of endangered and/or slow-growing species. This ultimately makes them a scarce resource and carries high cost burdens for our health care systems. One prominent example is the anticancer drug vincristine from Madagascar periwinkle which costs up to 1000 Euro per gram and needs to be extracted from 2 tons of the crude drug. Further instances are the antimalarial drug artemisinine, the cytostatic podophyllotoxin, the antitumoral paclitaxel, as well as various mono- and sesquiterpenoids serving as fragrances (like patchulol).

To improve PNP production, the first step to undertake is widening our understanding of the biosynthetic and genetic processes underlying their formation. During the last decades European researchers were at the forefront regarding the identification of structurally diverse PNPs or their precursors, regarding the characterization of enzymes involved in specific pathways and also regarding the regulation of PNP formation. The focus on specific plant systems, on defined substances or even on specific enzymatic reactions has generated a patchwork of research foci and expertise which could only partially be unified by concerted research initiatives like European Framework Programs and interdisciplinary approaches. Significant knowledge has been accumulated concerning a number of important PNP pathways: the biosynthesis of the antimicrobial

berberine has been characterized from the primary metabolite precursor through the end product; a great deal is known about the biosynthesis and regulation of the anticancer alkaloids vinblastine and vincristine; there is extensive molecular information about many important parts of the phenylpropanoid pathway, including the antioxidant flavonoids, with several genetic manipulation success stories; much is known about terpenoid metabolism, which also offers already several success stories of metabolic engineering, like the improvement of the ratio of the beneficial tocopherol isomers (vitamin E) in oilseeds, or the improvement of the levels of carotenoids (namely the vitamin A precursor beta-carotene) in rice and corn.

Currently, there are a few examples in which the production of high value PNP is translocated to novel host organisms and efforts to understand the basis of PNP formation in their natural hosts on a systemic level are under way. However, these efforts are largely non-European, taking place in the USA or Japan. For example, the genetics and molecular biology of the pathway leading to the precursor (artemisinic acid) of the antimalaria drug artemisinine have been investigated in detail and first attempts were successful to paste a similar pathway in yeast.

Beside these few initial success stories little progress on a large scale basis has been achieved regarding the metabolic engineering of other highly interesting pathways to significantly enhance the yield of the desired products or to engineer these pathways in heterologous hosts. Essential steps in multiple-step pathways are still elusive and await further investigation or the definition and testing of substitutes (heterologous enzymes) to by-pass tedious identification processes. The influences of both, precursor flux and transcription factors as well as the pathway compartmentalization on a cellular and organism level (e.g. tissue differentiation, developmental stage) are also still far from being understood.

To bring PNP research to the next level, meaning to generate designer organisms which are enabled to produce higher quantities of desired products or even to generate novel structures and compounds, a concerted action of individual research efforts is aimed for.

B.3 Reasons for the Action

The Action will not only provide an interdisciplinary framework for European scientists involved in the greater field of PNP research. It will moreover define a status-quo of the highly diverse field regarding plants, compounds, and pathways with the potential for metabolic engineering. This objective is far beyond the scope of individual researchers or small groups usually participating in defined research consortia. The Action will also integrate end-users (e.g. applied scientists and chemical engineers of pharmaceutical companies) who will define current and novel needs and target structures, synthetic biologists, and computational biologists/chemists who contribute novel approaches for plant metabolic engineering, and phytochemists, plant molecular biologists and physiologists who provide the tools for the aimed goal. This pan-European network will provide the unique opportunity of a holistic approach to PNP formation with the aim of its rational redesign to provide non-restricted resources of valuable pharmaceuticals and fine chemicals. This transformation of scientific knowledge and inventions into applied techniques and products matches the "European strategy for Life Science and Biotechnology" (COM(2002) 27).

The objectives are:

- To generate a highly interdisciplinary platform of European scientists and research institutions.
- Training, education, and motivation of skilled young scientists in the diverse area to build the basis for the further interdisciplinary development of the field of PNP research.
- To define a *status quo* of scattered research on PNP biosynthesis and its modification and to point out future avenues of research.
- To bring together European scientists, finding new intersections of research areas, and ultimately assemble strong sub-teams enabled to propose new collaborative research programs.
- To transfer results regarding plant metabolic engineering to an application level which could benefit companies as well as patients and therefore the European society on its way to eco-efficient and bio-based production of high value products

The expected results are:

- A long lasting, multidisciplinary group of scientists, which will act far beyond the duration of the Action and promote PNP research and metabolic engineering in Europe.
- A core group of young motivated scientists with multidisciplinary training to further develop PNP research.
- Development of integrated (from gene-to-product and vice versa) PNP knowledge centers and corresponding databases for ontogenic data access. Today´s databases are rigid and bottom up constructed. Information is available for the gene and its product, but starting from the metabolite, i.e. the desired final product, and to go upstream is still problematic.
- The development of a roadmap by which new research strategies could be defined.
- The definition of new target structures of high interest for new research foci.

The specific means are:

- Promoting the personal contact between researchers from different fields.
- Forming disciplinary and interdisciplinary subgroups working either on data mining and its evaluation or as “think-tanks” for the development of new strategies.
- Involving young scientists not only in training but also in conceptual work to profit from their unbiased perspective and approaches which has a great potential to speed development.
- Forming a subgroup of a learned society to establish a long-lasting promotion of research in plant secondary metabolism engineering.
- Developing conceptual publications to have the greatest impact and to attract European researchers to the group.

B.4 Complementarity with other research programmes

Europe has a long standing history in the exploration of PNPs and their biosynthetic pathways. However, the application of modern biotechnological methods to this field is still in its infancy. Currently, many leading groups in plant metabolic engineering are mainly located in the USA or Japan. A recently launched European consortium under FP7 (SmartCell) provides a first nucleus for a concerted European research effort towards plant metabolic engineering as it defines its mission as rational design of plant systems for the sustainable generation of industrial products.
In addition, the FP7 consortium Metapro (www.isoprenoid.com) is composed of experienced and young researchers focused on the development new tools and strategies for the optimization of secondary metabolites *in planta*.

Both, SmartCell and Metapro - although pursuing specific objectives concerning plant metabolic engineering - actually focus on specific targets (monoterpene iridoid pathway / tobacco / *Catharanthus* and astaxanthin / crocin / Solanaceae host platforms, respectively). The COST Action will be complementary and synergistic in that it aims to identify novel targets, processes, and tools to develop a base for broader plant metabolic engineering research in Europe. Moreover, this Action, based on its products, could add to the COST Action CM0804 (Chemical Biology with natural products) which deals predominantly with the chemical synthesis of NPs mostly of microorganism origin.

Another COST Action termed Molecular Farming (FA0804) has certain interaction potentials (e.g. generation of transgenic plants, information on plant promoters, sub-cellular targeting), but its focus is completely different in that it deals with protein molecules of pharmaceutical interest, while the here described Action is solely devoted to plant natural products which are by definition small molecules produced by complex biosynthetic pathways.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objective of the Action is cross-linking within a multi-disciplinary network European scientists with diverse expertise on PNP chemistry, plant metabolic engineering, plant enzymology, systems biology and computational biology, and chemistry to define and develop rational design strategies to produce known and novel PNP of pharmaceutical and industrial interest in a sustainable, economical, and ecological way.

C.2 Secondary objectives

- Generate a map of plant secondary metabolic pathways from different organisms currently under investigation (alkaloids, terpenes, isoprenoids, polyphenols, stilbenoids and phenylpropanoids) with an emphasis on engineering and on the identification of overlaps including use of synergy effects.
- Define target research avenues based on the global knowledge accumulated and expertise gathered – e.g. definition of target pathways / plants, host organisms, around which efforts could be congregated and ambitious objectives be formulated.
- Investigate alternatives for unknown reaction steps in biosynthetic pathways which could be substituted by heterologous genes/enzymes to bridge knowledge gaps and/or by-pass current bottlenecks.
- Define biocatalytic reactions currently not feasible by chemosynthesis but that carry a potential to be solved by biotransformations, and defining bottlenecks in current strategies.
- Investigate precursor flux from primary to secondary metabolism, regulation by transcription factors and their applicability in heterologous systems.
- Investigate temporal and spatial distribution, accumulation and storage mechanisms of PNP in their host plants, or find ways to modify compounds for improved exudation, storage or separation.
- Define criteria for a modular handling of datasets that are underlying PNP production (including the generation of ontologies for PNPs and the organisms and enzymes involved in their production, and the generation of publicly available data standards).

– Strengthen the ability to produce PNP based drugs or raw materials from non-European plants (or such PNPs with a high uncertainty in availability) reliably within Europe.
– Connect academic and institutional research with key-players from industry. Identify lead compounds and structures with potential economical interest.

C.3 How will the objectives be achieved?

High-quality research on PNP biosynthesis and its application as well as modification is represented by numerous European scientists but scattered on a spatial and disciplinary level. To fully utilize this scientific potential the following deliverables are planned for the Action:

– Scientific kick-off meeting for the definition of the status quo of the European PNP research.
– Regular network meetings (MC, WGs) to coordinate research activities and future strategies.
– Launching of a web-site as a basis for members of the COST Action as a dissemination tool for easy access of data, publications, and files as well as a discussion forum.
– Development of databases and ontologies including PNPs and (correlated) enzymes/genes as building blocks for synthetic biology approaches to modify NP biosynthesis.
– Implementation of a database infrastructure to link all aspects of metabolic pathways and PNPs of interest, as defined by the COST participants.
– Organization of young scientist´s workshops.
– Cross-disciplinary exchange of young researchers via STSM.
– Crosstalk between scientist and representatives of pharmaceutical and chemical industries as well as plant breeders. Delegates will be invited to participate in the Action or attend meetings.
– Enhancing public awareness by publications in popular science media and the internet, preventing or at least reducing negative effects as observed by the introduction of transgenic food plants.

C.4 Benefits of the Action

The successful improvements of methods and tools as well as the definition of priority targets in plant metabolic engineering with the aim of producing valuable pharmaceuticals and fine chemicals will have benefits beyond the mere scientific value. First, providing new means to produce otherwise rare or expensive pharmaceuticals will ultimately benefit the patients demanding therapy with natural product-derived medicines at reasonable prices and in nearly unlimited supplies. Second, generating knowledge in plant metabolic engineering also strengthen European industries with emphasis on life science and biotechnology, as outlined the paper “Life Science and Biotechnology –A strategy for Europe” by the European Commission (COM(2002) 27). Last but not least, engineering PNP biosynthesis in plants will circumvent the extensive use of petroleum-based chemicals and the high-energy demanding fermentation and synthesis techniques, potentially reducing the environmental impact of the manufacturing of complex chemicals.

C.5 Target groups/end users

The primary target group consists of individual researchers from Europe and partnering countries, including universities, national and independent research institutes. This includes not only researchers actively or passively participating in this Action but every researcher in the broader field of PNP research and beyond who accesses data and strategies developed by the Action. Also researchers from chemical, pharmaceutical, food and feed and related industries will be beneficiaries from the scientific results generated by this Action. Last but not least also the European public will be a target group since information on medicinal plant biotechnology will be made available to govern an informed choice about life-science and biotechnology. Eventually agriculture and engineering might also benefit if high value production in the long term will be based directly on plants and their work-up (downstream processing) would be alleviated.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

Most PNPs can be grouped in a handful of categories regarding their biosynthetic precursors or their core structural features, e.g. alkaloids, polyketides, phenylpropanoids, or terpenoids. In contrast to this simplified picture the total number of described structures is enormous and the large number of plant species producing valuable compounds even makes the field more diverse. Hence, as diverse as the subject is, as diverse is the research community doing PNP studies in its broader sense. It is of utmost importance to define in the beginning of the Action the status quo of PNP worldwide and especially in Europe. Within this initiative a small number of PNP pathways and/or lead compounds will be identified which will serve key components and strategies for the scientific program.

There are three most important research tasks to be coordinated by this Action; i) the definition of a *status quo* of PNP research and engineering world-wide and especially in Europe, including the standard definition for a database providing tools and information for engineering PNP production; ii) the assessment, development and distribution of tools and methods for plant secondary metabolism manipulation in host and non-host species; iii) the assessment and development of a systems approach for plant metabolic engineering.

D.2 Scientific work plan methods and means

Over the duration of the COST Action the scientific program will be subdivided into Working Groups (WG) dealing with at least three different aspects of improving PNP metabolic engineering. By this approach experts with different scientific expertise will work in close proximity to guarantee an interdisciplinary approach and to maximize output of the WGs. Moreover, cross-linking of the different WGs will be of high importance since outcomes generated by one will directly influence another.

***Status quo* and road map (WG1)**

Numerous plant species produce and accumulate highly diverse compounds, many of scientific interest, but not all of medicinal or commercial value. WG1 is mainly of conceptual focus in that it generates data as well as tools to access these data, in that it identifies lead structures and pathways and in that it sets standards for the development of computational tools to focus and enhance PNP engineering.

- A census of identified biosynthetic pathways and of the utilization of medicinal and industrial relevant PNPs will be a major task. This approach will guarantee that not only pathways and compounds which are already under strong development come into focus of the Action, but also those which so far have an orphan status but hold high potential will be considered.
- Based on an initial meeting as well as on intensive literature search a database of PNP pathways under investigation, of plants and plant cell cultures thereof, and of relevant genes and encoded enzymes will be generated. This list will evolve over time since it takes outcomes of the accompanying WGs into account. Additionally, pathway intermediates and end products might be identified which are of high interest from a medicinal or industrial point of view.
- At the same time, the initial meetings will be used to develop and set standards on which a web based software will be established which would provide the following features: i) the possibility of finding ontologies for the PNP of interest; ii) definition of an online platform and its underlying data structure to allow access to achieved results, cross-links to existing outside resources, and achieved status of discussions among the COST Action participants.
- Based on the global information gathered, target research avenues will be defined, such as target pathways, plants and host organisms, around which efforts should be congregated, and ambitious objectives will be formulated.

The aim of this Action is to develop methods and means to generate designer medicinal plants or plant cell cultures. Although this is in complete accordance to the EU strategy to develop the European biotechnology sector, the underlying technology is still controversial for the general public. As seen for the introduction of transgenic crop plants, lack of understanding of the underlying technology along with unsubstantiated fear of potential impact on health and environment has brought the implementation of the technology in Europe almost to a halt.

It is highly advisable to use this early stage of development of medicinal plant genetic engineering to generate a public awareness of the rational of the initiative. Beside the information for the scientific community provided by the web page, also an information platform for policy makers and informed lays will be established.

Molecular tools (WG2)

The metabolic engineering of PNP biosynthesis in a given plant requires a set of techniques and tools, which are basically at hand but need to be evolved for this specific application. In a very reductionist manner they can be named i) identification, ii) engineering, and iii) transfer.

–*Identification***:** In a given plant the conversion of a substrate to a product is mediated by an enzyme, an enzyme complex or an enzyme cascade. Hence, a concerted series of enzymatic reactions starting from a precursor leading to the PNP constitutes the pathway. Other than in bacteria and some fungi, in plants the genes encoding the relevant enzymes are not clustered but scattered over the whole genome. Therefore, identification of the relevant genes, necessary for metabolic engineering is tedious und still lacking in many important pathways. However, some of the missing steps in certain pathways could be substituted by heterologous enzymes, either from different plants of even from completely different organisms (bacteria, fungi, animals). One task of this WG will be the establishment of a database compiling enzymes (cloned genes) active in PNP biosynthetic pathways leading to high value products. Data linked to genes and enzymes should include their activity regarding different substrates and their potential to be produced in heterologous systems and their localization in cellular

compartments or plant organs. Another important aspect of this WG is the potential generation of metabolites which are not naturally occurring in their hosts or only in minute amounts. These are especially functionalized derivatives of core structures, e.g. hydroxylation steps in terpene backbones. An extensive compilation of metabolizing enzymes which are candidates for such catalytic steps will be made available and utilized by the partners for their specific needs.

–*Engineering:* While numerous enzymes are known which catalyze specific reactions, their performance is often sub-optimal regarding turnover rates, substrate recognition or expression properties and stability in a given host. Here, engineering of genes, enzymes, and pathways to address these issues will be the main topic. Enzymes exhibiting a high degree of promiscuity (i.e. accepting various substrates) or liberality (i.e. producing various products from a single substrate) may be good starting points for assembling synthetic pathways. Candidate genes could come from either related or unrelated plant species as well as from different organisms. Here, candidate genes and enzymes will be grouped and evaluated for their suitability and made available for testing. Beside codon optimization for maximum expression in a given host also other factors like pre-sequences, stabilizing sequences, gene fusions, or compartmentalization will be evaluated and recommendations and tools developed. Tools available for the optimization include those currently used for protein-redesign, which includes rational redesign based on mechanistic or ontological enzyme analysis followed by (site-directed) mutagenesis, directed evolution, or targeted evolutionary strategies, or – in a more visionary sense – even de novo design. With suitable enzymes for the desired (bottleneck or diverting) step at hands, reverse molecular engineering to the genes and their expression.

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–*Transformation***:** For successful metabolic engineering of PNP pathways, not only the catalysts themselves are of importance but also the methods and tools to introduce them into a given host. WG2 will also compile and provide engineering tools (vectors, promoters, transformation, and regeneration protocols) for projects in metabolic engineering. While transformation of model plants and a few crop species is straight forward and well established in numerous laboratories, transformation of medicinal plants is much more sophisticated and

restricted to few specialized groups. In this WG a database with established transformation and regeneration protocols will be generated and distributed. Another crucial point is the use of transformation vectors, especially regarding their regulatory elements (promoters and terminators), putative targeting pre-sequences for translocation into sub-cellular compartments and their resistance marker for transgene selection. Introduction of single genes into model plants is usually straight forward, the combination of genes usually necessary for the modification or establishment of pathways is by far more challenging. Hence, tools and methods for transgene stacking will be evaluated and if possible disseminated. A centralized vector and strain depository would be ideal but require too many resources for their enduring operation. Therefore, a database directing requests to the individual researchers would be more suitable. With focus on medicinal plants, a training network for young scientists in transgenic techniques with rare and/or medicinal plants will help to disseminate the expertise throughout research labs in Europe.

System engineering approach (WG3)

The plant is a biological system that is characterized by a highly complex genetic and cellular network, locked together by dynamic, parallel and nonlinear feedback interactions. This poses a formidable challenge to a rational engineering approach of any PNP pathway but basic issues will be addressed here. The focus of this WG lies in the consideration of a plant as a complex system and in the development of approaches which take into account that on a system level a number of actuating variables could be changed to obtain the desired result.

– *Elicitor control***.** Plants utilize a limited number of signaling compounds for the regulation of totally different biosynthetic pathways, a process which is only partially understood but which needs to be taken into account when rational pathway engineering is a goal. Elicitor molecules like jasmonic acid or methyl jasmonate can be used to induce the expression of a desired product in a large number of diverse PNP pathways by switching on key genes regulating a specific pathway. This eventually might result in an increased accumulation of the desired product and this process is of special interest in tissue cultures since it defines the production stage and allows separating their growth and production phases. However, for an engineered pathway no such master switches do exist today. A major task of this WG will be

to identify general key switches which could be integrated into a synthetic pathway to make it controllable. This could be promoter elements and/or chemical inducible promoters which in combination with a key-component could regulate a synthetic PNP pathway. Also a key task will be the identification of factors which could modulate the half-life of an elicitor and therefore, might enable their more efficient and controllable use. Such factors could be either modifying or degrading enzymes which directly influence the stability and functionality of the elicitor.

– *Control of plant metabolic networks*. Transcription factors (TFs) are considered viable alternatives to 'single enzyme' approaches for the manipulation of plant metabolic pathways. Because of the ability to control multiple, if not all steps in a particular metabolic pathway, TFs provide attractive tools for overcoming flux bottlenecks involving multiple enzymatic steps, or for deploying pathway genes in specific organs, cell types or even plants other than their origin. Despite that several TFs have been intensely described and already been used to boost the synthesis of selected plant secondary metabolites, there are numerous PNPs for which no controlling TF has been identified so far. For the TFs already described it is necessary to gather more information on pathway branch specificity and cross-talking with other major biosynthetic pathways. It will also be accessed if TFs could be generically used in different systems.

– *Flux control*. Cellular response to genetic and environmental perturbations is often reflected by and/or mediated through changes in the general metabolism, because the latter plays a key role in providing energy and precursors for biosynthesis. Such metabolic changes are often exerted through transcriptional changes induced by complex regulatory mechanisms coordinating the activity of different metabolic pathways. Systems level modelling of cellular metabolism has proven to be indispensable for the design of rational genetic modification strategies for the redistribution of the metabolic flux network towards desired end-products. The immense versatility of the phenotypes observed in plants imposes certain limitations regarding the extent of genericalness in the use of the constructed models. In order to study network properties of plant metabolism and to assess the regulatory role of each reaction, various methods have to be applied, including Flux Balance Analysis, Principal Component Analysis of the flux space and reaction deletion studies which simulate the effect of knock-out studies. Such models will be evaluated for the target pathways defined and for their applicability in pathway engineering to increase the production of PNPs of interest.

Overall, the three WGs will generate valuable data which will be largely accessible. This will give the option of generating building blocks for approaches in synthetic biology to generate novel pathways for the production of PNPs or even non-natural substances. The strong interconnection of all research groups usually working in isolated fields in natural product synthesis (this is a certain plant species or compound class) will have the opportunity to cross-link their expertise with a large synergistic effect.

E. ORGANISATION

E.1 Coordination and organisation

Research work conducted in this Action is founded by various sources like national funding agencies, European Framework Programme initiatives as well as through charitable organizations and companies.

With the beginning of the Action, an organizational structure will be implemented according to the "Rules and Procedures for implementing COST Actions (COST 4159/10)". Function owners like Action Chair and vice-Chair will be elected on the first Management Committee (MC) meeting. The designated bodies like MC, WG and subgroups are sufficient to fulfil the tasks and no further structural diversification will be implemented from the first. If the MC identifies the need for further substructures during the Action (e.g. STSM manager, dissemination groups or others) it might implement such during a MC meeting. MC meetings will be held at least once (maximum two times) per year and preferably adjacent to other Action activities (workshops and WG-meetings) to minimize travel load and expenses.

The responsibilities and tasks of the MC are:

- Ensuring the high scientific standard of the Action
- Enabling and implementing the integration of early stage researchers in all structures of this COST Action
- Communication with COST Office and Domain Committees
- Supervising the organization of Workshops, meetings and STMS

– Considering gender aspects and family compatibility in all aspects of the Action
– Dissemination of results
– Communication with the public, with policy makers and industry representatives
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In the beginning a web-based platform will be implemented which not only serves as an information tool but also as communication platform for the participants and also will be expanded according to the progress of the scientific work in the different WGs.

Annual workshops organized by the MC and held by the WGs will assure that up-to-date information about individual and national research will be the basis for the work carried out in this Action.

Especially to support students and early stage researchers but also to strengthen the connection between the research laboratories the following instruments will be implemented:

*Short Term Scientific Mission (STSM)**:*** To use scarce equipment or to learn specific techniques students will be enabled to perform scientific work in another laboratory. It is intended that this research stay might also happen in a cross-disciplinary manner to broaden the scope of the young researchers and to integrate other disciplines (e.g. exchange between analytical chemistry, molecular biology, and computational biology).

*Training Schools**:*** With this instrument a group of students and young researchers will be introduced into a novel technique or discipline. Moreover, such an event will strengthen the connection between young European researchers. Topics will be identified in the beginning of the Action and two Training Schools will be offered, in the second and the fourth year.

E.2 Working Groups

The scientific program of this Action will be carried out by three Working Groups, coordinated by a Working Group leader which reports to the MC. WGs are:

- *Status quo* and road map (WG1)
- Molecular tools (WG2)
- System engineering approach (WG3)

WGs will meet on a regular basis and organize workshops, training schools and STSMs.

As outlined in their detailed program (D2) WG1-3 deal with scientific and technical issues and need to be highly interconnected. To ensure a continuous flow of information regarding the current status and progress in WG1-WG3 close interaction is mandatory. This will be achieved in that active members of one WG are also participating members in another WG, allowing the fastest and most efficient transfer of information. Moreover, changes of the specific work program might evolve during the duration of the Action and due to the joining of new participants and will be included in a flexible manner. WGs are organized by a WG leader, to be nominated at the first MC meeting, who is responsible for the reporting to the MC as well as for distribution of outcomes and results from their work.

To minimize fragmentation a limited number of WG is envisaged. However, in the beginning of the Action it might be necessary to form subgroups (SG) inside a WG to address more specific questions in a smaller group and work more efficiently. In this case, a SG leader will be nominated who reports to the WG leader.

All WGs will encourage a significant number of early stage researchers not only to participate but also to serve as WG or SG leaders.

E.3 Liaison and interaction with other research programmes

By its dedicated topic as well as by its interdisciplinary character this Action has intersections to some minor degree with a number of EU-Programmes and initiatives.

- COST Action FA0804 “Molecular Farming: plants as a production platform for high value proteins”
- COST Action CM0804 “Chemical biology with natural products”
- FP7 Program “SmartCell”
- FP7 Program “Metapro”

The commitment of this Action is to integrate all different approaches and to bring plant metabolic engineering in Europe to the next level. To obtain the greatest impact and to generate the maximum synergetic effect, members of other programs are either already supporters or participants of this Action or will be consulted if appropriate. However, this Action has a much broader scope and addresses a more diverse set of researchers.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.

In fact, a significant part of the supporting and participating PIs of the Action (currently more than 1/3) are female and it is envisaged to reach an even ratio over the duration of the Action. All partners belong to employers supporting equal opportunities and are actively promoting the recruiting of women.

Within the Action, special attention will be turned to the involvement of female researchers in the MC as well as in all WGs. Early Stage Researchers will not only benefit from skills training in workshops and STSM but also will actively be involved in commissions and in groups developing goals and roadmaps for future research efforts. This will ensure that they can early on in their career bring in own positions for the development of a scientific field.

F. TIMETABLE

The anticipated duration of the Action is four years but it is intended that the fruitful collaboration will continue well beyond this period. For building the network and fulfil the objectives a series of events is necessary.

Activity	Year 1				Year 2				Year 3				Year 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Kick-off meeting	x															
MC-meeting		x		x		x		x		x		x		x		x
Progress report				x				x				x				
Final report																x
WG meeting		x		x		x		x		x		x		x	x	
Workshop				x				x				x			x	
STSMs			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Training Schools							x								x	
Website launch			x													

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, CH, DE, DK, EL, ES, FI, FR, IL, IT, NL, PL, PT, RO, SE, TR and UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 72 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The aim of the proposed project is the bundling of the so far scattered research in plant metabolic engineering for high value products across Europe and to define standards and procedures for the development of designer plants for the production of PNPs. Therefore, major target audiences for the dissemination of results, guidelines and tools are from the following areas:

- Researchers working in plant physiology and biochemistry, phytochemistry, computational biology/chemistry and bioinformatics, system biology, and synthetic biology.
- Scientists and technical personnel in companies dealing with the production of pharmaceuticals and fine chemicals from natural sources, in seed companies and breeder associations as well as in food and feed production.
- Policy makers and stakeholders. Since biotechnology is an integral part of the European strategy for a knowledge-based bio-economy, information and tools gathered by this Action will be of high importance for current and future research strategies.
- Scientific and learned societies as multipliers of novel findings and techniques.
- The general public, especially interested laypersons and pupils. Part of the outcome of this Action will be prepared to serve as information for the public and will inform about technology, innovations, as well as risk assessment.

H.2 What?

Personal communication during meetings and workshops will be the most efficient tool not only for obtaining scientific results but also for their dissemination. Besides that, the most efficient method for the dissemination of information today is a web-based platform. Therefore, the development and implementation of a web-based information system is the core tool and dissemination method. But also traditional print forms will be utilized depending on the nature of the documents.

- For the scientists participating in the Action a password-accessible platform will be provided in which web-based tools, a topic-related Wiki, key documents, and procedures can be found. General documents and developed guidelines will be accessible for all interested scientists.
- Open-access information, reports as well as documents for a lay-audience will be made available via the web-based platform.
- Training schools and workshops will form a base for communication.

H.3 How?

To gain the highest visibility it is of utmost importance to direct attention of target audiences to the COST Action and its information platform. Therefore, all participants will act as multipliers in that they promote the Action during scientific meetings, student courses, and all suitable events. According to the status of the Action information material will be made available (flyers, brochures and others) which draws the attention to more detailed information on the Action website.